

Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party

David Grimwade, Andrea Biondi, Marie-Joëlle Mozziconacci, Anne Hagemeyer, Roland Berger, Michael Neat, Kathy Howe, Nicole Dastugue, Joop Jansen, Isabelle Radford-Weiss, Francesco Lo Coco, Michel Lessard, Jesus-Maria Hernandez, Eric Delabesse, David Head, Vincenzo Liso, Danielle Sainty, Georges Flandrin, Ellen Solomon, Françoise Birg, and Marina Lafage-Pochitaloff, on behalf of Groupe Français de Cytogénétique Hématologique, Groupe Français d'Hématologie Cellulaire, UK Cancer Cytogenetics Group, and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies"

Acute promyelocytic leukemia (APL) is typified by the t(15;17), generating the *PML-RAR α* fusion and predicting a beneficial response to retinoids. However, a sizeable minority of APL cases lack the classic t(15;17), prompting the establishment of the European Working Party to further characterize this group. Such cases were referred to a workshop held in Monza, Italy and subjected to morphologic, cytogenetic, and molecular review, yielding 60 evaluable patients. In the majority (42 of 60), molecular analyses revealed underlying *PML/RAR α* rearrangements due to insertions (28 of 42) or more complex mechanisms, including 3-way and simple variant translocations (14 of

42). Metaphase fluorescence in situ hybridization (FISH) demonstrated that insertions most commonly led to formation of the *PML-RAR α* fusion gene on 15q. In 11 of 60 workshop patients, *PLZF/RAR α* rearrangements were identified, including 2 patients lacking the t(11;17)(q23;q21). In one case with a normal karyotype, FISH analysis revealed insertion of *RAR α* into 11q23, and *PLZF-RAR α* was the sole fusion gene formed. Two patients were found to have t(5;17), one with a diffuse nuclear *NPM* staining pattern and with *NPM-RAR α* and *RAR α -NPM* transcripts detected. In the other with an unbalanced der(5)t(5;17)(q13;q21) and a nucleolar *NPM* localization pattern, an

NPM/RAR α rearrangement was excluded, and FISH revealed deletion of one *RAR α* allele. In the remaining 5 workshop patients, no evidence was found for a rearrangement of *RAR α* , indicating that in rare instances, alternative mechanisms could mediate the differentiation block that typifies this disease. This study highlights the importance of combining morphologic, cytogenetic, and molecular analyses for optimal management of APL patients and better understanding of the pathogenesis of the disease. (Blood. 2000; 96:1297-1308)

© 2000 by The American Society of Hematology

Introduction

Acute promyelocytic leukemia (APL) is defined by particular morphologic features (see the accompanying article in this issue, by Sainty et al¹). A number of key clinical features set APL apart from other forms of acute myeloid leukemia (AML), which underlie the need for accurate diagnosis. These include a potentially devastating coagulopathy, which carries a high risk of mortality unless specifically addressed (reviewed by Tallman and Kwaan²; Barbui et al³), and sensitivity to retinoid differentiating agents including all-*trans* retinoic acid (ATRA) (reviewed by

Degos et al⁴) and to novel agents such as arsenic trioxide (As₂O₃).^{5,6} Early studies suggested that retinoids reduce the hemorrhagic complications of APL, whereas use of ATRA in combination with chemotherapy has been shown to confer significant improvements in overall survival compared with treatment with chemotherapy alone.⁷⁻¹¹ Hence, combination therapy with ATRA and chemotherapy has now been adopted as the standard treatment approach for this disease. For the majority of APL patients achieving complete remission (CR), the long-term outlook

From the Division of Medical and Molecular Genetics, Guy's, King's, and St. Thomas' School of Medicine, London, United Kingdom; Centro di Ricerca M. Tettamanti, Monza, Italy; Institut Paoli-Calmettes, INSERM U119, IFR 57 and Université de la Méditerranée, Marseille, France; Center for Human Genetics, Leuven, Belgium; INSERM U434, Institut de Génétique Moléculaire, Paris, France; St. Bartholomew's and the Royal London School of Medicine, London, United Kingdom; Laboratoire de Génétique des Hémopathies, CHU Purpan, Toulouse, France; Institute for Hematology, Erasmus University, Rotterdam, The Netherlands; Hôpital Necker Enfants Malades, Paris, France; Dipartimento di Biotechnology Cellulare ed Ematologia, Università La Sapienza, Roma, Italy; Laboratoire de Cytogénétique, CHU de Brest, France; Hospital Universitario de Salamanca, Salamanca, Spain; St. Jude Children's Hospital, Memphis, TN; and Cattedra di Ematologia i Policlinico di Bari, Bari, Italy.

Submitted December 9, 1999; accepted June 7, 2000.

Other participants are listed in the appendix of the accompanying manuscript by Sainty et al.¹

Support: D.G. is supported by the Leukaemia Research Fund of Great Britain; K.H. by the Imperial Cancer Research Fund; E.S. by European Community

(BMH4-CT98-3745); A.B. by Fondazione M. Tettamanti, Associazione Italiana Ricerca sul Cancro (AIRC) and MURST; F.L.C. by CNR, Target Project on Biotechnology, AIRC, and MURST; and M.L.P. by the Comité des Bouches-du-Rhône de la Ligue Nationale Française contre le Cancer. This work was supported by the European Community Biomed Concerted Action "CT94-1703," Molecular Cytogenetic Diagnosis in Haematological Malignancies, by the Flemish Government in the frame of action Kom op tegen Kanker/Vlaamse Kankerliga. This study includes research results of the Belgian program of Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming; scientific responsibility for this work is assumed by the authors.

Reprints: Marina Lafage-Pochitaloff, Laboratoire de Cytogénétique Hématologique, Institut Paoli-Calmettes et INSERM U119, 232 bd Sainte Marguerite, 13009 Marseille, France; e-mail: cytoген@arseille.fnclcc.fr.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2000 by The American Society of Hematology

is now favorable because of a relatively low risk of relapse, and routine use of bone marrow transplantation (BMT) in first CR is no longer recommended.

APL is characterized by the reciprocal translocation t(15;17)(q22;q21), disrupting the *PML* and *RAR α* genes, which are localized to chromosomes 15q and 17q, respectively (reviewed by Melnick and Licht¹²). The t(15;17) generates 2 chimeric genes: *PML-RAR α* is formed on the derivative 15 [der(15)], whereas the reciprocal *RAR α -PML* fusion is located on the derivative 17 [der(17)]. *PML* and *RAR α* have both been implicated in normal hemopoiesis.¹³⁻¹⁶ *PML* possesses growth suppressor and proapoptotic activity¹⁶⁻¹⁹ and is predominantly localized to discrete multiprotein nuclear structures (*PML* nuclear bodies), which become disrupted in the presence of the *PML-RAR α* fusion protein^{20,21} (reviewed by Hodges et al²²). *RAR α* is a transcription factor that mediates the effect of retinoic acid (RA) at specific response elements; high-affinity binding of the receptor to DNA is achieved through heterodimerization with a member of the distinct family of retinoid X receptors (RXR; reviewed by Chambon²³). Previous studies suggested that integrity of the retinoid signaling pathways is necessary for normal myeloid differentiation.¹³⁻¹⁵ The *PML-RAR α* protein retains key functional domains of both *PML* and *RAR α* , suggesting that it plays an important role in leukemogenesis. *PML-RAR α* may impair the growth suppressor and proapoptotic functions of *PML*, contributing to leukemic transformation, and also may induce a block in myeloid differentiation by repression of RA target genes through recruitment of co-repressor molecules and histone deacetylase; the latter phenomenon may also be compounded by sequestration of RXR (reviewed by Melnick and Licht¹²; Grimwade²⁴). The important role played by *PML-RAR α* in leukemogenesis has been confirmed recently using transgenic mice (reviewed by He et al²⁵; Westervelt and Ley²⁶). However, it should be noted that in these studies, less than a third of the animals expressing *PML-RAR α* ultimately developed APL. Furthermore, a latent period of several months was observed before manifestation of the leukemia, leading to the suggestion that additional oncogenic events are required and arousing interest as to whether the reciprocal *RAR α -PML* fusion product plays a role in this process.²⁷

Previous studies suggested that the *PML-RAR α* fusion protein not only induces the differentiation block that characterizes APL, but paradoxically is also important for mediating the differentiation response to ATRA.^{12,24} Hence, APL patients with cryptic formation of the *PML-RAR α* fusion gene share the beneficial response to retinoids and the favorable prognosis associated with the group with documented t(15;17).^{11,28,29} This finding highlights the importance of establishing the presence of the *PML/RAR α* rearrangement in patients with morphologic APL, not only for optimal management such that all patients who could benefit are not denied treatment with ATRA, but also for meaningful analysis of clinical trials involving retinoids.

Over the last few years, considerable reliance has been placed on conventional cytogenetics to confirm a morphologic diagnosis of APL, as a means of determining the treatment approach. In the majority of cases the t(15;17) is detected³⁰; however, more recently a series of alternative chromosomal aberrations have been reported, including t(11;17)(q23;q21),^{31,32} t(5;17)(q35;q12-21),³³ t(11;17)(q13;q21),³⁴ and der(17),³⁵ whereby *RAR α* is fused to the *PLZF*, *NPM*, *NuMA*, and *STAT5b* genes, respectively. In common with *PML-RAR α* -associated APL, patients with fusion genes involving *NPM* and *NuMA* appear to be sensitive to ATRA.^{34,36} In contrast, APL associated with a *PLZF/RAR α* rearrangement is typified by lack of a differentiation response to retinoids, and

patients with this disease treated with ATRA alone have a poor prognosis.³⁷ Recent studies have correlated ATRA sensitivity with ligand-dependent dissociation of the co-repressor complex from the APL-associated chimeric fusion proteins (reviewed^{12,24}). At pharmacologic levels of ATRA, the co-repressor complex is released from the retinoid receptor moiety of *PML-RAR α* , *NPM-RAR α* , *PLZF-RAR α* , and presumably *NuMA-RAR α* fusion proteins; however, the *PLZF-RAR α* fusion additionally binds co-repressors to its *PLZF* moiety in a retinoid-insensitive fashion. This latter phenomenon has been proposed to account for the lack of response to ATRA that characterizes cases of APL with the t(11;17)(q23;q21). However, it remains a possibility that the reciprocal derived *RAR α -PLZF* fusion could also contribute to retinoid resistance in this subtype of APL because its expression is potentially up-regulated by ATRA, which could induce persistent deregulation of the cell cycle.^{12,37} Clearly, molecular characterization of cases of APL with alternative translocations has provided insights not only into the pathogenesis of the disease, but also into the mechanisms underlying the response to retinoids. In the present study, the European Working Party performed morphologic, cytogenetic, and molecular review of 60 evaluable APL patients lacking the classic t(15;17) and sought to determine the frequency of such cases.

