Highly Purified Primitive Hematopoietic Stem Cells Are PML-RARA Negative and Generate Nonclonal Progenitors in Acute Promyelocytic Leukemia

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The hierarchical level of stem cell involvement in acute promyelocytic leukemia (APL) characterized by the pathognomonic PML-RARA fusion gene is unknown. To determine if the cells of the primitive hematopoietic stem cell compartment are involved in the leukemic process, we have used molecular and cell sorting techniques in peripheral blood and bone marrow (BM) cells at diagnosis from three patients with APL and t(15;17). In two of them, clonality analysis was also possible using the BsrX1 polymorphic site of the PGK gene. The PML-RARA fusion gene was readily identified by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of BM cells obtained at diagnosis in all three patients. These same samples were then used to sort CD34+ and their CD38+ and CD38- subsets by fluorescence-activated cell sorting using nonpurified cells, both types of enzymes (A and B) were found in platelets and red blood cells (RBCs) during active leukemia, but RBCs were not studied before transfusion. With regard to the hematopoietic progenitors, only colony-forming unit–granulocyte-macrophage (CFU-GM) growth was obtained and some of these progenitors were nonclonal. Moreover, they did not contain the abnormal PML-RARA fusion gene.

Materials and Methods

Case reports. Three patients were included in this study. All blood and bone marrow (BM) samples were obtained after institutional approval. Clinical and biologic characteristics of the patients are shown in Table 1. Patient no. 1 is a 46-year-old woman who was diagnosed with APL in April 1993.

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HEMATOPOIETIC STEM CELLS IN APL

A complete blood count (CBC) showed 23 × 10^9/L white blood cells (WBCs) with 80% circulating blast cells. Decreased fibrinogen levels (1.0 g/L) and increased D-dimers (5 μg/ml, N < 0.5) indicated the presence of a disseminated intravascular coagulation (DIC), but there was no bleeding. Cytogenetic analysis showed a typical t(15;17) in 26 of 27 mitoses. The patient was treated with daunorubicin, cytosine arabinoside, and all-trans retinoic acid (ATRA). Remission was obtained and she is currently undergoing maintenance chemotherapy. Patient no. 2 is a 74-year-old woman who presented with leukocytoclasia in May 1994. There was no circulating leukaemic cells. APL was diagnosed on a BM aspirate showing 92% blast cells with a t(15;17) on cytogenetic analysis. She was treated with ATRA alone and a complete remission was obtained in June 1994. She is currently on remission with maintenance chemotherapy. Patient no. 3 was 43 years old and presented in September 1994 with severe bleeding. The CBC showed 63 × 10^9/L WBCs (93% circulating leukaemic cells with an AML FAB subtype). There was evidence of severe DIC with reduced levels of fibrinogen (0.68 g/L) and factor V (40%), whereas the level of D-dimers was increased to 20 μg/ml. The patient was admitted to the intensive care unit, but died 24 hours later from hemorrhagic complications. Molecular analysis showed a PML-RARA rearrangement with a 3’ breakpoint.

**Cells.** Mononuclear cells from total BM obtained at diagnosis were separated on a Ficoll-Hypaque gradient. Cell studies were performed on BM mononuclear cells (BMMNCs) frozen at diagnosis except in patient no. 3, in whom fresh BM cells were used.

