RAPID COMMUNICATION

Molecular Evaluation of Response to All-Trans-Retinoic Acid Therapy in Patients With Acute Promyelocytic Leukemia

By Francesco Lo Coco, Giuseppe Avvisati, Daniela Diverio, Maria Concetta Petti, Myriam Alcalay, Pier Paolo Pandolfi, Daniela Zangrilli, Andrea Biondi, Alessandro Rambaldi, Maria Luisa Moleti, Franco Mandelli, and Pier-Giuseppe Pelicci

The advent of retinoic acid (RA) in the treatment of acute promyelocytic leukemia (APL) has led to a high frequency of short-lasting complete remissions (CR). We studied the response to RA by molecularly analyzing the RA receptor α (RARα) locus, which has recently been shown to be rearranged in all APLs. Southern blot analysis demonstrated that the RARα rearrangements persisted in the APL samples containing maturing myeloid cells 2 to 3 weeks after the start of RA treatment, but disappeared after 5 to 8 weeks, when the patients achieved CR.

Our investigations provide clear evidence that CR occurs at molecular level and that there is reconstitution of an apparently normal, nonclonal hematopoiesis. Further, it shows that RA acts by triggering differentiation rather than by exerting a cytotoxic effect on the leukemic clone.

© 1991 by The American Society of Hematology.

From the Hematology, Department of Human Biopathology, University "La Sapienza" of Rome; Clinica Medica 1, University of Perugia; Clinica Pediatrica, S. Gerardo Hospital-Monza, University of Milano; Hematology Section, Ospedali Riuniti of Bergamo; and Istituto Mario Negri, Bergamo, Italy.

Submitted December 7, 1990; accepted February 6, 1991.

Supported in part by an Associazione Italiana per la Ricerca sul Cancro grant to P.G.P. A. B. is supported by "Fondazione Tettamanti." Address reprint requests to Francesco Lo Coco, MD, Ematologia, Dipartimento di Biopatologia Umana, Via Benevento 6, 00161 Roma, Italy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.

0006-4971/91/7708-0636$3.00/0


1657
**Table 1. Clinical and Biologic Characteristics of APL Patients**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age/ Sex</th>
<th>Disease Status</th>
<th>Karyotype</th>
<th>WBC ( \times 10^9/L )</th>
<th>Hb g/dL</th>
<th>Plts ( \times 10^9/L )</th>
<th>% BM Blasts</th>
<th>Coagulopathy*</th>
<th>Therapy</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67/F</td>
<td>Onset</td>
<td>t(15;17)</td>
<td>1.9</td>
<td>8.3</td>
<td>54</td>
<td>70</td>
<td>Yes</td>
<td>All-t-RA</td>
<td>NE†</td>
</tr>
<tr>
<td>2</td>
<td>63/F</td>
<td>Onset</td>
<td>NA</td>
<td>0.8</td>
<td>9.6</td>
<td>9.0</td>
<td>92</td>
<td>Yes</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>18/F</td>
<td>Relapse</td>
<td>46,XX</td>
<td>1.7</td>
<td>12.9</td>
<td>177</td>
<td>80</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>51/F</td>
<td>Relapse</td>
<td>t(15;17)</td>
<td>2.4</td>
<td>14.9</td>
<td>140</td>
<td>70</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>39/M</td>
<td>Relapse</td>
<td>NA</td>
<td>1.9</td>
<td>14.9</td>
<td>79</td>
<td>76</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; NE, not evaluable; CR, complete remission.

*Defined as fibrinogen less than 150 mg/dL and FDP greater than 40 \( \mu g/mL \).
†Died of myocardial infarction after 20 days from All-t-RA start.

**RESULTS**

Southern blot analysis of BM samples collected at diagnosis identified RARα rearrangements in all five cases after hybridization with the H18 probe.

