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 Hagemeijer, Roland Berger, Michael Neat, Kathy Howe, Nicole Dastugue, Joop Jansen, Isabelle Radford-Weiss, Francesco Lo Coco, Michel Lessard, Jesús-Maria Hernandez, Eric Delabesse, David Head, Vincenzo Liso, Danielle Sainty, Georges Flandrin, Ellen Solomon, Françoise Birg, and Marina Lafage-Pochitaloff, on behalf of Groupe Francais de Cytogénétique Hématologique, Groupe Francais 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-RARα 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/RARα 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-RARα fusion gene on 15q. In 11 of 60 workshop patients, PLZF/RARα 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 RARα into 11q23, and PLZF-RARα 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-RARα and RARα-NPM transcripts detected. In the other with an unbalanced der(5)t(5;17)(q13;q21) and a nucleolar NPM localization pattern, an NPM-RARα rearrangement was excluded, and FISH revealed deletion of one RARα allele. In the remaining 5 workshop patients, no evidence was found for a rearrangement of RARα, 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)

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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 RARα genes, which are localized to chromosomes 15q and 17q, respectively (reviewed by Melnick and Licht23). The t(15;17) generates 2 chimeric genes: PML-RARα is formed on the derivative 15 [der(15)], whereas the reciprocal RARα-PML fusion is located on the derivative 17 [der(17)]. PML and RARα 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-RARα fusion protein20,21 (reviewed by Hodges et al22). RARα 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-RARα protein retains key functional domains of both PML and RARα, suggesting that it plays an important role in leukemogenesis. PML-RARα 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-RARα 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-RARα 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 derived RARα-PML fusion product plays a role in this process.17

Previous studies suggested that the PML-RARα 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-RARα 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-RARα 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 detected20; however, more recently a series of alternative chromosomal aberrations have been reported, including t(11;17)(q23;g21),31,32 t(5;17)(q35;q12-21),33 t(11;17)(q13;g21),34 and der(17).35 whereby PML-RARα is fused to the PLZF, NPM, NuMA, and STAT5b genes, respectively. In common with PML-RARα-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/RARα 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 (reviewed12,24). At pharmacologic levels of ATRA, the co-repressor complex is released from the retinoid receptor moiety of PML-RARα, NPM-RARα, PLZF-RARα, and presumably NuMA-RARα fusion proteins; however, the PLZF-RARα 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 RARα-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 i(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/RARα rearrangements were generally performed using ICRF PML and RARα 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 RARα breakpoint regions are shown in Figure 1; methods used by the participating laboratories have been fully described previously.18,38 The genomic map of RARα and exon numbering were according
semi-nested RT-PCR according to one of the previously described methods, using an XCyte 5 kit (MetaSystems, GmbH, Altlussheim, Germany). Multicolor banding (Table 1) were used for amplification of reciprocal R2 transcripts. To detect NPM-RAR a probe set 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 RARa 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 RARa 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-RARa 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 RARa 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 karyotyping was carried out on the unique case presenting with RARa-PML as the sole fusion gene using a 24XCyte kit (MetaSystems, GmbH, Altlussheim, Germany). Multicolor banding (mBAND) of chromosome 5 was carried out in the 2 (t(5;17)) cases as described recently, 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-RARa and RARa-PML fusion genes were detected by nested or semi-nested RT-PCR according to one of the previously described methods. PLZF-RARa and RARa-PLZF fusion transcripts were detected as described previously, however, for cases found by PLZF-RARa RT-PCR to have a 3’ breakpoint in PLZF (leading to retention of 3 PLZF zinc fingers in the PLZF-RARa fusion protein), PLZF primers R1 and R2 (Table 1) were used for amplification of reciprocal RARa-PLZF transcripts. To detect NPM-RARa and RARa-NPM fusion genes, we performed nested RT-PCR using NPM primers detailed in Table 1 in conjunction with previously described external and internal RARa primers. Identical RARa primers were used with STAT5b primers (Table 1) and previously described NuMA primers (N2a [external], Alt1b, Alt2b, N2b [internal]) for nested RT-PCR to screen for STAT5b-RARa and NuMA-RARa 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.