**Cell surface staining, flow cytometric analysis, and cell sorting.** For staining with CD34-fluorescein isothiocyanate (FITC) and CD38-phycocerythrin (PE) monoclonal antibodies (MoAbs; Becton Dickinson, Sunnyvale, CA), a one-step direct immunofluorescent procedure was performed according to standard techniques. Unstained cells and cells labeled with conjugated isotype-matched non-specific mouse Ig conjugated to FITC and PE obtained from Coulter (Coultronics, Margency, France) were used as negative controls. Fluorescence analysis and cell sorting were performed on a two-laser, air-cooled EPICS Elite cytometer (Coultronics). Cells were analyzed and sorted on the basis of their light scatter properties and fluorescence intensity, as published elsewhere. Briefly, a lymphoid-blast gate, which normally contains all the hematopoietic progenitors, was drawn according to a low to moderate forward scatter (SS) and to a very low side scatter (SS). Within this gate, the proportion of CD34+ CD38- cells and CD34+ CD38+ cells was estimated by determining first the channel number at which the negative control and the test sample curves crossed each other. Leukemic cells, which represented more than 70% of the analyzed cells, appeared out of this progenitor gate and were immunophenotypically CD34+CD38+. Thus, a CD34+CD38- cell fraction, a CD34+CD38+ cell fraction, and a CD34+ cell fraction, which contained leukemic cells, T cells, and presumably B cells, were sorted at 1,500 to 2,000 cells per second and collected in phosphate-buffered saline (PBS) containing 50% fetal calf serum (FCS). Under these conditions, the purity of sorted cells attained is greater than 96%.

**Hematopoietic progenitor assay.** Progenitor assays for patient no. 1 were performed from unsorted and cell-sorted subpopulations using standard methylcellulose culture. Briefly, cells were plated in 35-mm dishes (Greiner, Nütingen, Germany) in 1.1 mL of Iscove’s modified Dulbecco’s medium (IMDM) containing 0.8% methylcellulose (Fluka, Buchs, Switzerland), 30% FCS, 1% bovine serum albumin (BSA), 100 μM L-2-mercaptoethanol (Sigma, St Louis, MO), 2 mM L-glutamine, 190 U/mL penicillin, 100 ng/mL streptomycin, 3 U/mL recombinant human erythropoietin (rhEpo; Boehringer, Mannheim, Germany), 100 U/mL recombinant human interleukin-3 (rhIL-3), 100 U/mL rIL-6 (Genzyme, Boston, MA), 200 U/mL recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF; Genetics Institute, Cambridge, MA), 100 U/mL rhIL-6 (a gift from Dr L. Aarden, Red Cross Hospital, Amsterdam, The Netherlands), 50 ng/mL rhG-CSF (Rhône-Poulenc, Antony, France), and 50 ng/mL recombinant human stem cell factor (rhSCF; Amgen, Thousand Oaks, CA).

Duplicate cultures were incubated in a humidified atmosphere containing 5% CO2 in air at 37°C. BMMNCs before sorting were plated at 105 cells/dish and CD34+CD38-, CD34+CD38+, and CD34- sorted fractions were plated at 5.5 × 103, 103, and 3 × 103 cells per dish, respectively. Burst-forming units-erythroid (BFU-E) and CFU-GM were counted between 16 and 18 days of culture. BFU-E were subdivided into primitive and mature subclasses according to their number of clusters. CFU-GM were subdivided into mature (<500 cells/colony) and primitive (>500 cells/colony) subclasses. In all cell fractions, both BFU-E and CFU-GM colonies were individually plated for further molecular analysis (see below).

**DNA analyses.** DNA was extracted from diagnosis and remission samples of patient no. 1 and diagnosis samples of patient no. 2 using standard procedures. For PGK analyses using the Southern blot technique, 10 to 15 μg of DNA was digested with Pst I and BstXI and then half of the sample was further digested with Hpa II using 20 μg of DNA. Samples were then electrophoresed, transferred to nylon membranes, and hybridized to an 800-bp PGK probe (kindly provided by B. Vogelstein, Johns Hopkins University, Baltimore, MD).