Patients and methods

Patient characteristics

The European Working Party sought to characterize AML cases classified as APL, but lacking the t(15;17). Overall, 42 institutions from 6 European countries in addition to Memphis, TN participated in this study, as detailed in the accompanying paper.¹ Ninety cases of suspected APL were reviewed in a workshop held in Monza, Italy in June 1997. Cases were subjected to central morphologic review.¹ The corresponding karyotypes and molecular data were reviewed simultaneously but separately. Morphologic reviewers were ignorant of the cytogenetic and molecular data; in a second step, the reviewed data were combined and considered in the context of the clinical features. Patients were considered eligible for inclusion in the study only if all the following criteria were satisfied: (1) Morphologic features were consistent with or evocative of FAB type M3 or M3v. (2) Karyotype analysis was successful and the t(15;17) had been excluded. For patients with a normal karyotype, at least an overnight culture had to be performed to avoid normal metaphases from erythroblasts. (3) Molecular analysis had been performed by at least one of the following techniques: fluorescence in situ hybridization (FISH), reverse transcriptase-polymerase chain reaction (RT-PCR), or Southern blot analysis. On this basis, 30 patients were excluded from further consideration: 4 lacked features of APL (1 was classified as FAB type M1, 3 as FAB M2); in 15 cases there was no suitable material for further molecular analyses; in 4 cases cytogenetic review revealed a minor clone with t(15;17); and 7 cases presented with ider(17q), which was deemed to be a secondary abnormality to the t(15;17). Morphologic and immunophenotypic features of the evaluable patients are considered in the accompanying manuscript,¹ which shares the same case numbers.

FISH analyses

Analyses documenting the occurrence of *PML/RAR α* rearrangements were generally performed using ICRF *PML* and *RAR α* cosmid probes (from Ellen Solomon, Guy's, King's, and St. Thomas' School of Medicine, London), which were distributed among workshop members (courtesy of K. Howe and F. Birg). Details of the probes used and their positions relative to the *PML* and *RAR α* breakpoint regions are shown in Figure 1; methods used by the participating laboratories have been fully described previously.^{38,39} The genomic map of *RAR α* and exon numbering were according

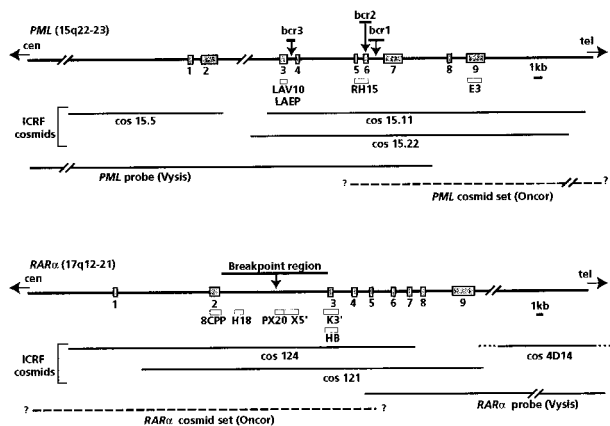


Figure 1. Map of the probes used for Southern blot and FISH analyses. Bars indicate Southern blot probes; lines indicate probes used for FISH analyses. The P63 probe, which includes the entire *RARα* cDNA, was also used in FISH experiments.

to Hjalt and Murray.⁴⁰ In some instances, commercially available probes were also used, in accordance with the manufacturer's instructions. The Oncor t(15;17) probe set (Gaithersburg, MD) contains digoxigenin-labeled cosmid specific for the 17q21 region and biotin-labeled cosmid specific for the 15q22 region. These probes are designed to detect the *RARα-PML* fusion gene on the der(17), but more precise mapping details are not provided by the manufacturer. Thus, we initially evaluated this probe set on a series of 5 patients with t(15;17); secondary *RARα* and *PML* signals were detected on the der(15) in 5 of 5 and 4 of 5 cases, respectively, leading to 2 fusion signals in metaphases and nuclei. This result suggests that the *RARα* probe spans the t(15;17) breakpoint, as does the *PML* probe in some instances.

The Vysis probe set (Downers Grove, IL), which is designed to detect the *PML-RARα* fusion gene, comprises a mixture of directly labeled probes: a *PML* probe, which begins in intron 7 and extends toward the centromere for 180 kb, and a *RARα* probe, which begins in intron 4 and extends toward the telomere for 400 kb (Figure 1).

In some instances, especially for complex karyotypes, whole chromosome painting (wcp) probes and centromeric probes (Cambio, Cambridge, UK; Oncor; Vysis) were used in single or dual-color FISH experiments.

Twenty-four-color FISH karyotyping⁴¹ was carried out on the unique case presenting with *RARα-PML* as the sole fusion gene using a 24Cyte kit (MetaSystems, GmbH, Altlußheim, Germany). Multicolor banding (mBAND) of chromosome 5 was carried out in the 2 t(5;17) cases as described recently,⁴² using an XCyte 5 kit (MetaSystems) according to the manufacturer's protocol. A DMRB epifluorescence microscope equipped with a motorized filter wheel and specific filters was used (Leica, Rueil-Malmaison, France). Images were captured and processed using the Isis/M-FISH (Multicolor FISH) imaging system (MetaSystems).

RT-PCR and Southern blot analyses

PML-RARα and *RARα-PML* fusion genes were detected by nested or semi-nested RT-PCR according to one of the previously described methods.⁴³⁻⁴⁵ *PLZF-RARα* and *RARα-PLZF* fusion transcripts were detected as described previously^{37,38,46}; however, for cases found by *PLZF-RARα* RT-PCR to have a 3' breakpoint in *PLZF* (leading to retention of 3 PLZF zinc fingers in the PLZF-RARα fusion protein),^{37,47} *PLZF* primers R1 and R2 (Table 1) were used for amplification of reciprocal *RARα-PLZF* transcripts. To detect *NPM-RARα* and *RARα-NPM* fusion genes, we performed nested RT-PCR using *NPM* primers detailed in Table 1 in conjunction with previously described external and internal *RARα* primers.⁴⁴ Identical *RARα* primers were used with *STAT5b* primers (Table 1) and previously described *NuMA* primers³⁴ (*N2a* [external], *Alt1b*, *Alt2b*, *N2b* [internal]) for nested RT-PCR to screen for *STAT5b-RARα* and *NuMA-RARα* fusion genes, respectively. Where availability of DNA permitted, workshop cases were also subjected to Southern blot analysis using the probes shown in Figure 1, as described previously.⁴⁸⁻⁵⁰

ATRA in vitro differentiation assays

Assays were performed according to a previously described method⁵¹ using ATRA at a final concentration of 10⁻⁶ mol/L.

Immunofluorescence

Immunofluorescence studies were performed as described previously using the monoclonal NA24 NPM antibody⁵² (gift from J. Cordell and D. Mason) and polyclonal^{38,53} or monoclonal (5E10⁵⁴ or PG-M3^{29,55}) *PML* antibodies.

Results

Central morphologic, cytogenetic, and molecular review undertaken at the Monza Workshop yielded 60 evaluable patients with confirmed APL lacking the t(15;17). The review process led to the definition of the following subgroups: (1) *PML/RARα* rearrangements (n = 42), including insertions (28 of 42) and complex chromosomal changes (14 of 42); (2) *PLZF/RARα* rearrangements (n = 11); (3) t(5;17) (n = 2); and (4) APL lacking rearrangement of *RARα* (n = 5). Central morphologic review revealed no major differences between the appearances of material derived from patients with *PML/RARα* rearrangements and from 20 control patients with documented t(15;17). Among the remaining patients, only those with *PLZF/RARα* rearrangements were found to have distinct morphologic features allowing their recognition, as described in the accompanying paper.¹

Characterization of APL workshop patients lacking the t(15;17), with underlying *PML/RARα* rearrangements

Insertion (15;17) or (17;15). In 28 patients including 16 with a normal karyotype, FISH and molecular findings were consistent with *PML/RARα* rearrangements being mediated by insertion (ins) events.

Metaphase FISH was performed in 20 of 28 patients (cases 1-20). In the majority (15 of 20), a fusion or co-localization signal reflecting the formation of *PML-RARα* was localized to 15q (cases 1-15). In one such patient (case 4), fusion signals were detected on both chromosomes 15, suggesting either loss of the normal 15 and duplication of the der(15) or recombination between the 2 homologs after the insertion event (Figure 2). Indeed, a mitotic recombination event leading to *BCR-ABL* fusion signals on both

Table 1. Primers used for RT-PCR analyses

Fusion gene target	Primer name and sequence
<i>RARα-PLZF</i> (3' <i>PLZF</i> breakpoint)	<i>PLZF-R1</i> (external): 5'-CTCTTGAGTGTGCTCTCATC-3'
	<i>PLZF-R2</i> (internal): 5'-CTTCCCACACAGCAGACAGA-3'
<i>NPM-RARα</i>	<i>NPM-F1</i> (external): 5'-GGAAGATTCGATGGCATGG-3'
	<i>NPM-F2</i> (internal): 5'-TCGGTGTGAACTAAAGGCC-3'
<i>RARα-NPM</i>	<i>NPM-R1</i> (external): 5'-TCTGTGGAACCTTGCTACCA-3'
	<i>NPM-R2</i> (internal): 5'-TGACTCTGCATCTTCTCCA-3'
<i>STAT5b-RARα</i>	<i>STAT5b-StgU</i> (external):*
	5'-GTTTGACGGTGTGATGGAAGTG-3'
	<i>STAT5b-F2</i> (internal): 5'-CAAGCCTCATTGGAATGATG-3'

*Primer derived from Arnould et al.³⁵

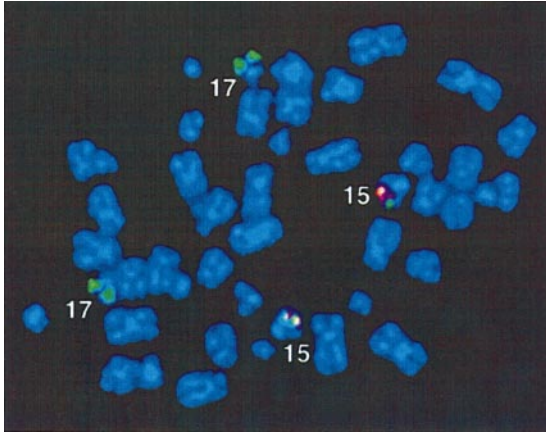


Figure 2. FISH analysis of case 4. The Oncor *RAR α* and *PML* probes showed fusion signals on both chromosomes 15; similar results were obtained with the Vysis probe set.

chromosomes 9 has been described in a Ph-negative case of chronic myeloid leukemia (CML) with submicroscopic *ins(9;22)*.⁵⁶ In 7 *ins(15;17)* cases, the Oncor probe set was used in parallel either with ICRF *PML* 15.5 and *RAR α* 121 cosmids (4 cases) or Vysis probes (3 cases), giving identical results, suggesting that the Oncor *RAR α* probe is not only centromeric, but also spans the 17q breakpoint (see Patients and methods and Figure 1). All of these *ins(15;17)* patients ($n = 15$) had apparently normal chromosomes 15 and 17 by conventional cytogenetic analysis and by FISH using wcp probes (7 of 7) and were thus cryptic. Furthermore, diagnostic karyotype was normal in the majority (9 of 15); in such patients, fusion signals were detected in the context of normal metaphases, thereby establishing that the results of cytogenetic analysis reflected sampling of leukemic cells rather than residual normal marrow elements. RT-PCR performed in 10 *ins(15;17)* patients revealed expression of *PML-RAR α* (Table 2). Reciprocal *RAR α -PML* transcripts were not detected in the 7 patients investigated, consistent with the occurrence of insertion events in these patients.