**ATRA in vitro differentiation assays**

Assays were performed according to a previously described method using ATRA at a final concentration of 10^{-8} mol/L.

**Immunofluorescence**

Immunofluorescence studies were performed as described previously using the monoclonal NA24 NPM antibody (gift from J. Cordell and D. Mason) and polyclonal or monoclonal (SE1054 or PG-M3955) 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/RARa rearrangements (n = 42), including insertions (28 of 42) and complex chromosomal changes (14 of 42); (2) PLZF/RARa rearrangements (n = 11); (3) t(5;17) (n = 2); and (4) APL lacking rearrangement of RARa (n = 5). Central morphologic review revealed no major differences between the appearances of material derived from patients with PML/RARa rearrangements and from 20 control patients with documented t(15;17). Among the remaining patients, only those with PLZF/RARa rearrangements were found to have distinct morphologic features allowing their recognition, as described in the accompanying paper.

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

**Insertion (15;17) or (17;15).** In 28 patients including 16 with a normal karyotype, FISH and molecular findings were consistent with PML/RARa 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-RARa 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

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**Table 1. Primers used for RT-PCR analyses**

<table>
<thead>
<tr>
<th>Fusion gene target</th>
<th>Primer name and sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RARa-PLZF (3’ PLZF breakpoint)</td>
<td>PLZF-R1 (external): 5’-CTTTGAGTGTGCTCTCATC-3’</td>
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<tr>
<td>RARa-NPM</td>
<td>NPM-F1 (external): 5’-CGAGATGCTGAGACATGG-3’</td>
</tr>
<tr>
<td>RARa-PLZF</td>
<td>NPM-R1 (external): 5’-CTGAGAACCTGTCTACCA-3’</td>
</tr>
<tr>
<td>RARa-NPM</td>
<td>NPM-R2 (internal): 5’-TGACTCTGGATCATCTGCA-3’</td>
</tr>
<tr>
<td>STAT5b-RARa</td>
<td>STAT5b-Sig (external): 5’-GGTGGAGCTTGAGTGAAGTG-3’</td>
</tr>
</tbody>
</table>

*Primer derived from Arnould et al.*

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consistent with the occurrence of insertion events in these patients. PML
PML-RAR
apparent by conventional cytogenetic analysis and was confirmed
consistent with insertion of chromosome 15 material into 17q. In 2
not detected by RT-PCR in either of the 2 patients tested.
PML-RAR
chromosomal abnormality at the level of resolution of conventional
small size of the insertion event and the absence of superimposed
on the 20 metaphases analyzed (data not shown), confirming the
formed on the latter patient did not reveal any chromosomal change
and 17 and expressed the
insertions because they presented with normal chromosomes 15
and t(11;17)-negative APL with morphologic features that were
reported previously.29,39 Twenty-four–multicolor karyotyping per-
Figure 2. FISH analysis of case 4. The Oncor RARα 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 RARα 121 cosmids (4 cases) or Vysis probes (3 cases), giving identical results, suggesting that the Oncor
RARα 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
diagnostic
because of the lack of evaluable metaphases. RT-PCR performed in
nuclei, but the chromosomal location could not be determined
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;
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 differ-
ent, as shown in Table 3. In both cases in which metaphase FISH
was performed, PML-RARα 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-RARα fusion on the der(15). Case 36 was previously reported to have a normal karyotype by R banding, to express a PML-RARα
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-RARα 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-RARα fusion gene.

Six patients were classified as very complex cases. In 2 of 6
cases (37 and 38), formation of the PML-RARα fusion gene was
due to a submicroscopic ins(15;17) demonstrated by FISH. In case 39,
PML-RARα resulted from a 4-way balanced translocation combined with an insertion of a chromosome 2p segment into the
der(17). In case 40, PML-RARα 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-RARα transcripts;
however, FISH analysis with Oncor probes did not show any fusion
signals, but rather duplication or triplication of RARα signals on the
der(17). Because these probes optimally detect the RARα-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 RARα-PML gene. Unfortunately,
in sufficient material was available to perform further metaphase
FISH documenting the location of the PML-RARα fusion gene.