PCR analysis from purified cell populations and from individual hemopoietic colonies was performed on crude cell lysates because of the limited number of cells analyzed. For sorted cell populations, we have used DNA from 660 to 2,000 cells for patient no. 1 and 550 to 4,800 for patient no. 2 that were lysed in ddH2O at 95°C for 5 minutes and then frozen immediately. For PCR-PGK analysis of individual colonies, the technique described by Gilliland et al9 was used. Briefly, each individual CFU-GM or BFU-E was plated from the methylcellulose dish into 20 μL ddH2O and then placed at 95°C for 5 minutes. In some later experiments, each colony was initially digested with proteinase K for 1 hour at 50°C to increase the access of HpaII to the crude cell lysate originating from the colony. These samples were either frozen at –20°C or processed immediately. Half of the crude cell lysate sample was digested with 10 U of HpaII in a total volume of 20 μL for 1 hour and half was left undigested.
PCR analysis was performed on both fractions using the external and internal PGK oligonucleotides as described.8 After two rounds of PCR, 20 μL of the amplified product was digested with 15 U of BstXI (New England Biolabs, Beverly, MA) for 6 hours or overnight at 55°C in the buffer recommended by the manufacturer. Amplified fragments were detected in 2% agarose gels after ethidium bromide staining.

**RNA analyses.** RNA was extracted from different cell populations at diagnosis in patients no. 1 and 3 using the technique of Chomczynski and Sacchi.9 Half of the sorted cell population for patient no. 1 (ie, 660 to 2,200 cells) and all sorted cells for patient no. 3 (8,000 to 150,000) were used for RNA extraction. No RNA could be obtained from the purified cell populations in patient no. 2. cDNA was synthesised using random hexamers (10 mmol/L), dNTP (1 mmol/L each), 20 U RNAsin, 10% DTT, and 200 U Moloney’s murine leukemia virus (MMLV) reverse transcriptase in diethyl pyrocarbonate-treated water at 37°C for 1 hour followed by an incubation at 70°C for 10 minutes. RT-PCR amplification was performed using a PML sense oligonucleotide (5' GTC TCC AAT ACA ACG ACA GC 3') specific for nucleotides 1543-1562 from Kastner et al.10 and an RARA-specific antisense oligonucleotide (5' TGA GGA CTT GTC CTG ACA GA) recognizing nucleotides 1735-1754 from Pandolfi et al.11 Both oligonucleotides were used at 0.4 μmol/L. A total of 5 to 12 μL of cDNA was amplified for 35 or 40 cycles with the following parameters: 30 seconds at 92°C, 1 minute at 55°C, and 1 minute at 72°C. Fifteen microliters of amplified product was diluted at 1:100 and 4 μL was then reamplified with internal primers (0.4 μmol/L; PML 3'-internal sense nucleotides 1574-1594 AGT GCA GCC AGA CCC AGT GCC and RARA internal antisense nucleotides 1676-1696 GAG GGA GGG CTG GGC ACT ATC) for a further 35 or 40 cycles using the same parameters. Fifteen microliters of amplified product was electrophoresed in a 2% agarose gel and the product was visualized with ethidium bromide. Quantity and quality of RNA was assessed by amplification of β actin12 or the porphobilinogen deaminase (PBGD)13 transcripts of the same cDNA.

The sensitivity of PML and RAR oligonucleotides under these conditions was determined by dilution of patient samples in HL60 cells with subsequent extraction of RNA. When the reaction was started with 5 μL of cDNA and two rounds of 35 cycles of PCR using internal primers, the sensitivity was approximately 10⁻². The latter could be increased consistently to 10⁻³ when the reaction was performed with 12 μL of cDNA followed by two rounds of 40 cycles of PCR. No increase in sensitivity was obtained by hybridization with an internal probe (data not shown).

**RESULTS**

**Patient no. 1.** Figure 1 shows the results of the clonality analysis performed on the diagnostic and remission marrow samples from patient no. 1. The BMMNC fraction analyzed from the diagnosis sample showed a typical monoclonal pattern with the disappearance of the 1.05-kb fragment in the sample digested with Hpa II (Fig 1, sample 1, lane b). When the same analysis was performed on the remission BM, a polyclonal pattern was identified with the reappearance of the 1.05-kb band, indicating the emergence of cells that did not belong to the leukemic clone (Fig 1, sample 2, lane b). This analysis also allowed us to determine the genotype of the leukemic cell population that had apparently originated from a cell in which the PGK allele lacking the BstXI site was active (Fig 1, sample 1, lane b).