In 5 of 20 patients (cases 16-20), metaphase FISH was consistent with insertion of chromosome 15 material into 17q. In 2 of these *ins(17;15)* patients (cases 18 and 19), this insertion was apparent by conventional cytogenetic analysis and was confirmed by FISH using wcp 15 and 17 probes, whereas in the others, chromosomes 15 and 17 were normal by conventional cytogenetic analysis. In 4 of 5 patients (cases 16-19), formation of the *PML-RAR α* fusion gene was confirmed and localized to 17q in 3 patients studied by metaphase FISH. In the remaining *ins(17;15)* patient (case 20), molecular analyses suggested that *RAR α -PML* was the sole fusion gene resulting from the insertion event, as reported previously.^{29,39} Twenty-four-multicolor karyotyping performed on the latter patient did not reveal any chromosomal change on the 20 metaphases analyzed (data not shown), confirming the small size of the insertion event and the absence of superimposed chromosomal abnormality at the level of resolution of conventional cytogenetics and M-FISH.

In 8 of 28 patients (cases 21-28), metaphase FISH was not performed. However, these cases were considered as probable insertions because they presented with normal chromosomes 15 and 17 and expressed the *PML-RAR α* transcript. Karyotype was normal in the majority (6 of 8), and *RAR α -PML* transcripts were not detected by RT-PCR in either of the 2 patients tested.

PML immunofluorescence was performed in 6 of 28 insertion cases. In 5 patients with cryptic formation of *PML-RAR α* fusion genes, the classic microparticulate nuclear staining pattern was

observed and correlated with a positive in vitro ATRA response in the 2 patients studied (Table 2). In contrast, in case 20, in which *RAR α -PML* was the sole fusion gene formed, a wild-type nuclear staining pattern was detected, correlating with a negative in vitro ATRA response as reported previously.^{29,39}

Complex rearrangements. In 14 patients, the *PML-RAR α* fusion gene was formed as a result of complex rearrangements involving at least 3 chromosomes, as detailed in Table 3. Such complex cases can be classified into 3 categories: (1) complex variant *t(15;17)* due to a 3-way balanced translocation involving 15q22, 17q21, and another chromosome; (2) simple variant *t(15;17)*, apparently involving either 15q22 or 17q21 with another chromosome; and (3) very complex cases.

In 6 patients, a complex variant due to 3-way balanced *t(15;17)* was defined; all partner chromosomal bands involved were different, as shown in Table 3. In both cases in which metaphase FISH was performed, *PML-RAR α* was found on the der(15).

In 2 patients, a simple variant *t(15;17)* was identified. Case 35 presented with a *t(5;15)(q13;q22)*, but FISH demonstrated a *PML-RAR α* fusion on the der(15). Case 36 was previously reported to have a normal karyotype by R banding, to express a *PML-RAR α* transcript, and was shown to have a *t(1;17)* by wcp.⁵⁷ Further analysis was performed by the workshop; DAPI banding permitted visualization of the *t(1;17)*, and FISH demonstrated formation of *PML-RAR α* on 1p34, as shown in Figure 3. These simple variant cases are likely to be due to the combination of a reciprocal translocation and a submicroscopic insertion, leading to the formation of the *PML-RAR α* fusion gene.

Six patients were classified as very complex cases. In 2 of 6 (cases 37 and 38), formation of the *PML-RAR α* fusion gene was due to a submicroscopic *ins(15;17)* demonstrated by FISH. In case 39, *PML-RAR α* resulted from a 4-way balanced translocation combined with an insertion of a chromosome 2p segment into the der(17). In case 40, *PML-RAR α* fusion signals were observed in nuclei, but the chromosomal location could not be determined because of the lack of evaluable metaphases. RT-PCR performed in cases 41 and 42 revealed expression of *PML-RAR α* transcripts; however, FISH analysis with Oncor probes did not show any fusion signals, but rather duplication or triplication of *RAR α* signals on the der(17). Because these probes optimally detect the *RAR α -PML* fusion gene on the der(17) in patients with the classic *t(15;17)*, the absence of detectable fusion signals in these patients is consistent with lack of formation of the *RAR α -PML* gene. Unfortunately, insufficient material was available to perform further metaphase FISH documenting the location of the *PML-RAR α* fusion gene.

Cases lacking *PML/RAR α* rearrangements

***PLZF-RAR α* cases.** In 11 of 60 workshop patients, APL was associated with a *PLZF/RAR α* rearrangement as determined by RT-PCR, including 5 patients that have not been reported previously (Table 4). Nine patients were found to have the reciprocal translocation *t(11;17)(q23;q21)*; in each of the 6 such patients analyzed, reciprocal *RAR α -PLZF* transcripts were detected in addition to *PLZF-RAR α* . *RAR α -PLZF* has been postulated to contribute to leukemogenesis and may play a role in the ATRA resistance associated with this subtype of APL.¹² Therefore, it was of interest to characterize 2 cases (cases 50 and 52) of *PML-RAR α* - and *t(11;17)*-negative APL with morphologic features that were typical of patients with the *t(11;17)*.¹ Case 50 presented with a *del(11)(q23)*, whereas case 52 had a normal karyotype. In both cases, FISH using wcp probes specific for chromosomes 11 and 17

Table 2. *PML/RAR α* rearrangements due to insertions

Case no.*	Karyotype†	<i>PML-RARα</i> FISH	<i>PML-RARα</i> RT-PCR	<i>RARα-PML</i> RT-PCR	PML IF	In vitro ATRA response	Reference
Insertions (15;17)‡							
1/M3	47,XY,+8/46,XY	Fusion on 15q§	ND	ND	ND	ND	
2/M3	47,XY,+8/47,idem,del(9)(q23q32)/46,XY	Fusion on 15q	ND	ND	ND	ND	
3/M3	46,XY	Fusion on 15q ¶	Positive (bcr3)	Negative	Microparticulate	Positive	29 (case 3)
4/M3	46,XX,der(19)t(8;19)(q21;p13 or q13)/46,XX	Fusion on both 15q§	Positive (bcr3)	Negative	ND	ND	
5/M3	46,XX	Fusion on 15q	ND	ND	ND	ND	
6/M3	46,XY	Fusion on 15q ¶	Positive (bcr1)	Negative	Microparticulate	ND	38 (case 2)
7/M3	46,XY	Fusion on 15q§	Positive (bcr1)	Negative	ND	ND	73
8/M3, M3r	45,X,-Y/46,XY	Fusion on 15q #	Positive (bcr3)	Negative	Microparticulate	Positive	29, 39 (case 1)
9/M3	47,XX,del(5)(q33q35),+i(8)(q10)/45,XX,del(5),add(7)(q32),-21	Fusion on 15q ¶	Positive (bcr3)	Negative	ND	ND	38 (case 1)
10/M3v	46,XY	Fusion on 15q§	Positive (bcr1)	ND	ND	ND	
11/M3v	46,XY	Fusion on 15q§	Positive (bcr3)	ND	ND	ND	
12/M3v	47,XX,+8/46,XX	Fusion on 15q§	Positive (bcr1)	ND	ND	ND	
13/M3v	46,XX	Fusion on 15q	ND	ND	ND	ND	
14/M3v	46,XY	Fusion on 15q ¶	Positive (bcr3)	Negative	ND	ND	38 (case 3)
15/M3	46,XY	Fusion on 15q¶	ND	ND	ND	ND	
Insertions (17;15)‡							
16/M3	46,XX,del(9)(q12q33)/46,XX	Fusion on 17q**	Positive (bcr3)	ND	ND	ND	
17/M3	47,XY,+21/48,idem,+8/46,XY	Fusion on 17q¶	ND	ND	ND	ND	
18/M3	46,XX,ins(17;15)(q21;q21q22)/46,XX	ND	Positive (bcr1-2)	ND	ND	ND	
19/M3	46,XY,t(4;16)(p14;q22),t(9;12)(q22;q24),ins(17;15)(q21;q15q22)/46,idem,t(6;8)(q13;q22)/46,XY	Fusion on der(17q) ¶	Positive (bcr1)	Negative	ND	ND	38 (case 4)
20/M3v	46,XX	<i>PML</i> and <i>RARα</i> signals on 17q#	Negative	Positive (bcr3)	Wild-type	Negative	29, 39 (case 2)
Probable insertions							
21/M3	45,XY,add(2)(q37),-7,add(9)(p22)/45,idem,add(10)(p14)	ND	Positive (bcr1)	ND	ND	Positive	
22/M3	46,XY	ND	Positive (bcr1)	ND	ND	ND	
23/M3	46,XX,del(7)(q22q32-34)/46,XX	Fusion on nuclei ¶	Positive (bcr3)	Negative	Microparticulate	ND	38 (case 5)
24/M3	46,XX	ND	Positive	ND	ND	ND	
25/M3	46,XX	Fusion on nuclei ¶	Positive (bcr3)	Negative	Microparticulate	ND	38 (case 6)
26/M3, M3r	46,XX	ND	Positive (bcr3)	ND	ND	ND	
27/M3	46,XY	ND	Positive	ND	ND	ND	
28/M3	46,XY	ND	Positive (bcr2)	ND	ND	ND	

IF indicates immunofluorescence; ND, not determined.

*Morphology as follows: M3, hypergranular classic M3; M3v, hypogranular variant M3; M3r, M3 with more than 30% regular nuclei as defined in the accompanying manuscript by Sainty et al.¹

†As obtained by conventional cytogenetics.