Cases lacking PML/RARα rearrangements

PLZF-RARα cases. In 11 of 60 workshop patients, APL was
associated with a PLZF/RARα rearrangement as determined by
RT-PCR, including 5 patients that have not been reported previ-
ously (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 RARα-PLZF transcripts were detected in
addition to PLZF-RARα. RARα-PLZF has been postulated to
contribute to leukemogenesis and may play a role in the ATRA
resistance associated with this subtype of APL.52 Therefore, it was
of interest to characterize 2 cases (cases 50 and 52) of PML-RARα–
and t(11;17)-negative APL with morphologic features that were
typical of patients with the t(11;17).5 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
observed and correlated with a positive in vitro ATRA response in
the 2 patients studied (Table 2). In contrast, in case 20, in which
RARα-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-RARα
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;
apparently involving either 15q22 or 17q21 with another chromosome; and (3) very complex cases.
did not show any exchange of material involving these 2 chromosomes. However, FISH using the ICRF RARα 121 probe demonstrated signals on chromosome 11q23 in case 52 (Figure 4A). RT-PCR confirmed formation of a PLZF-RARα fusion gene in both patients. In case 52, there was sufficient diagnostic material to evaluate whether PLZF-RARα was the sole fusion gene formed, and indeed RARα-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-RARα) 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-RARα-positive patients, as described above and previously.29,38 No terminal granulocytic morphologic differentiation was observed in any patient, contrasting with the characteristic microparticulate distribution detected in PML-RARα-positive patients, as described above and previously.29,38

Table 2. PML/RARα rearrangements due to insertions

<table>
<thead>
<tr>
<th>Case no.*</th>
<th>Karyotype†</th>
<th>PML-RARα FISH</th>
<th>RT-PCR</th>
<th>RARα- PML</th>
<th>PML IF</th>
<th>In vitro</th>
<th>ATTRA</th>
<th>response</th>
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<td>Fusion on 15q§</td>
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<td>ND</td>
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<tr>
<td>3/M3</td>
<td>46,XY</td>
<td>Fusion on 15q¶</td>
<td>Positive (bcr3)</td>
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<td>Negative</td>
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<tr>
<td>17/M3</td>
<td>46,XY, -21/48, idem, + 8/46,XY</td>
<td>Fusion on 17q¶</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/M3</td>
<td>46,XX,ins(17;15)(q21q21q22)/46,XX</td>
<td>Fusion on der(17q)¶</td>
<td>Positive (bcr1)</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>38 (case 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19/M3</td>
<td>46,XY,ins(17;15)(q21q21q22)/46,XX</td>
<td>Fusion on der(17q)¶</td>
<td>Positive (bcr1)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>Positive 29 (case 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/M3v</td>
<td>46,XX</td>
<td>PML and RARα signals on 17q#</td>
<td>Negative</td>
<td>Positive (bcr3)</td>
<td>Wild-type</td>
<td>Negative</td>
<td>29, 39 (case 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable insertions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21/M3</td>
<td>45,XY,add(2)(q37), –7,add(9)(p22)/45,idem,add(10)(p14)</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr1)</td>
<td>ND</td>
<td>ND</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22/M3</td>
<td>46,XY</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23/M3</td>
<td>46,XX,del(7)(q22q34)/46,XX</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24/M3</td>
<td>46,XX</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/M3</td>
<td>46,XX</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26/M3, M3r</td>
<td>46,XX</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27/M3</td>
<td>46,XY</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28/M3</td>
<td>46,XY</td>
<td>Fusion on nuclei¶</td>
<td>Positive (bcr3)</td>
<td>Negative</td>
<td>Microparticulate</td>
<td>ND</td>
<td>38 (case 6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IF indicates immunofluorescence; ND, not determined.
*As obtained by conventional cytogenetics.
†As demonstrated by metaphase FISH.
‡FISH performed with Oncor probe set.
§FISH performed with PML E3 and RARα cDNA P63 probes.
¶FISH performed with PML 15.5 and RARα 121 probes.
#FISH performed with Oncor probe set.
**FISH performed with PML 15.22 and RARα 4D14 probes.
Table 3. PML-RARα cases due to complex rearrangements

