High-risk acute promyelocytic leukemia with unusual T/myeloid immunophenotype successfully treated with ATRA and arsenic trioxide-based regimen

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Abstract

We describe two patients with acute promyelocytic leukemia (APL) with an unusual immunophenotype with co-expression of myeloperoxidase (MPO) with cytoplasmic CD3 (cCD3) representing myeloid and T-lineage differentiation. Both harbored *FLT3*-ITD mutations. One additionally had a deletion in the *PML* gene affecting the primer binding site, thus limiting measurable residual disease (MRD) analysis during follow-up. Both patients achieved durable remission with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO)-based therapy, thus mitigating the need for repetitive conventional chemotherapy cycles and allogeneic stem cell transplantation. Our report highlights the complexity and challenge of diagnosis and management of APL due to the variant immunophenotype and genetics and underscores the importance of synthesizing information from all testing modalities. The association of the unusual immunophenotype and *FLT3*-ITD mutation illustrates the plasticity of the hematopoietic stem cell and the pathobiology of leukemia with mixed lineage or lineage infidelity.

Keywords Acute promyelocytic leukemia (APL) · Mixed phenotype acute leukemia (MPAL) · Measurable (minimal) residual disease (MRD) · All-*trans* retinoic acid (ATRA) · Arsenic trioxide (ATO)

Introduction

Acute promyelocytic leukemia (APL) is an oncologic emergency due to the high mortality rate from hemorrhage and disseminated intravascular coagulation. Its exquisite sensitivity to all-trans retinoic acid (ATRA) mandates rapid diagnosis and initiation of therapy. Morphology and

immunophenotype allow a preliminary diagnosis in most cases while awaiting confirmation by fluorescent in situ hybridization (FISH) or conventional karyotyping for the chromosomal translocation t(15;17). In the majority of cases, the leukemic promyelocytes express myeloid antigens CD117, CD33 (homogeneous), CD13 (heterogeneous), and myeloperoxidase (MPO) and are negative for CD34 and HLA-DR [1–4]. The blasts in APL consistently lack or have a very dim expression of CD14, CD15, CD11a, CD11b, CD11c, CD18, CD66b, and CD66c [1, 5]. This immunophenotype in experienced hands has been reported as 100% sensitive and 99% specific for predicting APL [3, 4]. Aberrant expression of T cell antigen CD2 and of CD34 may be seen in the microgranular variant (M3v) of APL [6, 7]. Other variant immunophenotypes have been described including expression of CD15, CD56 [8], and variable expression of CD11b and CD11c [9].

According to the 2008 and 2016 revised World Health Organization (WHO) classification, acute leukemias expressing stringent lineage-defining markers of more than one lineage are characterized as mixed phenotype acute leukemias

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(MPAL) [8]. A diagnosis of MPAL also requires exclusion of acute leukemia with recurrent cytogenetic abnormalities including t(15;17). Previously, European Group for the Immunological Classification of Leukemia (EGIL), a scoring system based on expression of lineage-specific antigens, was applied to define biphenotypic acute leukemia (BAL) [9]; some of the high-scoring antigens were subsequently determined not specific—for example, CD79a, which may be present on normal thymocytes, relegating the EGIL system to historical reference. Expression of cytoplasmic CD3 (cCD3) defines T-lineage. The expression of cCD3 in APL is unusual and extremely rare. In a comprehensive analysis of the T-lymphoid genetic program in APL, Chapiro et al. [10] reported cCD3 expression in 2 out of 13 samples from patients with microgranular variant (M3v) of APL, which were selected from a cohort of 36 APL patients. The impact of this phenotype on APL outcome is unknown. Here we describe two patients diagnosed with APL, confirmed for t(15;17) promyelocytic leukemia/retinoic acid receptor α (*PML/RARA*) by FISH, with a T/myeloid immunophenotype. Both were treated successfully with ATRA- and arsenic trioxide (ATO)-based therapy, resulting in complete morphological and cytogenetic remission. Molecular remission was confirmed in one patient.

Methods

The morphological evaluation was performed on Wright-Giemsa stained peripheral blood and bone marrow aspirate smears, and hematoxylin and eosin-stained core biopsy sections.