After staining with CD38-FITC and CD38-PE MoAbs, this same diagnosis marrow sample was sorted by flow cytometry into three separate fractions as described in the Materials and Methods. CD34⁺/CD38⁺ cells and CD34⁻/CD38⁻ cells represented 3.25% and 0.8% of the cells analyzed, respectively. Half of the sorted cell population was used for clonogenic assays, whereas the remaining half was used for DNA and RNA analyses. Table 2 shows the clonogenic potential of each purified cell populations scored at day 16 of the culture. Very few colonies were obtained from the starting cell sample and none were obtained from the CD34⁺ cell fraction, whereas both granulocytic and erythroid colonies with a normal morphology were obtained from the purified CD34⁺/CD38⁻ and CD34⁻/CD38⁺ cells. Individual hematopoietic colonies from these dishes were then plucked and stored at −20°C for PCR/PKG analysis.

Both purified fractions (CD34⁺/CD38⁻ and CD34⁻/CD38⁻) were then used for PCR/PKG analyses and for detection of PML-RAR rearrangement by RT-PCR. DNA analysis of both fractions showed a polyclonal pattern (Fig 2). A small difference could be shown between the CD34⁺/CD34⁻ and CD34⁺/CD38⁻ fractions in terms of 530/433 band ratios, but this was most likely caused by the occurrence of heteroduplexes favoring the amplification of the 530-bp band. Indeed, the comparison of lanes a and b in both fractions did not show any difference of band intensity and it is very likely that both cell populations had a polyclonal cell content (Fig 2). Similarly, the CD34⁻ fraction seemed also to have a polyclonal content, but we could show a clear shift towards the lower molecular weight 433-bp band in the presence of the methylation-sensitive enzyme. This finding is therefore consistent with the presence of a predominant population of cells that are clonal for the allele containing the BstXI site as suggested by the results of the
Southern blot data at diagnosis (Fig 1). This fraction contained essentially leukemic cells but also CD34- cells in the progenitor window, including T and B cells. The persistence of the upper band is probably caused by the presence of polyclonally derived T cells in the presence of heteroduplexes favoring the amplification of the 530-bp band (Fig 2).

The fluorescence-activated cell sorter (FACS)-purified samples as well as the unsorted diagnostic BMMNC sample were then analyzed for the presence of a PML-RARA rearrangement using an RT-PCR technique. Initial nonnested RT-PCR analysis of diagnostic RNA using two sets of PML primers flanking the 5' and the 3' breakpoints in the PML gene showed a typical 3' breakpoint corresponding to bcr 1 of Lo Coco et al14 (data not shown). Further analyses were therefore performed with the bcr-1 3'-specific primers. As shown in Fig 3, RT-PCR analysis using two rounds of 35 cycles of PCR and nested primers close to the PML-RAR junction allowed identification of the abnormal transcript in the leukemic monoclonal sample (Fig 3, lane 4). To determine the sensitivity of our technique, we then performed dilution analysis of leukemic cells in HL60 cells. This allowed us to determine that, under the conditions used, 1 cell in $10^5$ could be detected (Fig 3, lanes 5 through 8).

The RNA analysis from the FACS-purified fractions was initially performed using 35 cycles of PCR after cDNA synthesis, followed by an additional 35 cycles using internal primers. This analysis showed both CD34+/CD38+ and CD34+CD38- fractions to be free of PML-RAR transcripts, whereas the actin transcript was readily identified (data not shown). To increase the sensitivity of our technique, we then increased the starting cDNA material from 5 µL to 12 µL and performed 2 rounds of 40 cycles of PCR under the same conditions. Using this approach, PML-RARA transcripts were detected in the CD34+/CD38- cell fraction (Fig 3, lane 11), whereas CD34+/CD38+ cell fraction remained PML-RARA negative (Fig 3, lane 10). Interestingly, using the same RT-PCR conditions, the unfractionated remission sample remained positive for PML-RARA transcripts (data not shown).