<table>
<thead>
<tr>
<th>Case no.*</th>
<th>Karyotype</th>
<th>PML-RARα FISH</th>
<th>PML-RARα RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three-way translocations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29/M3</td>
<td>46.XY.t(1;17;15)(p32;q21;q22)/46.idem.add(21)(p13)/46,XY</td>
<td>ND</td>
<td>Positive</td>
</tr>
<tr>
<td>30/M3v</td>
<td>46.XX.t(7;17;15)(q22;q21;q22)</td>
<td>Fusion on der(15)</td>
<td>ND</td>
</tr>
<tr>
<td>31/M3v</td>
<td>46.XY.t(6;17;15)(p21;q22;46,XY</td>
<td>ND</td>
<td>Positive (bcr1)</td>
</tr>
<tr>
<td>32/M3, M3r</td>
<td>46.XX.t(8;17;15)(q22;q21;q22);t(12;14)(q13;q32)/47.idem.+/8/46,XX</td>
<td>ND</td>
<td>Positive (bcr1)</td>
</tr>
<tr>
<td>33/M3v</td>
<td>46.XX.t(13;17;15)(p13;q21;q22)/46,XX</td>
<td>ND</td>
<td>Positive (bcr3)</td>
</tr>
<tr>
<td>34/M3</td>
<td>46.XX.t(5;17;15)(q14;q21;q22)/48.idem.+8,+21</td>
<td>Fusion on der(15)</td>
<td>Positive (bcr1/2)</td>
</tr>
<tr>
<td>Simple variant cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35/M3</td>
<td>46.XX.t(5;15)(q13;q22);ins(15;17)(q22;q21q21)+/46,XX</td>
<td>Fusion on der(15)+</td>
<td>ND</td>
</tr>
<tr>
<td>36/M3</td>
<td>46.XY.t(11;17)(p34;q21)+/ins(1;15)(p34;q22q22)+</td>
<td>Fusion on der(1)+</td>
<td>Positive (bcr1)</td>
</tr>
<tr>
<td>Very complex cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37/M3v</td>
<td>46.XY.add(4)(p16);ins(15;17)(q22;q21q21)+/add(17)(q25)/46,XY</td>
<td>Fusion on der(15)</td>
<td>Positive (bcr3)</td>
</tr>
<tr>
<td>38/M3v</td>
<td>46.XY.t(5;15;17)(q35;q22;q23)+/ins(15;17)(q22;q21q21)+/der(20)</td>
<td>Fusion on der(15)</td>
<td>ND</td>
</tr>
<tr>
<td>39/M3v</td>
<td>46.XX.t(2;19;17;15)(q24;p13;q21;q22)+/ins(17;2)(q21p16p24)+/47.idem.+8/46,XX</td>
<td>Fusion on der(15)</td>
<td>ND</td>
</tr>
<tr>
<td>40/M3</td>
<td>46.XY.del(2)(p12?)+/add(15)(q22);add(16)(q?1);add(17)(q71)/47.idem.+8/46,XX</td>
<td>Fusion in nuclei</td>
<td>ND</td>
</tr>
<tr>
<td>41/M3</td>
<td>46.XX.del(7)(q10q10)+/add(15)(q25);dup(17)(q11q21)+/46,XX</td>
<td>No fusion, but RARα x2 on dup(17q)</td>
<td>Positive (bcr1)</td>
</tr>
<tr>
<td>42/M3</td>
<td>46.XX.del(6)(q17)(q23q27)+/–13,der(14)(q14;16)(p11;7)+/–16,hsr(17)</td>
<td>No fusion, but RARα x3 on hsr(17q)</td>
<td>Positive</td>
</tr>
</tbody>
</table>