Flow cytometry

Four-color flow cytometry was performed on the BD FACSCantos™ II flow cytometer. Typically 20,000 events were acquired. Peripheral blood and bone marrow sample processing and antibody staining were performed by a standard wash/lyse method per manufacturer's suggestion. The following antibodies were used for analysis (all obtained from BD Biosciences unless otherwise specified): anti-CD34, CD117, CD13, CD33, CD15, CD16 (BD Pharminogen), CD14, CD11b, CD11c, MPO, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD79a, kappa/lambda combination (Dako), CD56, CD30, CD1a (Bio-Rad), terminal deoxynucleotidyl transferase (TdT), and HLA-DR. They were conjugated with one of four fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), or allophycocyanin (APC). The amount of antibodies used was based on the manufacturer's instructions. For intracytoplasmic staining (TdT, cytoplasmic CD3, cytoplasmic CD79a, MPO), prior fixation and permeabilization

were performed. The analysis was performed using the BD FACS Diva v8.0.1 software. The dim CD45, low, or variable side scatter cell population was selected for analysis.

Immunohistochemistry

Immunohistochemical stains were performed on formic acid-decalcified, formalin-fixed, paraffin-embedded, 4- μ m-thick bone core biopsy sections. Immunohistochemistry was performed on the Bond-III automated immunohistochemistry (IHC) platform (Leica Microsystems) using primary Bond ready-to-use antibodies to CD34, CD2, CD3, CD5, CD7, TdT, and MPO antigens. The tissue bound primary antibodies were visualized using Bond Polymer Refine Detection according to the manufacturer's instructions.

Cytogenetics

Conventional metaphase karyotyping was performed by GTG-banding, and FISH was performed on interphase cells using dual-color, dual-fusion probes specific for the *PML* (15q22) and *RARA* (17q21) loci, which identify the *PML/RARA* gene fusion.

Quantitative reverse transcription-polymerase chain reaction

qRT-PCR for the *PML-RARA* mRNA (*bcr1*, *bcr2*, and *bcr3* isoforms) was performed on pre- and post-treatment samples at a commercial laboratory. RNA was isolated, reverse transcribed into complementary DNA (cDNA), amplified using primers specific for the *PML* and *RARA* genes, and subjected to qRT-PCR analysis using primers previously described in the Europe Against Cancer (EAC) guidelines [11] designed to amplify across *PML-RARA* breakpoints. There are three common breakpoints within the *PML* gene, *bcr1* (intron 6), *bcr2* (exon 6), and *bcr3* (intron 3). All breakpoints fuse a portion of the *PML* gene to a consistent breakpoint region within the *RARA* gene to generate the long, short, or variable-fusion isoforms. The level of *PML-RARA* fusions in the patient sample was expressed as copy numbers further normalized to the reference gene *ABL1* and resulted in a normalized ratio of *PML-RARA* transcripts to *ABL1* transcripts present in the sample.

Sequencing

Sequencing was performed at the translational genomics laboratory of the University of Maryland School of Medicine. To amplify the *PML-RARA* fusion gene, a two-step RT-PCR analysis was performed as described by Biondi et al. [12]. The PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide and

visualized under ultraviolet light. Following purification of the PCR products, Sanger sequencing was performed on an automated DNA sequencer [(ABI 3730 DNA analyzer, (Life Technologies, Carlsbad, CA)]. The nucleotide sequence of the different PCR fragments was assessed by using the sense primers M2 and M5 for the M2/R5-R8 and M5/R5-R8 *PML-RARA* fusion products as described previously [12]. The sequencing data were further analyzed using the human BLAST-like alignment tool (BLAT) search tool from the University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu/cgi-bin/hgBlat?hgsid=662024597_5FG1jOCdchki6XggN5IPRH5oOaIT&command=start).

