Hybrid fusion genes are specific tumor markers of several leukemic subtypes. The use of reverse transcription-polymerase chain reaction (RT-PCR) to amplify chimeric cDNAs allows sensitive detection of the neoplastic clone for diagnostic and monitoring studies in these leukemias. Nonetheless, the clinical relevance of minimal residual disease (MRD) evaluation by PCR remains controversial. In this study, 9 patients (pts) with acute promyelocytic leukemia (APL) in long-term remission for 4 to 12 years were analyzed for the presence of MRD by RT-PCR amplification of the specific PML/RAR-α fusion gene. Seven pts had been treated with conventional chemotherapy (CHT) alone, 1 had undergone allogeneic bone marrow transplantation (BMT), and 1 autologous BMT as consolidation therapy after CHT. In 8 cases, the presence of the t(15;17) rearrangement could be documented in diagnostic BM specimens by cytogenetic and/or molecular analysis. A two-rounds “nested” RT-PCR assay with sensitivity levels of 1 in 10⁴ was used to analyze BM samples collected at 32 to 141 months from the achievement of complete remission (CR). In no cases were residual PML/RAR-α transcripts detectable in these remission controls. All patients are in unmaintained CR at 48 to 154 months from CR and at 6 to 17 months from PCR evaluation. These results suggest that long-term survival of APL is associated with eradication of cells carrying the specific PML/RAR-α rearrangement, indicating that PCR negativity should be considered the therapeutic goal in these patients. Our findings further strengthen the clinical relevance of PCR monitoring studies in APL, as opposite to other leukemic subtypes (chronic myeloid leukemia and acute myeloid leukemia-M2) in which the prognostic significance of PCR evaluation is unclear.

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Chimeric fusion genes resulting from nonrandom chromosome translocations are consistent and specific features of several human leukemic subtypes. Relevant examples of leukemia-associated hybrid genes include the BCR/ABL rearrangement of Ph+ leukemias, the PML/RAR-α hybrid characteristic of acute promyelocytic leukemia (APL), and the AML1/ETO fusion gene found in t(8;21)-positive French-American-British (FAB) M2 acute myeloid leukemia (AML). One of the clinical advantages derived from the elucidation of these molecular abnormalities resides in the availability of ideal tumor-specific markers, which are detectable even if present in small amounts by reverse transcription-polymerase chain reaction (RT-PCR). This technique is being extensively exploited for both diagnostic and monitoring studies of these and other leukemic subtypes, and a number of clinical studies have been undertaken that are aimed at verifying the prognostic significance of PCR analysis as a predictor of patient outcome and its role in better addressing treatment strategies.

Despite the interesting premises, the clinical relevance of PCR monitoring studies in leukemia has been recently questioned. For example, in Ph+ chronic myeloid leukemia (CML) and in t(8;21) AML patients, cells carrying the abnormal fusion gene are frequently documented after several years of long-term remission. Although various factors may account for such unpredicted findings, including technical problems (false PCR positive), biologic diversity within the distinct leukemic subsets (acute v chronic, different FAB subtypes), and variability of clinical context (allogeneic/autologous bone marrow transplantation [BMT] and chemotherapy), the impact of PCR monitoring studies on treatment strategies, at least in the above mentioned leukemic subtypes, remains controversial.

In the past few years, we have focused attention on the molecular features of APL and on the clinical relevance of the specific PML/RAR-α rearrangement. An RT-PCR strategy to amplify this fusion gene was developed by our group and by others, and this has proved in independent studies to be useful for rapid diagnosis as well as for predicting response of APL to differentiation therapy with all-trans retinoic acid (ATRA). In addition, our preliminary data on treatment monitoring indicate that PCR positivity detected starting from 4 months after remission induction significantly correlates with clinical relapse, therefore representing a reliable prognostic indicator.

In this study, we analyzed by RT-PCR a selected series of 9 APL patients in long-term remission (4 to 12 years) and found no evidence of residual rearrangement in all cases. These data further highlight the clinical relevance of PCR monitoring analyses in APL and suggest that cure of this disease is associated with eradication of the neoplastic
clone. In light of the findings reported in other leukemic subtypes, our results also imply that minimal residual disease (MRD) studies have variable clinical impact in different leukemic subsets.