ND indicates not determined.
†Abnormalities demonstrated by FISH analyses.
‡der(15) consists of 15pter–?15q22: 15q22–?5qter.
§der(1) consists of 17pter–?17q21: 17q21–?1qter.
|der(15) consists of 15pter–?15q22: 15q22–?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 Vyssis or ICRF RARα 121 probes showed an additional RARα signal on the der(5) (Figure 6A), and RT-PCR demonstrated expression of NPM-RARα and RARα-NPM fusion transcripts (Figure 7). The NPM breakpoint (Figure 7) was identical to that associated with formation of the NPM-RARα fusion in the 2 previously reported cases of APL with the t(5;17)13,65 and also to that of the NPM-ALK fusion associated with the t(2;5)(p23q35) in anaplastic large-cell lymphoma.66 In case 55, previously reported,57,68 the molecular review performed in the present study ruled out a RARα 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 RARα 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 RARα allele. These 2 cases could also be distinguished by NPM immunofluorescence using the NA24 antibody,52 which recognizes NPM-RARα as well as NPM. In the NPM-RARα-positive patient,52 diffuse nuclear staining was observed, as distinct from the nucleolar staining52 detected in the patient lacking the NPM-RARα 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 RARα. In 5 patients, FISH, Southern blot, and RT-PCR analyses did not reveal rearrangements of RARα (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. Investigations are in progress to exclude mutations of RARα in these patients, although to date, no leukemias have been reported in mice expressing mutant RARα.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
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. However, it is now clear from the present study that a sizeable fraction of cases do not have this translocation. In the 611 patients evaluated, 82 patients (13%) were t(15;17)-negative. The frequency of t(15;17) varied significantly from 44% to 57% among the various participating institutions. This variability reflects differences in the methods used to detect chromosomal breakpoints and in the diagnostic criteria for APL. It is important to note that the diagnostic criteria for APL have evolved over time, and it is possible that some of the t(15;17)-negative cases may actually have t(15;17) rearrangements that were not detected by the methods used in this study. Further research is needed to clarify the significance of t(15;17)-negative APL and to determine whether these cases have distinct clinical characteristics.

**Table 4. Clinical and biologic data in the PLZF-RARα-positive patients**

<table>
<thead>
<tr>
<th>Case* (country)</th>
<th>Sex/ Age X 10^9/L</th>
<th>WBC, (3ZF)/ (2ZF)/ (1ZF)</th>
<th>DIC</th>
<th>Karotype</th>
<th>PLZF-RARα (ZF)/ RARα (2ZF)/ PLZF†</th>
<th>ATRA treatment</th>
<th>Chemotherapy (protocol)‡</th>
<th>CR1, mo</th>
<th>Current status (from time of diagnosis)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>43§ (France)</td>
<td>M/32 11.6</td>
<td>+ 45X.1Y.1Q23(11;17)</td>
<td></td>
<td>46X4Y</td>
<td>+ (2ZF)/+</td>
<td>D1-5</td>
<td>(APL9352)</td>
<td></td>
<td>+36 Alive in clinical and molecular CR at 37 mo (allo-BMT at 5 mo)</td>
<td>New case</td>
</tr>
<tr>
<td>44§ (France)</td>
<td>M/34 2.4</td>
<td>+ 45X.1Y.1Q23(11;17)</td>
<td></td>
<td>46X4Y</td>
<td>+ (2ZF)/+</td>
<td>D1-31</td>
<td>Dauno-Ara-C (APL9352), Amsa/Ara-C as 2nd line to achieve 1st CR</td>
<td>47</td>
<td>Dead at 56 mo in relapse</td>
<td>Licht et al, 1995 (case 5)‡, Koken et al, 199958</td>
</tr>
<tr>
<td>45§ (Belgium)</td>
<td>M/68 6.9</td>
<td>+ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/+</td>
<td>D60-75</td>
<td>Dauno D1-31/Aracytine</td>
<td>2</td>
<td>Dead at 15 mo in relapse</td>
<td>Guidez et al, 199459, Licht et al, 1995 (case 2)‡</td>
</tr>
<tr>
<td>46 (UK)</td>
<td>M/53 4.5</td>
<td>++ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/+</td>
<td>D1-31</td>
<td>Dauno/Ara-C/Eto-G-CSF, 3 consolidation courses (MRC AML 1)11</td>
<td>42</td>
<td>Alive in CR1 at 43 mo</td>
<td>Grimwade et al, 1997 (case 7)30, Culligan et al, 199840</td>
</tr>
<tr>
<td>47 (USA)</td>
<td>F/37 45.2</td>
<td>+ 46XX.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/-</td>
<td>D1-18</td>
<td>No</td>
<td>3</td>
<td>CR2, lost to follow-up and dead at 11 mo (brain stem hemorrhage)</td>
<td>Scott et al, 1994 (case 16)56, Licht et al, 1995 (case 4)‡</td>
</tr>
<tr>
<td>48 (USA)</td>
<td>M/81 7.6</td>
<td>+ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (3ZF)/-</td>
<td>D1-18</td>
<td>No</td>
<td>No</td>
<td>Dead at day 18 (brain stem hemorrhage)</td>
<td>Licht et al, 1995 (case 3)‡</td>
</tr>
<tr>
<td>49 (Italy)</td>
<td>M/43 10.4</td>
<td>+ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/-</td>
<td>D1-46</td>
<td>(AIDA 049362)</td>
<td>15</td>
<td>Dead in 2nd relapse at 30 mo (auto-BMT at 23 mo)</td>
<td>New case</td>
</tr>
<tr>
<td>50§ (Italy)</td>
<td>M/34 20.0</td>
<td>- 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/-</td>
<td>D51-60</td>
<td>Dauno/Ara-C/Eto (EORTC GIMEMA AML 1064), Ara-C/lda/ATRA as 2nd line, CR1 obtained after HU</td>
<td>28</td>
<td>Alive in CR1 at 33 mo (allo-BMT in CR1 at 5 mo)</td>
<td>New case</td>
</tr>
<tr>
<td>51§ (Netherlands)</td>
<td>M/30 69.5</td>
<td>+ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (2ZF)/+</td>
<td>D1-7</td>
<td>HOVON 29,46 CR2 obtained with ATRA/G-CSF,46 consolidation with IDAC</td>
<td>11</td>
<td>Alive in clinical and molecular CR2 at 51 mo (allo-BMT in CR2 at 23 mo)</td>
<td>Jansen et al, 199946</td>
</tr>
<tr>
<td>52§ (Italy)</td>
<td>M/62 9.9</td>
<td>+ 46XY.I11;17(12;17)/</td>
<td></td>
<td>46XY</td>
<td>+ (3ZF)/-</td>
<td>D3-23</td>
<td>DAT 2+7/DAT 2+7MACC</td>
<td>16</td>
<td>Alive in CR1 at 17 mo</td>
<td>New case</td>
</tr>
</tbody>
</table>