Case reports

Patient 1

A 29-year-old previously healthy African-American woman presented with new-onset gum bleeding and a petechial rash. White blood cell (WBC) count was $23 \times 10^3/\text{mL}$ (normal range 4.5–11), hemoglobin (Hgb) 4.6 g/dL (normal range 12–17), and platelet count $32 \times 10^3/\text{mL}$ (normal range 150–450) (Table 1). The peripheral blood smear had 92% immature cells with folded or bilobed nuclei, scant to occasionally dense azurophilic cytoplasmic granules, and Auer rods, morphologically typical of microgranular variant of APL (Fig. 1a). The blasts co-expressed cytoplasmic MPO, along with cCD3 and cytoplasmic TdT, consistent with a T/myeloid immunophenotype (Fig. 1b). The complete immunophenotype is shown in Table 2. The expression of T-lineage antigens (CD2, CD3, CD5, and CD7), TdT, CD34, and MPO was also investigated by immunohistochemical (IHC) stains on the core biopsy section (Fig. 1c–h), which confirmed CD3 expression in a subset of leukemic promyelocytes. The expression of CD3 and other pan T cell antigens CD2 and CD7 was dimmer in the leukemic promyelocytes when compared to the residual normal T cells in the sections (Fig. 1e, f, h). The abnormal t(15;17) *PML/RARA* translocation was identified by metaphase cytogenetics and confirmed by FISH in 92% of nuclei. qRT-PCR identified the bcr3 *PML/RARA* transcript in peripheral blood (155.568%) and bone marrow (246.369%). The FMS-like tyrosine kinase 3 receptor internal tandem duplication (*FLT3*-ITD) mutation was identified by sequencing.

The patient was treated per the APML4 regimen for remission induction with ATRA, ATO, and idarubicin for high-risk APL with dexamethasone prophylaxis for differentiation syndrome [13]. The induction course was complicated by disseminated intravascular coagulation (DIC) and neutropenic fever. Follow-up bone marrow biopsy on day 35 after the initiation of induction chemotherapy (D+35) showed trilineage hematopoiesis without morphological evidence of APL. *PML/*

Table 1 Patient characteristics

	Age (years)/sex	WBC at diagnosis (/mL)	Platelets at diagnosis (/mL)	Immunophenotype	Karyotype	Oncoprotein	Myeloid mutations	Outcome
Patient 1	29/F	23,000	32,000	CD34, CD117, HLA-DR, CD38, CD71, CD33, CD11b, CD11c, CD13, CD7, cMPO, cTdT, cCD3	46,XX,t(15;17)(q24;q21) [20]. nuc ish(PML/RARA)x3, (PML con RARA)x2[183/200]	<i>PML/RARα</i> bcr3 isoform	<i>FLT3</i> -ITD (p.Glu604_Phe605ins6, C.1794_1811dup)	Ongoing CR (26 months after diagnosis)
Patient 2	50/M	20,000	11,000	CD34 (partial), CD117, HLA-DR, CD38, CD71, CD64, CD33, CD13, CD11c, cMPO (dim), cCD3	46,XY,t(15;17)(q24;q21) [20]. nuc ish(PML/RARA)x3, (PML con RARA)x2[183/200],(RARAx2) (5'RARA sep 3' RARAx1)[179/200]	–	<i>FLT3</i> -ITD (p.Tyr599_Pro606dup, c.1795_1818dup, ASXL1 (p.Pro779Leu)	Ongoing CR (23 months after diagnosis)

Values are defining T and myeloid lineages of the leukemia cells

ASXL1 additional sex combs like 1, c cytoplasmic, CR complete remission, F female, *FLT3*-ITD FMS-like tyrosine kinase 3 receptor internal tandem duplication, M male, *PML/RARα* promyelocytic leukemia/retinoic acid receptor alpha, TdT terminal deoxynucleotidyl transferase, WBC white blood cells

RARA transcript in the bone marrow had decreased to 2.424%. Subsequently, the patient underwent consolidation therapy with ATRA and ATO and maintenance therapy with ATRA, methotrexate, and mercaptopurine. The PML-RARA transcript was undetectable ($<0.001\%$) on D+139, as was *FLT3*-ITD. The patient remains in complete remission (CR) at last follow-up and PML-RARA transcript remains undetectable 26 months after diagnosis.