‡As demonstrated by metaphase FISH.

§FISH performed with Vysis probe set.

||FISH performed with Oncor probe set.

¶FISH performed with *PML* 15.5 and *RAR α* 121 probes.

#FISH performed with *PML* E3 and *RAR α* cDNA P63 probes.

**FISH performed with *PML* 15.22 and *RAR α* 4D14 probes.

did not show any exchange of material involving these 2 chromosomes. However, FISH using the ICRF *RAR α* 121 probe demonstrated signals on chromosome 11q23 in case 52 (Figure 4A). RT-PCR confirmed formation of a *PLZF-RAR α* fusion gene in both patients. In case 52, there was sufficient diagnostic material to evaluate whether *PLZF-RAR α* was the sole fusion gene formed, and indeed *RAR α -PLZF* transcripts were not detected by RT-PCR, consistent with a submicroscopic insertion event (Figure 4B). Overall, *PLZF* breakpoints were determined in 10 patients: 7 had a 5' (intron 2) breakpoint (2 *PLZF* zinc fingers retained in *PLZF-RAR α*) and 3 had a 3' (intron 3) breakpoint (3 *PLZF* zinc fingers retained); introns were numbered according to Zhang et al.⁴⁷

PML immunofluorescence was performed in 6 patients, revealing in

each case discrete nuclear dots in leukemic blasts (Figure 4C,D), indistinguishable from the pattern observed in non-APL controls.³⁸ This contrasted with the characteristic microparticulate distribution detected in *PML-RAR α* -positive patients, as described above and previously.^{29,38} No terminal granulocytic morphologic differentiation was observed in the presence of 10⁻⁶ mol/L ATRA in vitro either in the 5 t(11;17) patients tested or in the ins(11;17) patient (case 52).

Clinical features of workshop patients with *PLZF/RAR α* rearrangements are presented in Table 4. In contrast to a previous study, which highlighted the adverse prognosis of t(11;17) patients treated with ATRA alone,³⁷ each of the 10 patients in the present study treated with combination chemotherapy achieved a CR, in 6 of whom induction chemotherapy was accompanied by ATRA. No

Table 3. *PML-RAR α* cases due to complex rearrangements

Case no.*	Karyotype	<i>PML-RARα</i> FISH	<i>PML-RARα</i> RT-PCR
Three-way translocations			
29/M3	46,XY,t(1;17;15)(p32;q21;q22)/46,idem,add(21)(p13)/46,XY	ND	Positive
30/M3v	46,XX,t(7;17;15)(q22;q21;q22)	Fusion on der(15)	ND
31/M3v	46,XY,t(6;17;15)(p21;q21;q22)/46,XY	ND	Positive (bcr1)
32/M3, M3r	46,XX,t(8;17;15)(q22;q21;q22),t(12;14)(q13;q32)/47,idem,+8/46,XX	ND	Positive (bcr1)
33/M3v	46,XX,t(13;17;15)(p13;q21;q22)/46,XX	ND	Positive (bcr3)
34/M3	46,XX,t(5;17;15)(q14;q21;q22)/48,idem,+8,+21	Fusion on der(15)	Positive (bcr1/2)
Simple variant cases			
35/M3	46,XX,t(5;15)(q13;q22),ins(15;17)(q22;q21q21)†/46,XX	Fusion on der(15)‡	Positive
36/M3	46,XY,t(1;17)(p34;q21)†,ins(1;15)(p34;q22q22)†	Fusion on der(1)§	Positive (bcr1)
Very complex cases			
37/M3v	46,XY,add(4)(p16),ins(15;17)(q22;q21q21)†,add(17)(q25)/46,XY	Fusion on der(15)	Positive (bcr3)
38/M3v	46,XY,t(5;15;17)(q35;q22;q23)†,ins(15;17)(q22;q21q21)†,der(20)t(8;20)(q12;p12)	Fusion on der(15)	Positive (bcr3)
39/M3v	46,XX,t(2;19;17;15)(p24;p13;q21;q22)†,ins(17;2)(q21;p16p24)†/47,idem,+8/46,XX	Fusion on der(15)	ND
40/M3	46,XY,del(2)(p1?p2?),add(15)(q22),add(16)(q?1),add(17)(q?1)/47,idem,+8/46,XY	Fusion in nuclei	ND
41/M3	47,XX,der(7)t(1;7)(q10;p10),+8,add(15)(q25),dup(17)(q11q21)†/46,XX	No fusion, but <i>RARα</i> x2 on dup(17q)	Positive (bcr1)
42/M3	46,XX,der(6)t(6;17)(p23;q2?)†,-13,der(14)t(14;16)(p11;?),-16,hsr(17)(q21)†,der(21),+der(21)x2/46,XX	No fusion, but <i>RARα</i> x3 on hsr(17q)	Positive

ND indicates not determined.

*Morphology as follows: M3, hypergranular classic M3; M3v, hypogranular variant M3; M3r, M3 with more than 30% regular nuclei as defined in the accompanying manuscript by Sainty et al.¹

†Abnormalities demonstrated by FISH analyses.

‡der(15) consists of 15pter→15q22::17q21::5q13→5qter.

§der(1) consists of 17qter→17q21::15q22::1p34→1qter.

||der(15) consists of 15pter→15q22::17q21::5q35→5qter.

cases of ATRA syndrome were observed, consistent with the hypothesis that this phenomenon is associated with modulation of surface adhesion molecules and cytokine release that is correlated with differentiation of the leukemic clone. Five patients are alive in first CR (range, 13-42 months; median, 28 months), including 2 receiving allogeneic BMT; and 5 patients relapsed, of whom 1 remains in remission after allogeneic BMT in second CR.

t(5;17). Two workshop patients were found to have a t(5;17); clinical and biologic data are summarized in Table 5. In case 54, conventional cytogenetics revealed t(5;17)(q34;q21) and a deletion of band 5q13 on the der(5). Multicolor banding of chromosome 5 allowed confirmation of the 5q translocation breakpoint and revealed that the del(5)(q13q13) was in fact an insertion of band 5q13 into 3q26 (Figure 5A,C). FISH analysis using Vysis or ICRF *RAR α* 121 probes showed an additional *RAR α* signal on the der(5) (Figure 6A), and RT-PCR demonstrated expression of *NPM-RAR α* and *RAR α -NPM* fusion transcripts (Figure 7). The *NPM* breakpoint

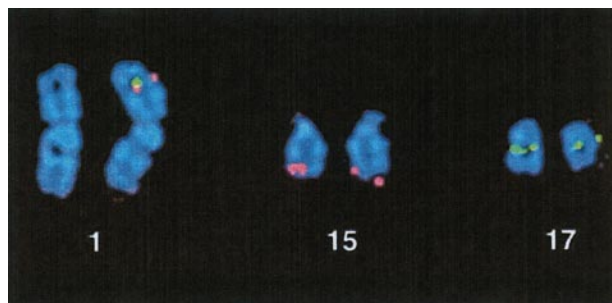


Figure 3. Case 36 with t(1;17)(p34;q21). FISH using ICRF *PML* 15.5 (red) and *RAR α* 121 (green) cosmid probes demonstrating *PML-RAR α* fusion signals on the der(1).

(Figure 7) was identical to that associated with formation of the *NPM δ -RAR α* fusion in the 2 previously reported cases of APL with the t(5;17)^{33,65} and also to that of the *NPM-ALK* fusion associated with the t(2;5)(p23;q35) in anaplastic large-cell lymphoma.⁶⁶ In case 55, previously reported,⁶⁷⁻⁶⁸ the molecular review performed in the present study ruled out a *RAR α* rearrangement. Because the translocation was unbalanced, the 5q breakpoint was difficult to assign by conventional cytogenetics; multicolor banding of chromosome 5 allowed this breakpoint to be refined to 5q13 (Figure 5B,C). FISH analyses using all the *RAR α* FISH probes shown in Figure 1 revealed signals only on the normal chromosome 17 (Figure 6B), suggesting deletion of the other allele. Work is currently in progress to exclude mutations in the remaining *RAR α* allele. These 2 cases could also be distinguished by *NPM* immunofluorescence using the NA24 antibody,⁵² which recognizes *NPM-RAR α* as well as *NPM*. In the *NPM-RAR α* -positive patient,⁵⁴ diffuse nuclear staining was observed, as distinct from the nucleolar staining⁵² detected in the patient lacking the *NPM-RAR α* fusion and NB4 and HL60 controls (Figure 8). In both t(5;17) patients, a wild-type *PML* localization pattern was detected (Figure 8).

Morphologic APL cases apparently lacking rearrangements of *RAR α* . In 5 patients, FISH, Southern blot, and RT-PCR analyses did not reveal rearrangements of *RAR α* (Table 6, Figure 9); in addition, *PML* immunofluorescence was performed in case 59, revealing a wild-type pattern. Morphologic review confirmed the diagnosis of APL as described by Sainty et al.¹ Investigations are in progress to exclude mutations of *RAR α* in these patients, although to date, no leukemias have been reported in mice expressing mutant *RAR α* .⁶⁹

Frequency of the classic t(15;17) in patients with APL

To establish the proportion of APL patients lacking the classic t(15;17), we derived epidemiologic data from centers participating