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 Sainz et al.1

†Breakpoints in PLZF intron 2 lead to the retention of 2 zinc fingers (2ZF) in the PLZF-RARα 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.}
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 karyotype, with the t(9;22) accounting for approximately 0.8% of cases. Identification of this group is extremely important because of the poor response to retinoids as single-agent therapy and in view of recent data suggesting that these patients are also resistant to As2O3. 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 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 therapy and in view of recent data suggesting that these patients are also resistant to As2O3. 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 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

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

<table>
<thead>
<tr>
<th>Case/M3</th>
<th>Sex/Age</th>
<th>WBC, x10^9/L</th>
<th>DIC</th>
<th>Karyotype after FISH analysis</th>
<th>RT-PCR*</th>
<th>Immunofluorescence</th>
<th>ATRA treatment</th>
<th>Chemotherapy (protocol)</th>
<th>Current status (from time of diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54/M3</td>
<td>F/9</td>
<td>17</td>
<td>−</td>
<td>46,XX,ins(3;5) (q26;q1313), t(5;17)(p24;q21)</td>
<td>NPM-RARα positive, RARα-NPM positive</td>
<td>PML: wild-type pattern</td>
<td>D27-</td>
<td>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/Amso</td>
<td>Alive in CR1 at 29 mo</td>
</tr>
<tr>
<td>55/M3r</td>
<td>F/76</td>
<td>43.1</td>
<td>−</td>
<td>46,XX,del(5)(q13:q221),del(8)(q22q24),del(17)§, 5−32 dmin</td>
<td>PML-RARα negative, PLZF-RARα negative</td>
<td>NPM-RARα negative, NuMA-RARα negative, STAT5β-RARα negative</td>
<td>D1-4†</td>
<td>Dauno/Ara-C</td>
<td>Died at D14, respiratory failure due to ATRA syndrome or Corynebacterium. 90% blasts in bone marrow before death</td>
</tr>
</tbody>
</table>