Treatment was generally well tolerated and toxicity was limited to mild bone pain and dryness of skin and lips. A significant hyperleukocytosis (> 20 \( \times 10^9/L \)) was observed in only one case (pt 1), and none of the patients displayed BM hypoplasia or coagulopathy during RA therapy.

Four patients (nos. 2, 3, 4, and 5) obtained CR within 30 to 50 days. The remaining patient (no. 1) died of myocardial infarction with associated hyperleukocytosis on the treatment day 20 and could not be evaluated for the response to RA.

Studies performed on BM specimens taken on the treatment day 14 to 16 documented partial response in three cases (nos. 2, 3, and 4), with a percentage of blasts that varied from 30% to 50%. DNA analysis showed that the rearranged RARα fragments persisted, but at a lower intensity than at diagnosis. Molecular studies performed at the time CR was achieved showed that the abnormal RARα hybridization fragments had totally disappeared (Fig 2, cases 2, 3, and 4).

The blood sample collected on day +15 from patient No. 1 was hyperleukocytic with a WBC 82.3 \( \times 10^9/L \) and a differential count of 5% promyelocytes, 29% myelocytes, 24% metamyelocytes, 36% neutrophils, and 6% lymphocytes. There was no variation in the intensity of the abnormal rearranged RARα fragment at Southern blot analysis with respect to the diagnostic BM control. The molecular picture remained unchanged in a further BM specimen aspirated on day +18 (Fig 2, case 1).

The intermediate BM control performed on day +15 in patient 5 showed no decrease in the intensity of the rearranged band. The associated picture in the BM was one of maturing cells and dysplastic elements that were not recognizable as typical leukemic promyelocytes. When this patient obtained CR on day +50, molecular analysis showed no abnormal RARα fragments.

**DISCUSSION**

By molecularly isolating the chromosome 15 and 17 breakpoints from cases of APL with the t(15;17), we and others have defined the architecture of the translocation and demonstrated the location of breakpoints within the RARα locus on chromosome 17 and the MYL transcription unit on chromosome 15. By the joining of the residual 15 with the translocated 17 fragment generates a chimeric MYL-RARα gene product. Whatever may be the role of RARα gene rearrangements play in the pathogenesis of APLs, they provide a specific molecular marker for identifying leukemic promyelocytes and monitoring of patient status during the course of the disease.

In the present study we provide molecular evidence of CR in RA-treated APL patients, and show that CR is accompanied by the reconstitution of an apparently normal, nonclonal hematopoiesis. In fact, Southern blot analysis of the four patients who achieved CR, demonstrated that the RARα rearrangements persisted 2 to 3 weeks after the start of RA treatment, but disappeared after 5 to 8 weeks.

In addition, our observations in patients 1 and 5 (persistence of the specific rearrangement pattern in maturing elements) indicate a differentiative effect of RA treatment. In particular, in patient 1 the presence of PB-maturing elements (almost 90% were metamyelocytes, myelocytes, and neutrophils) showing the same intensity RARα rearrangement as BM blasts suggested their origin from leuke-
mic promyelocytes. A similar picture was observed in the intermediate BM control of case 5, in which the persistence of the abnormal band at day +16 was seen in maturing elements and finally disappeared at day +50.

In conclusion, our findings provide further evidence that RA treatment may induce CR in APL, as recently demonstrated by other investigators. Moreover, the results of our molecular analysis suggest a “normal” hematopoietic reconstitution following RA treatment.

Therefore, the possibility of eliminating the leukemic clone of APL (even if temporarily) by maturation and without an ablative approach may further contribute to define this disease as a distinct one within acute leukemias, as recently discussed by Wiernik.

REFERENCES

Molecular evaluation of response to all-trans-retinoic acid therapy in patients with acute promyelocytic leukemia

F Lo Coco, G Avvisati, D Diverio, MC Petti, M Alcalay, PP Pandolfi, D Zangrilli, A Biondi, A Rambaldi and ML Moleti