Table 4. Clinical and biologic data in the PLZF-RAR α -positive patients

Case* (country)	Sex/ Age	WBC, $\times 10^9/L$	DIC	Karyotype	PLZF-RAR α (ZF)/RAR α -PLZF†	ATRA treatment	Chemotherapy (protocol)‡	CR1, mo	Current status (from time of diagnosis)	References
43§ (France)	M/32	11.6	+	45,X,-Y,t(11;17)(q23;q21)	+(2ZF)/+	D1-5	(APL93 ⁶²)	+36	Alive in clinical and molecular CR1 at 37 mo (allo-BMT at 5 mo)	New case
44§ (France)	M/34	2.4	+	45,X,-Y,add(2)(q33),t(11;17)(q23;q21)/46,XY	+(2ZF)/+	D1-31	Dauno/Ara-C (APL93 ⁶²), Amsa/Ara-C as 2nd line to achieve 1st CR	47	Dead at 56 mo in relapse	Licht et al, 1995 (case 5) ³⁷ ; Koken et al, 1999 ⁵⁸
45§ (Belgium)	M/68	6.9	+	46,XY,t(11;17)(q23;q21)/47,idem,+8	+(2ZF)/+	D60-75 D150-240	Dauno D1-3/Ara-C D1-7, Mitox/Ara-C as 2nd line at D240 to achieve 1st CR	2	Dead at 15 mo in relapse	Guidez et al, 1994 ⁵⁹ ; Licht et al, 1995 (case 2) ³⁷
46 (UK)	M/53	4.5	++	46,XY,t(11;17)(q23;q21)/46,XY	+(2ZF)/+	D1-31	Dauno/Ara-C/Eto/G-CSF, 3 consolidation courses (MRC AML 12 ¹¹)	+42	Alive in CR1 at 43 mo	Grimwade et al, 1997 (case 7) ³⁸ ; Culligan et al, 1998 ⁶⁰
47 (USA)	F/37	45.2	+	46,XX,t(11;17)(q23;q21)	+(2ZF)/ND	No	(SWOG 8600 ³⁷)	3	CR2, lost to follow-up and dead at 11 mo	Scott et al, 1994 (case 16) ⁶¹ ; Licht et al, 1995 (case 4) ³⁷
48 (USA)	M/81	7.6	+	46,XY,t(11;17)(q23;q21)	+(3ZF)/ND	D1-18	No	No	Dead at day 18 (brain stem hemorrhage)	Licht et al, 1995 (case 3) ³⁷
49 (Italy)	M/43	10.4	+	46,XY,i(7)(q10),t(11;17)(q23;q21)	+(2ZF)/ND	D1-46	(AIDA 0493 ⁶³)	15	Dead in 2nd relapse at 30 mo (auto-BMT at 23 mo)	New case
50§ (Italy)	M/34	20.0	-	46,XY,del(11)(q23)/45, idem,-Y/46,XY	+/ND	D51-60	Dauno/Ara-C/Eto (EORTC GIMEMA AML 10 ⁶⁴), Ara-C/Ida/ATRA as 2nd line, CR1 obtained after HU	+28	Alive in CR1 at 33 mo (allo-BMT in CR1 at 5 mo)	New case
51§ (Netherlands)	M/30	69.5	+	46,XY,t(11;17)(q23;q21)	+(2ZF)/+	D1-7	HOVON 29, ⁴⁶ CR2 obtained with ATRA/G-CSF, ⁴⁶ consolidation with HIDAC	11	Alive in clinical and molecular CR2 at 51 mo (allo-BMT in CR2 at 23 mo)	Jansen et al, 1999 ⁴⁶
52§ (Italy)	M/62	9.9	+	46,XY,ish ins(11;17)(q23;q21q21)	+(3ZF)/-	No	Ida/Ara-C/Eto (EORTC GIMEMA AML 13), CR obtained after MICE, NOVIA consolidation	+13	Alive in CR1 at 15 mo	New case
53 (UK)	M/75	2.0	++	46,XY,t(11;17)(q23;q21)/46, idem,del(12)(p1?)/46, idem,-6,+7/46,XY	+(3ZF)/+	D3-23	DAT 2+7/DAT 2+7/MACE	+16	Alive in CR1 at 17 mo	New case

D indicates day. DIC indicates disseminated intravascular coagulation: -, none; +, moderate; ++, severe; ish, in situ hybridization.

*Only cases 44 and 46 could be classified as classic M3 and basophilic M3, respectively; all cases could be classified as M3r according to the accompanying manuscript by Sainy et al.¹

†Breakpoints in PLZF intron 2 lead to the retention of 2 zinc fingers (2ZF) in the PLZF-RAR α fusion, whereas breakpoints in intron 3 lead to retention of 3 zinc fingers (3ZF); introns numbered according to reference 47.

‡Dauno indicates daunorubicin; Ara-C, cytosine arabinoside; Amsa, m-amsacrine; Mitox, mitoxantrone; Eto, etoposide; G-CSF, granulocyte colony-stimulating factor; ida, idarubicin; HU, hydroxyurea.

§No terminal granulocytic morphologic differentiation with in vitro ATRA alone.

||Wild-type pattern with PML immunofluorescence.

in the workshop. For the purposes of this analysis, data collection was restricted to 18 of 42 laboratories that permitted determination of the frequency of specific cytogenetic changes in a completely unselected patient group. Overall, cytogenetic analyses were performed successfully in 611 patients with newly diagnosed APL over an 8-year period, as summarized in Table 7.

Discussion

The t(15;17) is the diagnostic hallmark of APL and initially had been considered to be present in all patients with this condition.³⁰ However, it is now clear from the present study that a sizeable

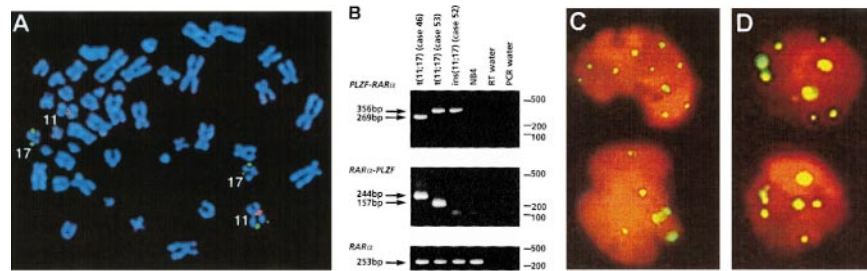


Figure 4. Molecular analyses of *PLZF-RARα* cases. (A) Case 52 with a normal karyotype and formation of *PLZF-RARα* as the sole fusion gene due to an insertion event. FISH using ICRF *RARα* 121 (green) cosmid probe and chromosome 11 centromeric probe (red) demonstrated insertion of *RARα* sequences into band 11q23. (B) RT-PCR revealed expression of *PLZF-RARα* (3ZF *PLZF* breakpoint) as the sole fusion transcript in case 52, whereas both fusion transcripts were detected in cases 46 (2ZF) and 53 (3ZF). (C,D) PML immunofluorescence using the PG-M3 antibody on cytosin preparations from case 43 (C) and case 52 (D, courtesy of Francesco Fazi) showed a wild-type pattern, as distinct from the microparticulate PML staining shown in NB4 cells in Figure 8. Images were captured on a Zeiss Axioplan fluorescence microscope.

minority actually lack this chromosomal aberration, with epidemiologic data from the Monza workshop indicating that the t(15;17) is not identified in 9% patients with APL after successful diagnostic cytogenetic analysis. Furthermore, this study shows that the majority of cases of morphologic APL lacking the t(15;17) are still associated with formation of the *PML-RARα* fusion gene, created by insertion events or more complex rearrangements. Such mechanisms occur in approximately 4% and 2% of cases of APL, respectively, and typically lead to the formation of *PML-RARα* at its usual location on 15q and, less commonly, at the site of the reciprocal fusion gene on 17q or alternative chromosomal locations. These findings are highly analogous to those previously reported in CML. In this condition, 90% of cases are associated with the t(9;22), leading to a rearrangement between the *BCR* and *ABL* genes. *BCR/ABL* rearrangements are also present in approximately half the CML patients lacking the classic t(9;22). In the majority of these patients, chromosomes 9 and 22 are of normal appearance with formation of the *BCR-ABL* fusion gene at its usual location on chromosome 22; more rarely, the fusion gene is located

on chromosome 9 or alternative chromosomal sites, reflecting the occurrence of more complex rearrangements (reviewed by Aurich et al⁷⁰). The striking similarity between the frequency of the classic translocation and complex and cryptic rearrangements involving the genes disrupted by each respective translocation in CML and APL raises the possibility that similar underlying mechanisms may be involved. This possibility is supported by a recent study documenting proximity of *BCR* and *ABL* and of *PML* and *RARα* genes at specific phases of the cell cycle in hemopoietic progenitors.⁷¹

The epidemiologic survey revealed *PLZF/RARα* as the second most common molecular rearrangement associated with APL, accounting for approximately 0.8% of cases. Identification of this group is extremely important because of the poor response to retinoids as single-agent therapy³⁷ and in view of recent data suggesting that these patients are also resistant to As₂O₃.⁵⁸ However, it is clear from the present study that CR is attainable in this group with combination chemotherapy, indicating that cases of *PLZF-RARα*-positive APL are not necessarily associated with an

Table 5. Clinical and biologic data relating to APL cases with t(5;17)

Case/M3	Sex/Age	WBC, ×10 ⁹ /L	DIC	Karyotype after FISH analysis	RT-PCR*	Immunofluorescence	ATRA treatment	Chemotherapy (protocol)	Current status (from time of diagnosis)
54/M3	F/9	17	–	46,XX,ins(3;5) (q26;q13q13), t(5;17)(q34;q21)	<i>NPM-RARα</i> positive <i>RARα-NPM</i> positive	PML: wild-type pattern NPM: diffuse nuclear pattern	D27- D120†	LAME 91. Ara-C/Mitox induction; no CR. Morphologic and cytogenetic CR (32 metaphases) documented at D40. Consolidation: Dauno/Ara-C/Eto, Ara-C/Aspar/Amsa	Alive in CR1 at 29 mo
55/M3r	F/76	43.1	–	46,XX,der(5)t(5;17) (q13;q21),del(8) (q22q24),der(17)§, 5~32 dmin	<i>PML-RARα</i> negative <i>PLZF-RARα</i> negative <i>NPM-RARα</i> negative <i>NuMA-RARα</i> negative <i>STAT5b-RARα</i> negative	PML: wild-type pattern NPM: nucleolar wild- type pattern	D1-4‡	Dauno/Ara-C	Died at D14, respiratory failure due to ATRA syndrome or Corynebacterium. 90% blasts in bone marrow before death.

Aspar indicates asparaginase; LAME, leucémie aiguë myéloïde de l'enfant; other abbreviations as for Tables 2 and 4.

*RNA integrity was confirmed in case 55 by amplification of *RARα* transcripts, as described previously.⁴⁴

†ATRA given at a dose of 40 mg/m²/d; no ATRA syndrome was observed.

‡In vitro ATRA sensitivity data for case 55 have been reported elsewhere⁶⁸; an atypical response was observed with spontaneous reduction of NBT in the absence of morphologic evidence of differentiation.

§der(17) consists of a small chromosome identified by FISH using chromosome 17 wcp and centromeric probes.

||dmin were labeled by chromosome 8 wcp and c-myc probes.