Aspar indicates asparaginase; LAME, leucemie aiguë myeloïde 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.
§del(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.
adverse prognosis, as suggested previously. In addition, the present study has identified cases of APL with cryptic PLZF/RARα rearrangements, including one patient with a normal karyotype in whom PLZF-RARα 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-RARα. Whereas the 2 previously reported patients with NPM-RARα 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/RARα rearrangement as a means of determining the subgroup of APL patients likely to benefit from retinoids and As2O3. 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-RARα 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 others29,38 that observation of a microparticulate nuclear staining pattern in leukemic blasts is specific to cases expressing the PML-RARα fusion protein and therefore is predictive of a beneficial response to ATRA and As2O3. This pattern is detected in patients with the classic t(15;17) as well as in those
patients in whom PML-RARα 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-RARα–associated APL. 20 However, this study and others 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 RARα-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/RARα rearrangements37 or with expression of RARα-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

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**Table 6. Morphologic APL cases lacking evidence for RARα rearrangements**

<table>
<thead>
<tr>
<th>Case/M3</th>
<th>Karyotype</th>
<th>PML-RARα FISH</th>
<th>RT-PCR*</th>
<th>Southern blot†</th>
</tr>
</thead>
<tbody>
<tr>
<td>56/M3r</td>
<td>45,X, + Y,der(7)(7;11)(q34;p15)ins(7;12)(q34;q24.3), der(11)(7;11)/46,XY</td>
<td>Normal pattern‡</td>
<td>PML-RARα negative, PLZF-RARα negative</td>
<td>RARα not rearranged</td>
</tr>
<tr>
<td>57/M3r</td>
<td>46,XX</td>
<td>ND</td>
<td>PML-RARα/PLZF-RARα negative</td>
<td>RARα not rearranged</td>
</tr>
<tr>
<td>58/M3r</td>
<td>46,XY</td>
<td>Normal pattern‡</td>
<td>PML-RARα negative</td>
<td>RARα not rearranged</td>
</tr>
<tr>
<td>59/M3, M3r</td>
<td>46,XY</td>
<td>Normal pattern‡</td>
<td>PML-RARα, RARα-PML negative</td>
<td>ND</td>
</tr>
<tr>
<td>60/M3, M3r</td>
<td>45,XX,−2,−12,+13,add(17)(q27)</td>
<td>ND</td>
<td>PML-RARα/PLZF-RARα negative</td>
<td>RARα not rearranged</td>
</tr>
</tbody>
</table>

| Abbreviations as indicated in Table 2.  
*RNA integrity was confirmed in each case by amplification of RARα 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 BamH1 and RARα probes PX20 and 8CPP were used. Mapping details of probes are provided in Figure 1.  
‡FISH results indicate no fusion signals, no deletion of RARα, and no translocation of RARα sequences to another chromosome.  

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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(15;17) NB4 cell line. NPM immunofluorescence using the NA24 antibody shows a wild-type nucleolar pattern in both cell lines and in the NPM-RARα-negative patient (case 55), and a diffuse nuclear pattern in the NPM-RARα-positive patient (case 54). Images were captured with a Leica TCS NT confocal microscope.

Figure 9. Case 56 with der(7) and lacking RARα rearrangement. FISH using ICRF PML 15.5 (red) and RARα 121 (green) cosmids probes, demonstrating normal locations of PML and RARα sequences in the malignant clone. See text and Table 6.
confirmed APL was identified in which RARα-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 RARα-PML under the control of the human cathepsin G (hCG) promoter did not induce leukemia in its own right, RARα-PML significantly increased the frequency of APL among mice expressing a bcr1 PML-RARα transgene; furthermore, co-expression of both fusion transcripts was suggested to lead to a more aggressive form of the disease.1,2 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 RARα-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.3 The RARα-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.4,5,6,7 Recently, interesting studies have also suggested that RARα-PLZF can modify the leukemic phenotype of PLZF-RARα transgenic mice.7,8 Expression of PLZF-RARα under the hCG promoter induced a myeloproliferative disorder in 100% of mice, whereas co-expression of PLZF-RARα and RARα-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.7,9 However, in the present study, a patient with a normal karyotype was identified in whom PLZF-RARα 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.10 Apparent differences between mouse models of APL and the disease in man could reflect the nature of the hemopoietic progenitor targeted by PML/RARα and PLZF/RARα 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 RARα 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.

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### References


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

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