Patient 2

A 50-year-old Caucasian man with no past medical history presented with fatigue, fever, and petechial rash. WBC was $20 \times 10^3/\text{mcL}$, Hgb 7.3 g/dL, and platelet count $11 \times 10^3/\text{mcL}$. On peripheral blood smear, 82% of the WBC were immature with folded or bilobed nuclei, fine azurophilic granules in the cytoplasm, and Auer rods, typical for the microgranular variant of APL (Fig. 2a). The blasts co-expressed cMPO and cCD3 by flow cytometry, consistent with a T/myeloid immunophenotype (Fig. 2b). The complete immunophenotype is shown in Table 2. The expression of T-lineage antigens (CD2, CD3, CD5, and CD7), TdT, CD34, and MPO was investigated by immunohistochemical (IHC) stains on the core biopsy section

(Fig. 2c–h), which confirmed CD3 expression, and additionally expression of CD2 and TdT in a subset of leukemic promyelocytes. The results of IHC are shown in Fig. 2c–h and Table 2. Conventional cytogenetics identified $t(15;17)$, and FISH confirmed the presence of *PML/RARA* in 91% of nuclei. However, qRT-PCR did not identify a *PML/RARA* transcript. Further sequencing analysis of the *PML/RARA* fusion gene revealed a deletion of exon 5 of the *PML* gene in its entirety on chromosome 15q. The breakpoints of the deletion were identified as seq[GRCh37] chr15:74324913–74325056 on the GRCh37/hg19 browser. Based on the Human Genome Variation Society (HGVS) (<http://varnomen.hgvs.org/>), the deletion nomenclature is *PML* c.1255_1398del p.(Pro419_Glu466del). Molecular studies identified *FLT3*-ITD and *ASXL1* mutations.

The patient was treated per the APML4 regimen for high-risk APL [13]. His hospital course was complicated with neutropenic fevers and fungal pneumonia, which were successfully treated with intravenous antibiotics and antifungal agents. On D+25, cells with morphological features of APL were absent from the bone marrow aspirate and biopsy. Subsequently, the patient received consolidation and maintenance therapy. Measurable residual

Fig. 1 Patient 1. **a** Leukemic promyelocytes in the bone marrow (Wright-Giemsa, 1000 $\times$). **b** Flow cytometry dot plot showing co-expression of myeloid (cMPO) and T-lymphoid (cCD3*) antigens; the blue dotted line and arrow indicate the dimmer expression of cCD3 in leukemic cells as compared with normal residual T cells. **c** MPO, immunoperoxidase, $\times$

2000. **d** TdT, immunoperoxidase, $\times$ 2000. **e** CD2, immunoperoxidase, $\times$ 2000. **f** CD3, immunoperoxidase, $\times$ 2000. **g** CD5, immunoperoxidase, $\times$ 2000. **h** CD7, immunoperoxidase, $\times$ 2000. Note in **e**, **f**, and **h** that the expression of CD2, CD3, and CD7 is dimmer in leukemic cells in contrast to the background normal T cells. Only normal T cells are positive for CD5

Table 2 Distribution of positive and negative different antigens by flow cytometry and immunohistochemistry

	Flow cytometry			Immunohistochemistry
	CD45 vs. SSC	Positive markers	Negative markers	
Patient 1	84.2% with dim CD45 and intermediate side scatter	CD34, CD117(p), CD33, CD13, CD11c, CD38, CD71, cMPO, HLA-DR(p), CD7(p), cCD3, and TdT(p).	CD11b, CD14, CD15, CD16, CD56, CD64, surface CD3, CD2, CD4, CD5, CD8, CD10, CD19, CD20, cCD79a	Blasts express MPO, CD34, CD117, CD2* (dim, subset), CD3 (dim), CD7 (dim), and TdT (subset, dim). Blasts negative for CD5.
Patient 2	89% with dim CD45 and intermediate side scatter	CD34(p), CD117, CD33, CD13, CD38, CD64, CD71, MPO(dim), HLA-DR (p), CD2, and cCD3	CD11b, CD11c, CD14, CD15, CD16, CD56, surface CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, cCD79a, TdT	Blasts express MPO, CD117, CD34 (focal), CD2, CD3 (subset, dim), TdT* (subset, dim). Blasts negative for CD5 and CD7.

*These antigens were dim positive by immunohistochemistry, but negative by flow cytometry

disease (MRD) could not be monitored by conventional PML/RARA qRT-PCR due to the deletion of the *PML* gene, but FISH for t(15;17) was negative at D+28 and D+58, and the *FLT3*-ITD mutation was undetectable at D+58, consistent with a response to therapy. The patient remains in morphologic and cytogenetic remission at 23 months from his initial diagnosis.