MATERIALS AND METHODS

Nine patients (pts) with APL in long-term remission (4 to 12 years) were selected for this study. Biologic and clinical characteristics at presentation are given in Table 1. A diagnosis of hypergranular M3 AML had been established in all cases by conventional FAB criteria.5 Five pts had a successful karyotypic examination performed at diagnosis on BM aspirates. This had showed the presence of a typical t(15;17) aberration in the blasts of 4 cases (nos. 1 through 4, Table 1) and an apparently normal 46,XX karyotype in the other (no. 7). In this latter case and in 3 other cases (nos. 3, 4, and 8), cryopreserved BM cells collected at diagnosis were available to extract total RNA for RT-PCR analysis. In pts no. 5 and 6, in which no adequate metaphases were obtained for karyotypic examination, McCoy's cytogenetic pellets were available and were used for DNA extraction and Southern blot analysis. Finally, in pt no. 9, neither cytogenetic data nor cryopreserved material were available from the time of diagnosis; therefore, the values for this patient relied on morphocytochemical analyses only.

All pts received as induction treatment conventional chemotherapy (CHT) including an anthracycline (daunorubicin or idarubicin) alone or in combination with cytosine arabinoside. Pt no. 7 underwent allogeneic BMT from an HLA-identical sibling after remission induction, whereas all other pts received conventional CHT as consolidation and maintenance. Pt no. 8, who relapsed after 11 months, was subsequently reinduced with CHT and underwent autologous BMT as consolidation.

After informed consent, remission BM aspirates were obtained at 32 to 141 months from the achievement of CR (Table 2) and used for molecular studies. These included Southern blot analysis of the RAR-α and/or PML gene configuration and RT-PCR amplification of the PML/RAR-α fusion cDNA. Southern blot and RT-PCR conditions, genomic probes to explore the PML and RAR-α genes, oligonucleotides used to amplify the PML/RAR-α cDNA, and primers used to hybridize the PCR blots have been reported elsewhere.11,15,16 Amplification of β-actin was performed as an internal control with 5 μL of the same cDNAs used to identify PML/RAR-α, as described.11

RESULTS

The specific PML/RAR-α rearrangement is detectable by Southern blot or RT-PCR in virtually 100% of FAB-defined AML-M3 cases, including those apparently lacking the t(15;17) cytogenetic aberration.18,19 Of the 9 pts included in this study, we were able to document in 8 cases the specific PML/RAR-α rearrangement in diagnostic BM cells. In 4 of these (2 of which also had a typical t15;17), the chimeric gene could be detected by RT-PCR amplification, whereas in 2 others with no available RNA, genomic RAR-α and PML rearrangements were detected by Southern blot analysis. Finally, 2 other cases (nos. 1, and 2, Table 1) had karyotypic evidence of a t(15;17) at the time of diagnosis.

The results of RT-PCR analysis in remission samples are shown in Table 2. Patients were evaluated after 32 to 141 months from the achievement of complete remission (CR). In all cases, no PML/RAR-α transcripts were visible on either the ethidium bromide gels or after hybridization of PCR products with specific PML exon II, exon III, and exon V oligonucleotides.11 Presently, these 9 pts are in unmaintained CR with a median follow up of 77 months (range, 48 to 154). The median time from RT-PCR evaluation and last follow-up is 12 months (range, 6 to 17). Figure 1 shows the results of RT-PCR analysis in diagnostic and remission BM samples of some representative cases.

To assess the sensitivity of our RT-PCR assay, total RNA isolated from pt no. 7 was serially diluted with mixing with the t15;17-negative myeloid cell line GF-D8 RNA, as reported.11 Our RT-PCR amplification assay could detect the PML/RAR-α transcript in the presence of less than 0.1 ng total RNA, which represented a final dilution between 10⁻⁴ and 10⁻³ (not shown).