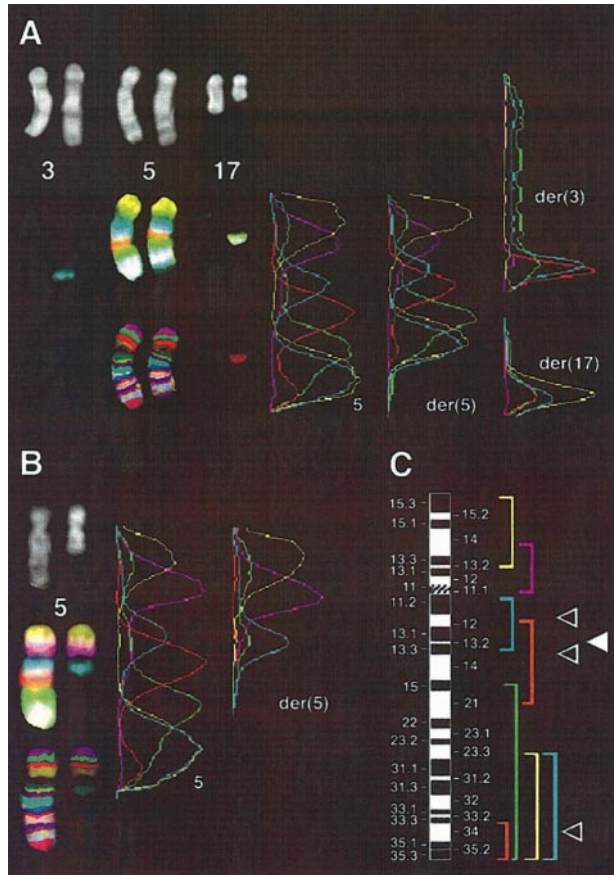


Figure 5. Multicolor banding of chromosome 5 in the t(5;17) cases. (A) Case 54: insertion of the 5q13 band into band 3q26 and translocation of segment 5q34-qter to band 17q21. Figure shows the translocation of 17q to the der(5q). Left panel: top, DAPI filter; middle, compilation of captures with each filter excluding the DAPI one; bottom, multicolor banding specific for chromosome 5 material obtained after image processing. Right panel: profile of fluorescence intensities along the chromosomal axes. Peaks on der(3) and der(17) are derived from chromosome 5 material. (B) Case 55: localization of the 5q breakpoint to 5q13. Analysis as described in panel A. (C) Location and labeling of chromosome 5 region-specific partial chromosome paints; breakpoints in case 54 (Δ) and in case 55 (\blacktriangle) are shown.

adverse prognosis, as suggested previously. In addition, the present study has identified cases of APL with cryptic *PLZF/RAR α* rearrangements, including one patient with a normal karyotype in whom *PLZF-RAR α* was the sole fusion gene formed as a result of an insertion event. Rearrangements disrupting *STAT5b*, *NuMA*, and *NPM* appear to be extremely rare, with only isolated case reports in the literature.^{33-35,65} Indeed, we detected no cases involving the former 2 fusion partners and only one case with t(5;17) expressing *NPM-RAR α* . Whereas the 2 previously reported patients with

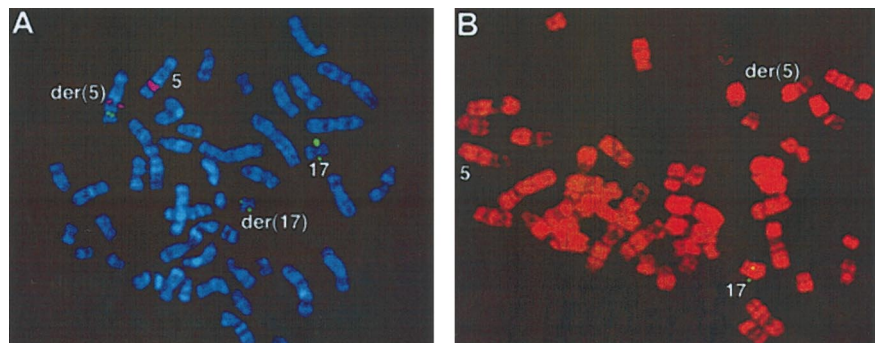


Figure 6. FISH using ICRF *RAR α* 121 cosmid probe in the t(5;17) cases. (A) Case 54: translocation of *RAR α* sequences (green) to the der(5), identified using a chromosome 5q31 Oncor probe (red) using DAPI banding. (B) Case 55: *RAR α* signals (green) were detected only on the normal chromosome 17. R banding using propidium iodide does not permit capture of double minute chromosomes.

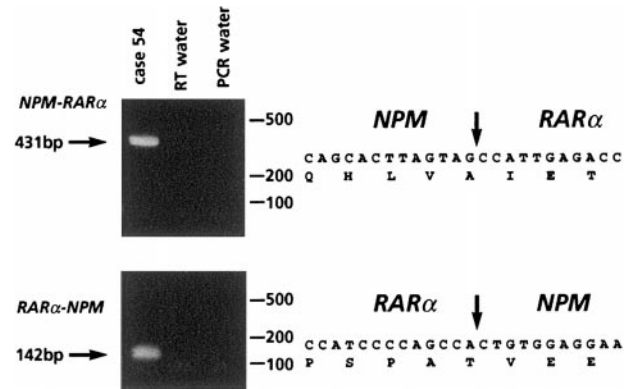


Figure 7. Molecular analysis of case 54 with t(5;17). Left panel: RT-PCR showing *NPM-RAR α* and *RAR α -NPM* transcripts. Right panel: sequence analysis of fusion transcripts, with location of cDNA fusion junction.

NPM-RAR α APL did not receive ATRA before relapse,^{33,65} our patient was treated with ATRA as part of induction therapy and is alive in first CR at 29 months.

An important aspect of the present study is that it permitted the evaluation of different techniques to establish the presence of the *PML/RAR α* rearrangement as a means of determining the subgroup of APL patients likely to benefit from retinoids and As_2O_3 . It is clear that long-established methods such as conventional cytogenetics are not invariably successful in this regard and must be supplemented by alternative approaches, such as FISH, RT-PCR, Southern blot analysis, or *PML* immunofluorescence. Nevertheless, cytogenetics should not be abandoned because it detects the t(15;17) in the majority of patients, identifies secondary cytogenetic changes, and has revealed novel translocations in APL, prompting subsequent molecular characterization of their respective breakpoint regions. In many respects, RT-PCR screening of cases of suspected APL affords a number of advantages: providing a rapid diagnostic test, distinguishing *PML* breakpoint patterns, and defining targets for residual disease monitoring, which has been shown to provide independent prognostic information (reviewed by Grimwade²⁴). Indeed, identification of the *PML-RAR α* fusion by molecular techniques in patients lacking the t(15;17) predicts a beneficial response to ATRA, and such patients share the favorable prognosis of those with the classic t(15;17).^{11,28,29}

PML immunofluorescence techniques are even more rapid than RT-PCR and in some institutions have been incorporated into the standard diagnostic approach to patients with suspected APL.²⁴ It is clear from this study and others^{29,38} that observation of a microparticulate nuclear staining pattern in leukemic blasts is specific to cases expressing the *PML-RAR α* fusion protein and therefore is predictive of a beneficial response to ATRA and As_2O_3 . This pattern is detected in patients with the classic t(15;17) as well as in those

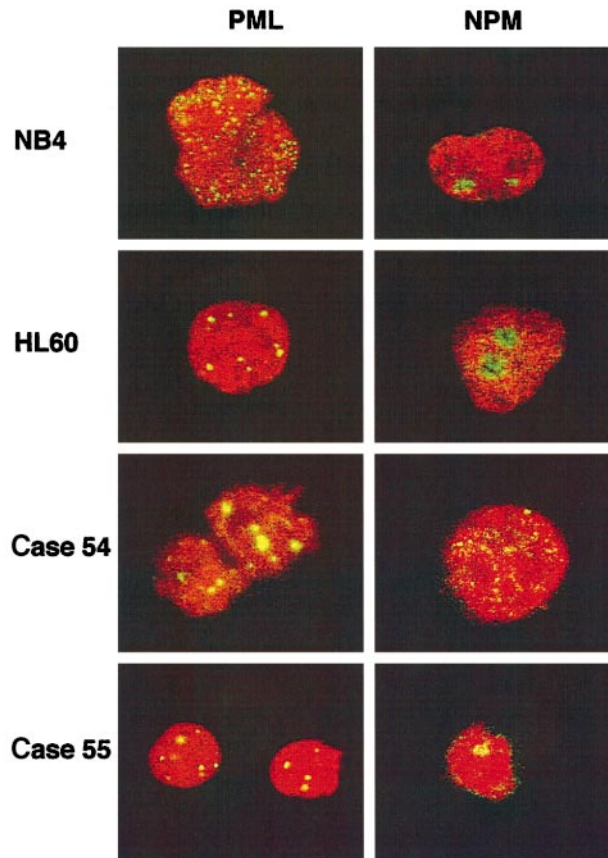


Figure 8. PML and NPM immunofluorescence (IF) in *t(5;17)* cases and cell-line controls. PML immunofluorescence using the PG-M3 antibody shows a wild-type pattern (discrete nuclear dots) in HL60 and in both patients, and a microparticulate diffuse nuclear pattern in the *t(5;17)* NB4 cell line. NPM immunofluorescence using the NA24 antibody shows a wild-type nucleolar pattern in both cell lines and in the *NPM-RAR α* -negative patient (case 55), and a diffuse nuclear pattern in the *NPM-RAR α* -positive patient (case 54). Images were captured with a Leica TCS NT confocal microscope.

patients in whom *PML-RAR α* is the sole fusion gene formed as a result of insertion events.³⁸ Disruption of PML nuclear bodies has been proposed to play an important role in the pathogenesis of *PML-RAR α* -associated APL.²⁰ However, this study and others

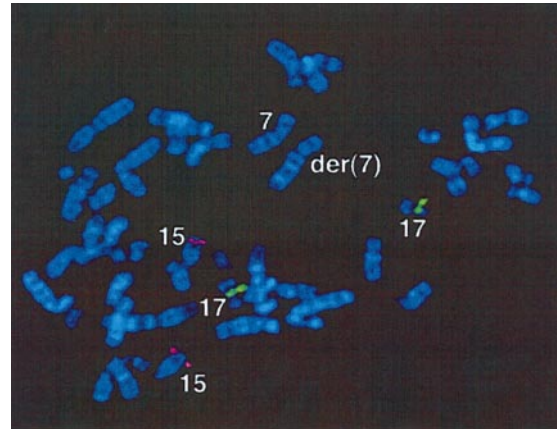


Figure 9. Case 56 with *der(7)* and lacking *RAR α* rearrangement. FISH using ICRF *PML* 15.5 (red) and *RAR α* 121 (green) cosmid probes, demonstrating normal locations of *PML* and *RAR α* sequences in the malignant clone. See text and Table 6.