To determine the clonogenic content of the purified cell populations, we then used PCR-PGK analysis. Using this technique, which was first developed by Gilliland et al, each colony should give a typical clonal pattern with the disappearance of either the 530-bp or the 433-bp PGK allele in the Hpa II-digested fraction because Hpa II-digested fragments will not be amplified during PCR. Table 3 shows the results obtained with cell populations at diagnosis as well as the BM sample obtained during remission. These data indicate that both CD34+/CD38- and CD34+/CD38+ cell populations contain colonies of both genotypes, ie, either with the 530-bp or the 433-bp PGK allele remaining active. More importantly, even in the CD34+/CD38+ cell population in which RT-PCR analysis showed the presence of PML-RARA transcripts, we were able to identify colonies with a genotype opposite to that found in the leukemic promyelocytes (Table 3). Similarly, the remission sample contained a normal distribution of colonies of both genotypes, with either the 530-bp or the 433-bp allele of PGK remaining active (Table 3 and Fig 4).

**Patient no. 2.** Clonality analysis was performed on diagnosis BM sample and from the FACS-purified CD34+/CD38+ and CD34+/CD38- fractions. As shown in Fig 5, PCR-PGK analysis of the BMMNC sample containing 92% of blasts showed a clonal pattern with the successful ampli-
Fig 3. RT-PCR analysis of RNA from diagnosis and remission samples as well as from the FACS-purified cell populations in patient no. 1. RNA was extracted using guanidium isothiocyanate-acid phenol procedure, reverse-transcribed, and amplified with actin primers (internal control for the integrity of RNA) or primers specific for 3' PML breakpoint and RAR. Two rounds of 40 cycles of amplification were performed using internal primers. Lane 1, size marker; lane 2, RNA from HL-60 cells (negative control); lane 3, water control; lane 4, blast cells at diagnosis, giving rise to a 270-bp PML-RAR fragment; lanes 5 through 8, PML-RAR-positive diagnostic sample diluted in HL-60 cells before being processed for RNA extraction and PCR at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions, respectively; lane 9, BM sample before cell sorting; lanes 10, 11, and 12, RNA from CD34+/CD38-, CD34+/CD38', and CD34' fractions, respectively. Leukemia-specific PML-RAR fusion gene was not found in the CD34+/CD38' cell population after two rounds of PCR of 35 cycles each and nested primers.

Table 3. Clonality of Primitive Hematopoietic Cells From Patient No. 1 at Diagnosis and at Remission Using Methylation Analysis of Polymorphic PGK Fragments Amplified by PCR

| PGK Genotype* | Sorted CD34' Subpopulations (diagnosis) | | | BMMNC (remission) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CD34+/CD38' | CD34+/CD38' | Total† | CD34+/CD38' | CD34+/CD38' | Total† | CD34+/CD38' | CD34+/CD38' | Total§ |
| Type A | 3 | 2 | 5 | 2 | 2 | 4 | 4 | 3 | 7 |
| Type B | 5 | 2 | 7 | 2 | 3 | 5 | 3 | 3 | 6 |

The results shown represent the number of colonies successfully amplified and considered to be interpretable given the fact that technical artefacts (plucking fibroblasts or more than 1 colony) can give ambiguous results, as previously described.

* Colonies designated as type A are the progenitors that originated from a cell in which the PGK allele lacking the BstXI site remained active (similar to the pattern seen in Southern blot analysis of blast cells), as opposed to colonies type B with the PGK allele possessing the BstXI site remained active.