DISCUSSION

In the present study, we show that long-term remission APL patients have no RT-PCR detectable residual PML/RAR-α rearrangement in their BM cells. Thus, it appears that cure of APL is accompanied by elimination (at least below our RT-PCR sensitivity levels) of cells carrying the t(15;17) aberration. We remark that in the 9 patients, 8 of whom are long-term survivors for more than 5 years (Table 2), all PCR analyses were performed in samples obtained from BM aspirates. Because APL is usually a leukopenic disease in most instances confined to BM, the analysis of peripheral blood for either diagnosis or assessment of resid-

### Table 1. Biologic and Clinical Features of Patients at Presentation

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Age (yr)/Sex</th>
<th>Year of Diagnosis</th>
<th>FAB</th>
<th>WBC (&lt;10⁹/L)</th>
<th>Hb (g/dL)</th>
<th>Pts (×10⁹/L)</th>
<th>DIC</th>
<th>Evidence of t(15;17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52/F</td>
<td>1983</td>
<td>M3</td>
<td>0.6</td>
<td>7.9</td>
<td>68</td>
<td>Yes</td>
<td>Karyotypic</td>
</tr>
<tr>
<td>2</td>
<td>24/F</td>
<td>1979</td>
<td>M3</td>
<td>0.9</td>
<td>9.0</td>
<td>97</td>
<td>No</td>
<td>Karyotypic</td>
</tr>
<tr>
<td>3</td>
<td>51/M</td>
<td>1988</td>
<td>M3</td>
<td>0.6</td>
<td>11.3</td>
<td>59</td>
<td>Yes</td>
<td>Karyotypic/PCR</td>
</tr>
<tr>
<td>4</td>
<td>40/M</td>
<td>1989</td>
<td>M3</td>
<td>4.9</td>
<td>9.1</td>
<td>52</td>
<td>No</td>
<td>Karyotypic/PCR</td>
</tr>
<tr>
<td>5</td>
<td>37/F</td>
<td>1988</td>
<td>M3</td>
<td>1.9</td>
<td>7.9</td>
<td>17</td>
<td>Yes</td>
<td>Southern</td>
</tr>
<tr>
<td>6</td>
<td>44/F</td>
<td>1985</td>
<td>M3</td>
<td>27.4</td>
<td>9.1</td>
<td>82</td>
<td>Yes</td>
<td>Southern</td>
</tr>
<tr>
<td>7</td>
<td>6/F</td>
<td>1988</td>
<td>M3</td>
<td>3.8</td>
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<td>59</td>
<td>No</td>
<td>PCR</td>
</tr>
<tr>
<td>8</td>
<td>26/F</td>
<td>1987*</td>
<td>M3</td>
<td>1.9</td>
<td>12.9</td>
<td>59</td>
<td>No</td>
<td>PCR</td>
</tr>
<tr>
<td>9</td>
<td>30/M</td>
<td>1984</td>
<td>M3</td>
<td>2.3</td>
<td>8.5</td>
<td>27</td>
<td>Yes</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Abbreviation: DIC, diffuse intravascular coagulation.

* Patient in first relapse at this time.
Table 2. RT-PCR Amplification of PML/RAR-α in Long-Term Remission BM Samples

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Treatment</th>
<th>Time of RT-PCR Analysis (mo from CR)</th>
<th>Results</th>
<th>Follow-Up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNR</td>
<td>112</td>
<td>Neg</td>
<td>124</td>
</tr>
<tr>
<td>2</td>
<td>DNR</td>
<td>141</td>
<td>Neg</td>
<td>154</td>
</tr>
<tr>
<td>3</td>
<td>IDA</td>
<td>50</td>
<td>Neg</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>IDA</td>
<td>32</td>
<td>Neg</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>IDA</td>
<td>54</td>
<td>Neg</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>DNR</td>
<td>95</td>
<td>Neg</td>
<td>105</td>
</tr>
<tr>
<td>7</td>
<td>DNR + AraC/All. BMT</td>
<td>54*</td>
<td>Neg</td>
<td>64*</td>
</tr>
<tr>
<td>8</td>
<td>DNR/Aut. BMT</td>
<td>61</td>
<td>Neg</td>
<td>77</td>
</tr>
<tr>
<td>9</td>
<td>DNR</td>
<td>105</td>
<td>Neg</td>
<td>111</td>
</tr>
</tbody>
</table>

Abbreviations: DNR, daunorubicin; IDA, idarubicin; AraC, cytosine arabinoside; All. BMT, allogeneic BMT; Aut. BMT, autologous BMT; Neg, negative.