have established that a wild-type PML nuclear staining pattern is associated with APL cases with alternative reciprocal translocations including *t(5;17)*,⁶⁵ *t(11;17)(q13;q21)*,³⁴ and *t(11;17)(q23;q21)*,^{38,58} as well as in the case in which *RAR α -PML* was the sole fusion gene formed as a result of an insertion event.²⁹ This finding implies that delocalization of PML does not provide a final common pathway to all molecular subtypes of APL. APL cases associated with *NPM* or *NuMA* rearrangements appear to be sensitive to retinoids,^{34,36,65} whereas cases with *PLZF/RAR α* rearrangements³⁷ or with expression of *RAR α -PML* alone²⁹ fail to differentiate with retinoids as the sole therapeutic agent. This suggests that identification of a normal PML staining pattern in cases of morphologically confirmed APL should not lead to treatment with ATRA alone in the first instance; indeed, whether to use ATRA at any stage (and in any combination) in these patients would await further cytogenetic and molecular characterization or the results of in vitro ATRA differentiation assays. Interestingly, a recent study has suggested that *t(11;17)*-associated APL may differentiate in response to ATRA in combination with granulocyte colony-stimulating factor.⁴⁶

There has been considerable interest in the potential contribution of reciprocal fusion gene products to the pathogenesis of APL, and in the course of this study, a single case of morphologically

Table 6. Morphologic APL cases lacking evidence for *RAR α* rearrangements

Case/M3	Karyotype	<i>PML-RARα</i> FISH	RT-PCR*	Southern blot†
56/M3r	45,X,-Y, <i>der(7)t(7;11)(q34;p15)ins(7;12)(q34;q24.3),der(11)t(7;11)/46,XY</i>	Normal pattern‡	<i>PML-RARα</i> negative <i>PLZF-RARα</i> negative	<i>RARα</i> not rearranged
57/M3r	46,XX	ND	<i>PML-RARα/PLZF-RARα</i> negative <i>NPM-RARα/NuMA-RARα</i> negative <i>STAT5b-RARα</i> negative	<i>RARα</i> not rearranged
58/M3r	46,XY	Normal pattern‡	<i>PML-RARα</i> negative	<i>RARα</i> not rearranged
59/M3, M3r	46,XY	Normal pattern‡	<i>PML-RARα/RARα-PML</i> negative <i>PLZF-RARα/RARα-PLZF</i> negative <i>NPM-RARα/NuMA-RARα</i> negative <i>STAT5b-RARα</i> negative	ND
60/M3, M3r	45,XX,-2,-12,+13, <i>add(17)(q2?)</i>	ND	<i>PML-RARα/PLZF-RARα</i> negative <i>NPM-RARα/NuMA-RARα</i> negative <i>STAT5b-RARα</i> negative	<i>RARα</i> not rearranged

Abbreviations as indicated in Table 2.

*RNA integrity was confirmed in each case by amplification of *RAR α* transcripts, as described previously.⁴⁴

†For Southern blot analyses, *EcoRI*- and *HindIII*-digested genomic DNA was hybridized with probes H18, X5', and HB for cases 57, 58, and 60, whereas for case 56, DNA was additionally digested with *Bam*HI and *RAR α* probes PX20 and 8CPP were used. Mapping details of probes are provided in Figure 1.

‡FISH results indicate no fusion signals, no deletion of *RAR α* , and no translocation of *RAR α* sequences to another chromosome.

Table 7. Frequency of cytogenetic and molecular subgroups of APL

Cytogenetic/molecular abnormality	Number of cases (%) (n = 611)
t(15;17)	559 (91.5%)
ider(17q)	10 (1.6%)
Uncharacterized (normal chromosomes 15 and 17)	7 (1.1%)
No t(15;17), underlying <i>PML/RARα</i> rearrangement	33 (5.4%)
Insertions	22 (3.6%)
Complex chromosomal rearrangements	11 (1.8%)
t(11;17)(q23;q21) and/or <i>PLZF-RARα</i> fusion	5 (0.8%)
t(5;17)	2 (0.4%)
<i>NPM/RARα</i> rearrangement	1 (0.2%)
<i>NPM/RARα</i> negative	1 (0.2%)
<i>RARα</i> not rearranged	5 (0.8%)

confirmed APL was identified in which *RAR α -PML* (bcr3) appeared to be the sole fusion gene formed. The role of reciprocal fusion genes has been investigated recently using transgenic mice. Whereas expression of bcr3 *RAR α -PML* under the control of the human cathepsin G (hCG) promoter did not induce leukemia in its own right, *RAR α -PML* significantly increased the frequency of APL among mice expressing a bcr1 *PML-RAR α* transgene; furthermore, co-expression of both fusion transcripts was suggested to lead to a more aggressive form of the disease.²⁷ However, it remains possible that the phenotype was influenced by the expression of nonreciprocal fusion transcripts with significant overlap of central portions of *PML*. In man, analysis of large clinical trials has revealed that *RAR α -PML* is not expressed in approximately 30% of cases, including the majority of insertions, and that expression of reciprocal transcripts has no influence on disease characteristics or outcome.¹¹ The *RAR α -PLZF* protein has also been the focus of some attention. This protein contains 6 or 7 zinc fingers, binds DNA, may deregulate the cell cycle, and is up-regulated by ATRA, potentially contributing to leukemogenesis and ATRA resistance.^{12,37} Interestingly, recent studies have also suggested that *RAR α -PLZF* can modify the leukemic phenotype of *PLZF-RAR α* transgenic mice.⁷² Expression of *PLZF-RAR α* under the hCG

promoter induced a myeloproliferative disorder in 100% of mice, whereas co-expression of *PLZF-RAR α* and *RAR α -PLZF* led to a morphologic picture more reminiscent of APL, implying that both fusion products arising from the t(11;17) are necessary for the leukemic phenotype.⁷² However, in the present study, a patient with a normal karyotype was identified in whom *PLZF-RAR α* was the sole fusion gene formed because of an insertion event, and in whom the morphologic appearances were indistinguishable from patients with the t(11;17), in which both fusion transcripts were expressed.¹ Apparent differences between mouse models of APL and the disease in man could reflect the nature of the hemopoietic progenitor targeted by *PML/RAR α* and *PLZF/RAR α* rearrangements. Results obtained so far from transgenic mouse models imply that more than one step is required to develop APL and that reciprocal fusion genes could influence the rate of development and behavior of leukemias. If this is indeed the case in man, further understanding of these processes may provide insights into the molecular events underlying APL in patients with nonreciprocal rearrangements and also in patients lacking rearrangements of *RAR α* that were identified by this study. Although it is clear that the latter represent a small subgroup of APL cases, their existence suggests that this disease may arise by mechanisms distinct from the formation of aberrant retinoid receptors. Characterization of the molecular changes underlying such cases may establish whether the APL phenotype is inextricably linked to deregulation of retinoid signaling pathways and could provide further insights into the processes mediating normal myeloid differentiation.

Acknowledgments

This work is dedicated to the memory of Pr Philippe Bernard, Secretary of the Groupe Français de Cytogénétique Hématologique and of the Société Française d'Hématologie. We are very grateful to Daniel Isnardon for technical help in confocal microscopy and to Drs Jackie Cordell, Nick Cross, Pierre Fenaux, Arthur Zelent, and Prof. David Mason for helpful advice.

References

- Sainty D, Liso V, Cantù-Rajoldi A, et al. A new morphological classification system for acute promyelocytic leukemia distinguishes cases with underlying *PLZF-RAR α* rearrangements. *Blood*. 2000;96:1287-1296.
- Tallman MS, Kwaan HC. Reassessing the hemostatic disorder associated with acute promyelocytic leukemia. *Blood*. 1992;79:543-553.
- Barbui T, Finazzi G, Falanga A. The impact of all-*trans*-retinoic acid on the coagulopathy of acute promyelocytic leukemia. *Blood*. 1998;91:3093-3102.
- Degos L, Dombret H, Chomienne C, et al. All-*trans* retinoic acid as a differentiating agent in the treatment of acute promyelocytic leukemia. *Blood*. 1999;85:2643-2653.
- Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As₂O₃) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*. 1997;89:3354-3360.
- Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N Engl J Med*. 1998;339:1341-1348.
- Fenaux P, Chomienne C, Degos L. Acute promyelocytic leukemia: biology and treatment. *Semin Oncol*. 1997;24:92-102.
- Tallman MS, Andersen JW, Schiffer CA, et al. All-*trans*-retinoic acid in acute promyelocytic leukemia. *N Engl J Med*. 1997;337:1021-1028.
- Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in *PML/RAR* alpha-positive acute promyelocytic leukemia by combined all-*trans* retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood*. 1997;90:1014-1021.
- Kanamaru A, Takemoto Y, Tanimoto M, et al. All-*trans* retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. *Blood*. 1995;85:1202-1206.
- Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-*trans* retinoic acid: result of the randomized MRC trial. *Blood*. 1999;93:4131-4143.
- Melnick A, Licht JD. Deconstructing a disease: *RAR α* , its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood*. 1999;93:3167-3215.
- Tsai S, Collins SJ. A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. *Proc Natl Acad Sci U S A*. 1993;90:7153-7157.
- Onodera M, Kunisada T, Nishikawa S, Sakiyama Y, Matsumoto S, Nishikawa SI. Overexpression of retinoic acid receptor α suppresses myeloid cell differentiation at the promyelocyte stage. *Oncogene*. 1995;11:1291-1298.
- Labrecque J, Allan D, Chambon P, Iscove NN, Lohnes D, Hoang T. Impaired granulocytic differentiation in vitro in hematopoietic cells lacking retinoic acid receptors α 1 and γ . *Blood*. 1998;92:607-615.
- Wang ZG, Delva L, Gaboli M, et al. Role of *PML* in cell growth and the retinoic acid pathway. *Science*. 1998;279:1547-1551.
- Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS. *PML*, a growth suppressor disrupted in acute promyelocytic leukemia. *Mol Cell Biol*. 1994;14:6858-6867.
- Quignon F, De Bels F, Koken M, Feunteun J, Ameisen JC, de Thé H. *PML* induces a novel caspase-independent death process. *Nat Genet*. 1998;20:259-265.
- Wang ZG, Ruggiero D, Ronchetti S, et al. *PML* is essential for multiple apoptotic pathways. *Nat Genet*. 1998;20:266-272.
- Dyck JA, Maul GG, Miller WH, Chen JD, Kakiyama A, Evans RM. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. *Cell*. 1994;76:333-343.
- Weis K, Rambaud S, Lavau C, et al. Retinoic acid regulates aberrant nuclear localization of *PML-RAR α* in acute promyelocytic leukaemia cells. *Cell*. 1994;76:345-356.