† Plucked, 46; amplified, 30; ambiguous, 18.
‡ Plucked, 42; amplified, 32; ambiguous, 23.
§ Plucked, 30; amplified, 29; ambiguous, 16.
HEMATOPOIETIC STEM CELLS

Involvement is of considerable interest in AML as a specific translocation could occur preferentially in a certain stage of hematopoietic differentiation. In this report, we have studied three patients with APL using cell purification and molecular biology techniques. Our findings are consistent with the hypothesis that the target cell for neoplastic amplification in APL has a CD34+/CD38' phenotype. In two patients, clonal analysis could be performed using methylation analysis of BstXI polymorphic fragments of the PGK gene; in a third patient, who was a male, only an RNA-based PCR analysis could be performed from the purified CD34+/CD38' and CD34+/CD38- cell populations. In the first patient, in whom clonal analysis could be performed at the level of hematopoietic progenitors, we were able to show that both CD34+/CD38' and CD34+/CD38- cell populations contained non-clonal progenitors, but the PML-RARA transcript was shown only in the CD34+/CD38' fraction. The latter finding could also be shown in the patient no. 3, with the presence of a faint band in the CD34+/CD38' fraction, whereas the CD34+/CD38- fraction was PML-RARA negative. This finding could be explained either by the selection of a small number of leukemic cells during the cell sorting process or, conversely, by the fact that some leukemic cells have the CD34+/CD38' phenotype. We are not able to distinguish at this time between these two possibilities. Our immunotyping data indicate that the leukemic cells obtained at diagnosis are essentially CD34+/CD38'. The presence of non-clonal progenitors in the PML-RARA-negative CD34+/CD38' cell fraction as well as in the PML-RARA-positive CD34+/CD38' cell fraction from patient no. 1 at diagnosis

DISCUSSION

The determination of target cell populations for neoplastic involvement is of considerable interest in AML as a specific

translocation could occur preferentially in a certain stage of hematopoietic differentiation. In this report, we have studied three patients with APL using cell purification and molecular biology techniques. Our findings are consistent with the hypothesis that the target cell for neoplastic amplification in APL has a CD34+/CD38' phenotype. In two patients, clonal analysis could be performed using methylation analysis of BstXI polymorphic fragments of the PGK gene; in a third patient, who was a male, only an RNA-based PCR analysis could be performed from the purified CD34+/CD38' and CD34+/CD38- cell populations. In the first patient, in whom clonal analysis could be performed at the level of hematopoietic progenitors, we were able to show that both CD34+/CD38' and CD34+/CD38- cell populations contained non-clonal progenitors, but the PML-RARA transcript was shown only in the CD34+/CD38' fraction. The latter finding could also be shown in the patient no. 3, with the presence of a faint band in the CD34+/CD38' fraction, whereas the CD34+/CD38- fraction was PML-RARA negative. This finding could be explained either by the selection of a small number of leukemic cells during the cell sorting process or, conversely, by the fact that some leukemic cells have the CD34+/CD38' phenotype. We are not able to distinguish at this time between these two possibilities. Our immunotyping data indicate that the leukemic cells obtained at diagnosis are essentially CD34+/CD38'. The presence of non-clonal progenitors in the PML-RARA-negative CD34+/CD38' cell fraction as well as in the PML-RARA-positive CD34+/CD38' cell fraction from patient no. 1 at diagnosis

FIGURES

FIGURE 4. PCR analysis of individual hematopoietic colonies in patient no. 1 from the CD34+/CD38 cell fraction (A) and from the remission BM (B). Note that in the two BFU-E shown here the absence of amplification of the PGK allele with the BstXl digest indicates a genotype that is different from the pattern observed in leukemic blasts that were clonal for the allele containing the BstXl site.

FIGURE 5. Clonal analysis of unpurified BMMNCs and FACS-purified CD34+/CD38 and CD34+/CD38 hematopoietic cells from patient no. 2. The unpurified fraction shows the presence of a predominant cell population that is clonal for the allele lacking the BstXl site, whereas in both purified fractions a shift towards the 433-bp band is seen, indicating the enrichment of both on polyclonal cells.