In a previous study in which early postremission APL samples were analyzed, we have shown that 11 of 13 pts who had tested PCR-positive were in relapse 1 to 4 months later, whereas 22 pts who had tested negative remained disease-free during the observation period of 4 months to 5 years. We concluded that PCR evaluation of residual disease provides information of considerable clinical importance in APL, suggesting the need of further treatment in PCR-positive cases.\(^{16}\) The predictive value of PCR positivity as an indicator of relapse in APL has recently been confirmed in two independent studies.\(^{20,21}\)

Here we provide additional evidence that, in APL, in contrast to other leukemic subtypes in which the prognostic significance of residual disease is not clear, PCR monitoring is extremely useful for the management of the disease. In fact, combined with data from previously reported studies,\(^{16,20,21}\) our data on long-term survivors strongly suggest that PCR-negativity should be considered a therapeutic goal in APL.

Two other leukemias characterized by chimeric fusion genes, ie, Ph\(^{+}\) CML and, more recently, t(8;21) AML, have been analyzed in clinical studies aimed at defining the prognostic relevance of PCR monitoring. As regarding CML, in which the BCR/ABL rearranged clone has been documented in a significant proportion of long-term remission pts,\(^{5-8}\) it is important to consider that residual disease studies have mainly been reported in the outcome of allogeneic BMT. This obviously implies a distinct biologic context, in which the major burden of donor cells would exert a control over the host’s abnormal clone through a graft-versus-leukemia effect. However, other investigators have also reported that neither PCR-positivity in CML patients undergoing interferon treatment\(^{6}\) nor autologous BMT\(^{22}\) is necessarily associated with immediate disease recurrence.

Although recent studies on titration of the RT-PCR amplification products (“quantitative” PCR) in Ph\(^{+}\) CML seem to provide interesting prognostic information, a wide application of this technique still awaits adequate standardization.

Concerning AML1/ETO-positive M2 AML, two groups have reported the persistence of residual rearranged cells in long-term survivors treated by CHT, suggesting that the complete elimination of leukemic cells is not necessary for the achievement of long-term remission.\(^{9,10}\) Strikingly, of these AML-M2 long-term survivors who tested PCR positive, one pt reported by Chang et al\(^{9}\) was in remission for 5 years, and two cases of the series described by Nucifora et al\(^{10}\) were in remission for 8 and 9 years, respectively. In an attempt to interpretate such intriguing findings, in both reports the investigators hypothesize that the specific translocation is probably not sufficient for the expression of a clinically aggressive disease, and that a second mutational event could be required for overtly manifested disease progression.\(^{6,9}\)

We believe that the discrepancies between our findings on APL and the above-mentioned studies most likely rely on the biologic diversity of these distinct diseases. In contrast to virtually all other leukemic subsets, in which additional genetic events would probably be needed for clonal expansion of transformed cells, both the t(15;17) translocation and its underlying molecular lesion are usually detected as the only genetic abnormality characterizing APL cells. Together with most recent findings on the pathogenetic role of the PML/RAR-α protein,\(^{24}\) this suggests that the PML/RAR-α rearrangement is a unique and per se sufficient event that confers on the APL cell a fully transformed phenotype.
SUMMARY

DETECTABLE RESIDUAL DISEASE

The predictive value of residual PML/RAR-α arrangement as an indicator of relapse and, on the other hand, the need of its eradication to the final cure of the disease.

REFERENCES


Absence of reverse transcription-polymerase chain reaction detectable residual disease in patients with acute promyelocytic leukemia in long-term remission

D Diverio, PP Pandolfi, A Biondi, G Avvisati, MC Petti, F Mandelli, G Pelicci and F Lo Coco