22. Hodges M, Tissot C, Howe K, Grimwade D, Freemont PS. Structure, organisation and dynamics of PML nuclear bodies. *Am J Hum Genet*. 1998;63:297-304.
23. Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J*. 1996;10:940-954.
24. Grimwade D. The pathogenesis of acute promyelocytic leukaemia: evaluation of the role of molecular diagnosis and monitoring in the management of the disease. *Br J Haematol*. 1999;106:591-613.
25. He LZ, Merghoub T, Pandolfi PP. In vivo analysis of the molecular pathogenesis of acute promyelocytic leukemia in the mouse and its therapeutic implications. *Oncogene*. 1999;18:5278-5292.
26. Westervelt P, Ley TJ. Seed versus soil: the importance of the target cell for transgenic models of human leukemias. *Blood*. 1999;93:2143-2148.
27. Pollock JL, Westervelt P, Kurichety AK, Pelicci PG, Grisolan JL, Ley TJ. A bcr-3 isoform of RAR α -PML potentiates the development of PML-RAR α -driven acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. 1999;96:15103-15108.
28. Miller WH, Kakizuka A, Frankel SR, et al. Reverse transcription polymerase chain reaction for the rearranged retinoic acid receptor α clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. 1992;89:2694-2698.
29. Mozziconacci MJ, Liberatore C, Brunel V, et al. In vitro response to all-trans retinoic acid of acute promyelocytic leukemias with nonreciprocal PML/RAR α or RARA/PML fusion genes. *Genes Chromosomes Cancer*. 1998;22:241-250.
30. Larson RA, Kondo K, Vardiman JW, Butler AE, Golomb HM, Rowley JD. Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia. *Am J Med*. 1984;76:827-841.
31. Chen SJ, Zelent A, Tong JH, et al. Rearrangements of the retinoic acid receptor alpha and promyelocytic zinc finger genes resulting from t(11;17)(q23;q21) in a patient with acute promyelocytic leukaemia. *J Clin Invest*. 1993;91:2260-2267.
32. Chen Z, Brand NJ, Chen A, et al. Fusion between a novel *Kruppel*-like zinc finger gene and the retinoic acid receptor- α locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. *EMBO J*. 1993;12:1161-1167.
33. Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. *Blood*. 1996;87:882-886.
34. Wells RA, Catzavelos C, Kamel-Reid S. Fusion of retinoic acid receptor α to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet*. 1997;17:109-113.
35. Amould C, Philippe C, Bourdon V, Grégoire MJ, Berger R, Jonveaux P. The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor α in acute promyelocytic-like leukemia. *Hum Mol Genet*. 1999;8:1741-1749.
36. Redner RL, Corey SJ, Rush EA. Differentiation of t(5;17) variant acute promyelocytic leukemia blasts by all-trans retinoic acid. *Leukemia*. 1997;11:1014-1016.
37. Licht JD, Chomienne C, Goy A, et al. Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). *Blood*. 1995;85:1083-1094.
38. Grimwade D, Gorman P, Duprez E, et al. Characterization of cryptic rearrangements and variant translocations in acute promyelocytic leukemia. *Blood*. 1997;90:4876-4885.
39. Lafage-Pochitaloff M, Alcalay M, Brunel V, et al. Acute promyelocytic leukemia cases with nonreciprocal PML/RAR α or RARA/PML fusion genes. *Blood*. 1995;85:1169-1174.
40. Hjalt TAH, Murray JC. Genomic structure of the human retinoic acid receptor-alpha1 gene. *Mamm Genome*. 1999;10:528-529.
41. Speicher MR, Ballard SG, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet*. 1996;12:368-375.
42. Chudoba I, Plesch A, Lörch T, Lemke J, Claussen U, Senger G. High resolution multicolor-banding: a new technique for refined FISH analysis of human chromosomes. *Cytogenet Cell Genet*. 1999;84:156-160.
43. Biondi A, Rambaldi A, Pandolfi PP, et al. Molecular monitoring of the myl/retinoic acid receptor- α fusion gene in acute promyelocytic leukemia by polymerase chain reaction. *Blood*. 1992;80:492-497.
44. Borrow J, Goddard AD, Gibbons B, et al. Diagnosis of acute promyelocytic leukaemia by RT-PCR: detection of PML-RARA and RARA-PML fusion transcripts. *Br J Haematol*. 1992;82:529-540.
45. Castaigne S, Balitrand N, de Thé H, Dejean A, Degos L, Chomienne C. A PML/retinoic acid receptor alpha fusion transcript is constantly detected by RNA-based polymerase chain reaction in acute promyelocytic leukemia. *Blood*. 1992;79:3110-3115.
46. Jansen JH, de Ridder MC, Geertsma WMC, et al. Complete remission of t(11;17) positive acute promyelocytic leukemia induced by all-trans retinoic acid and granulocyte colony-stimulating factor. *Blood*. 1999;94:39-45.
47. Zhang T, Xiong H, Kan LX, et al. Genomic sequence, structural organization, molecular evolution, and aberrant rearrangement of promyelocytic leukemia zinc finger gene. *Proc Natl Acad Sci U S A*. 1999;96:11422-11427.
48. Lo Coco F, Diverio D, D'Adamo F, et al. PML/RAR-alpha rearrangement in acute promyelocytic leukemia apparently lacking the t(15;17) translocation. *Eur J Haematol*. 1992;48:173-176.
49. Diverio D, Lo Coco F, D'Adamo F, et al for the Italian Cooperative Study Group "GIMEMA": Identification of DNA rearrangements at the retinoic acid receptor- α (RAR- α) locus in all patients with acute promyelocytic leukemia (APL) and mapping of APL breakpoints within the RAR- α second intron. *Blood*. 1992;79:3331-3336.
50. de Thé H, Chomienne C, Lanotte M, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus. *Nature*. 1990;347:558-561.
51. Chomienne C, Ballerini P, Balitrand N, et al. All-trans retinoic acid in acute promyelocytic leukemias. II. In vitro studies: structure-function relationship. *Blood*. 1990;76:1710-1717.
52. Cordell JL, Pulford KAF, Bigerna B, et al. Detection of normal and chimeric nucleophosmin in human cells. *Blood*. 1999;93:632-642.
53. Daniel MT, Koken M, Romagné O, et al. PML protein expression in hematopoietic and acute promyelocytic leukemia cells. *Blood*. 1993;82:1858-1867.
54. Stuurman N, de Graaf A, Floore A, et al. A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies. *J Cell Sci*. 1992;101:773-784.
55. Flenghi L, Fagioli M, Tomassoni L, et al. Characterization of a new monoclonal antibody (PG-M3) directed against the aminoterminal portion of the PML gene product: immunocytochemical evidence for high expression of PML proteins on activated macrophages, endothelial cells, and epithelia. *Blood*. 1995;85:1871-1880.
56. Hagemeyer A, Buijs A, Smit E, et al. Translocation of BCR to chromosome 9: a new cytogenetic variant detected by FISH in two Ph-negative, BCR-positive patients with chronic myeloid leukemia. *Genes Chromosomes Cancer*. 1993;8:237-245.
57. Eclache V, Benzacken B, Le Roux G, Casassus P, Chomienne C. PML/RAR α rearrangement in acute promyelocytic leukaemia with t(1;17) elucidated using fluorescence in situ hybridisation. *Br J Haematol*. 1997;98:440-443.
58. Koken MH, Daniel MT, Gianni M, et al. Retinoic acid, but not arsenic trioxide, degrades the PLZF/RAR alpha fusion protein, without inducing terminal differentiation or apoptosis, in a RA-therapy resistant t(11;17)(q23;q21) APL patient. *Oncogene*. 1999;18:1113-1118.
59. Guidez F, Huang W, Tong JH, et al. Poor response to all-trans retinoic acid therapy in a t(11;17) PLZF/RAR alpha patient. *Leukemia*. 1994;8:312-317.
60. Culligan DJ, Stevenson D, Lin Chee Y, Grimwade D. Acute promyelocytic leukaemia with t(11;17)(q23;q12-21) and a good initial response to prolonged ATRA and combination chemotherapy. *Br J Haematol*. 1998;100:328-330.
61. Scott AA, Head DR, Kopecky KJ, et al. HLA-DR-, CD33+, CD56+, CD16- myeloid/natural killer cell acute leukemia: a previously unrecognized form of acute leukemia potentially misdiagnosed as French-American-British acute myeloid leukemia-M3. *Blood*. 1994;84:244-255.
62. Fenaux P, Chastang C, Chevret S, et al. A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. *Blood*. 1999;94:1192-1200.
63. Avvisati G, Lo Coco F, Diverio D, et al. AIDA (all-trans retinoic acid + idarubicin) in newly diagnosed acute promyelocytic leukemia: a Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) pilot study. *Blood*. 1996;88:1390-1398.
64. Keating S, Zittoun R, Suci S, et al. Postremission therapy for patients with AML in first CR in the AML 10 trial of the EORTC LCG and GIMEMA [abstract]. *Blood*. 1998;92(suppl 1):494a.
65. Hummel JL, Wells RA, Dubé ID, Licht JD, Kamel-Reid S. Deregulation of NPM and PLZF in a variant t(5;17) case of acute promyelocytic leukemia. *Oncogene*. 1999;18:633-641.
66. Morris S, Kirstein M, Valentine M, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in a non-Hodgkin's lymphoma. *Science*. 1994;263:1281-1284.
67. Brunel V, Sainy D, Carbuccia N, et al. Unbalanced translocation t(5;17) in an atypical acute promyelocytic leukemia. *Genes Chromosomes Cancer*. 1995;14:307-312.
68. Mozziconacci MJ, Liberatore C, Grignani F, et al. Atypical response to all-trans retinoic acid in a der(5)t(5;17) acute promyelocytic leukemia. *Leukemia*. 1999;13:862-868.
69. Kogan SC, Hong S-H, Shultz DB, Privalsky ML, Bishop JM. Leukemia initiated by PML/RAR α : the PML domain plays a critical role while retinoic acid-mediated transactivation is dispensable. *Blood*. 2000;95:1541-1550.
70. Aurich J, Dastugue N, Duchayne E, Schlaifer D, Rigual-Huguet F, Rosa-Caballin M. Location of the BCR-ABL fusion gene in two cases of Ph-positive chronic myeloid leukemia. *Genes Chromosomes Cancer*. 1997;20:148-154.
71. Neves H, Ramos C, Gomes da Silva M, Parreira A, Parreira L. The nuclear topography of ABL, BCR, PML, and RAR α genes: evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. *Blood*. 1999;93:1197-1207.
72. He LZ, Zelent A, Pandolfi PP. RAR α -PLZF is a critical determinant of the leukemic phenotype in APL [abstract]. *Blood*. 1999;94(suppl 1):368a.
73. Gutiérrez NC, Garcia JL, Chillón C, Muntion S, Gonzalez M, Hernandez JM. Cryptic insertion (15;17) in a case of acute promyelocytic leukemia detected by fluorescence in situ hybridization. *Haematologica*. 1999;84:88-90.