FIGURE 6. PML-RARA analysis of hematopoietic cells from patient no. 3 using PML-RARA (upper panel) and PBGD (lower panel) primers. Lane 1, CD34+ cells obtained after cell sorting; lane 2, CD34+/CD38- fraction; lane 3, CD34+/CD38' fraction; lane 4, BMMNCs before cell sorting; lane 5, negative control HL60 cells; lane 6, 1-kb molecular weight marker.
strongly suggests that the primitive hematopoietic stem cell compartment (CD34+/CD38-) is free of leukemic cells in APL. Although clonal analysis at the level of hematopoietic progenitors could not be performed in patients no. 2 and 3, FACs-purified CD34+/CD38- cell populations were either enriched on polyclonal progenitors (patient no. 2) or PML-RARA negative (patient no. 3) compared with unpurified BMMNCs or with blast-enriched CD34+ cell populations (patient no. 3). One other possibility to explain the absence of PML-RARA transcripts in the CD34+/CD38- cell fraction could be the absence of transcription of PML-RARA gene in this particular subset of progenitors despite the presence of the t(15;17), a situation that has been recently described in some patients with CML.15 The methodology used in our study cannot address this possibility, which has never been described in APL. One reason that makes it unlikely is the nonclonal nature of FACs-purified CD34+/CD38- cells in two of the three patients that have been analyzed in this study. In both patients no. 1 and 2, BMMNCs obtained at diagnosis showed a clear clonal pattern that was different from the pattern found in CD34+/CD38- fractions. Moreover, in patient no. 1, we have shown that 50% of informative clonogenic progenitors originating from the CD34+/CD38- fraction were of type B, which is different from the type A pattern found in the leukemic cells. However, a minor fraction of CD34+/CD38- cells could have a t(15;17) without transcription of the PML-RARA hybrid gene and without detectable clonal amplification. This finding would then imply either the absence of self-renewal capacity in these cells that represent the most primitive hematopoietic stem cell fraction described in humans in terms of long-term culture initiation potential16 or the occurrence of clonal amplification only during hematopoietic differentiation towards a CD34+/CD38- phenotype. Until the demonstration of the absence of transcription of a PML-RARA hybrid gene in CD34+/CD38- cell populations in APL, these assumptions remain essentially hypothetical. A recent report using SCID mouse reconstitution model has shown the feasibility of an in vivo assay for stem cells initiating AML17 with the demonstration, in a single patient with an FAB M1 subtype, of the origin of the leukemia in the CD34+/CD38- fraction. Clearly, the use of both clonality and SCID mouse reconstitution experiments in other subtypes of AML at the level of hematopoietic stem cells could be extremely useful in the future. The results obtained with the single AML M1 patient in SCID model, compared with those obtained by X-chromosome inactivation analysis data,3 highlight the heterogeneity in AML at the level of stem cell involvement. As opposed to other subtypes of AML, APL has been characterized as a distinct entity with typical molecular18 and clinical19 features, including the unique in vitro and in vivo differentiative capacity with ATRA.20 The ability to distinguish primitive stem cells with nonneoplastic characteristics in APL has important research implications, especially in studying PML-RARA-associated leukemogenesis. Our results could also have important clinical implications. It is generally accepted that 20% to 30% of patients with APL will become resistant to ATRA and will eventually relapse. If the most primitive hematopoietic stem cell compartment is not involved, this would suggest that the employment of autografting strategies using purified CD34+ cell populations may be valid in APL. The use of CD34+ cell subpopulations for grafting are also being evaluated in some experimental settings.21 Finally, the CD34+/CD38- cell population in APL could be used for stem cell amplification or genetic manipulation purposes, when these techniques become available, in relapsed patients.

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