Discussion

We present two patients with an unusual T/myeloid immunophenotype in t(15;17) APL and successful treatment of both with an ATRA and ATO-based regimen. Interestingly, one patient had a deletion in the *PML* gene (exon 5) that involved the primer binding site and precluded generation of

Fig. 2 Patient 2. **a** Leukemic promyelocytes in the bone marrow (Wright-Giemsa, 1000×). **b** Flow cytometry dot plot showing co expression of myeloid (CD33) and T-lymphoid (cCD3) antigens; the blue dotted line and arrow indicate the dimmer expression of cCD3 in leukemic cells as compared with normal residual T cells. **c** MPO, immunoperoxidase, × 2000. **d** TdT, immunoperoxidase, × 2000. **e** CD2, immunoperoxidase, ×

2000. **f** CD3, immunoperoxidase, × 2000. **g** CD5, immunoperoxidase, × 2000. **h** CD7, immunoperoxidase, × 2000. Note in **e** and **f** that the expression of C and CD3 is dimmer in leukemic cells in contrast to the background normal T cells. Only normal T cells are positive for CD5 and CD7

the abnormal PML/RARA transcript. This resulted in discordant FISH and RT-PCR results precluding MRD detection by qRT-PCR.

Aberrant or cross antigen expression in leukemic blasts may result from lineage infidelity resulting from genetic aberrations, which may affect transcription factors, cytokines, or growth factors that regulate lineage commitment in hematopoietic cells or from lineage promiscuity occurring as a consequence of mutations that cause maturational arrest at an early, multilineage potential progenitor stage. Gene expression studies have shown a distinct profile for acute leukemia with a T/myeloid phenotype characterized by silencing of myeloid transcription factor CCAAT-enhancer-binding protein α (C/EBP α) and enhanced expression of Tribbles homolog 2 (TRIB2) [14]. TRIB2 is a direct target of the T cell commitment factor NOTCH1, implicating aberrantly activated Notch signaling in the pathogenesis [14]. Although large studies with mutational data are lacking in this patient population, *NOTCH1* mutations appear to be relatively common in T/myeloid MPAL, along with mutations in *DNMT3A*, *NRAS*, *KRAS*, *EZH2*, *TP53*, and *RUNX1* genes [15, 16]. *FLT3*-ITD mutations, as seen in our two patients, have also been reported in patients with MPAL [16]. This mutation is more commonly seen in APL, where it is associated with marked leukocytosis, microgranular morphology, expression of CD2, CD34, HLA-DR, and CD11b surface antigens, and a short PML/RARA (BCR3) isoform [17, 18]. Unlike other non-promyelocytic acute myeloid leukemia (AML), however, in which *FLT3*-ITD mutations are associated with a high propensity for relapse, the role of *FLT3*-ITD mutations in APL is controversial [17, 19–25], and most multicenter studies suggest that any association with poorer outcomes may be mitigated by the addition of ATO [22, 23, 25].

A diagnosis of MPAL according to the WHO requires expression of more than one lineage-determining antigens on the blasts and exclusion of recurrent cytogenetic abnormalities seen in AML such as *inv*(16), *t*(8;21), and *t*(15;17). Although cCD3 expression, which establishes T-lineage, is commonly heterogeneous, per WHO, for diagnosis of a mixed T-phenotype, the brightest cCD3-positive blasts should reach the intensity of the normal residual T cells present in the sample [8]. In both cases described in this report, the intensity of cCD3 expression is dimmer than the control T cells in the specimen (Figs. 1b and 2b). The cases do not fulfill either of the two WHO requirements for MPAL. The pattern of expression of cCD3 on the APL cells in our study is similar to that observed by Chapiro et al. [10]; cCD3 in their study was considered positive if the relative fluorescent intensity (RFI) on the leukemic promyelocytes was greater than 2 but lower than the background T cells within the CD45 leukemic gate. Interestingly, both the cCD3 expressing APL cases in their study expressed pre-T cell receptor α , an invariant glycoprotein associated with the β T cell receptor chain and CD3

protein that is considered specific for the T cell lineage [26]. Due to the very limited data about cCD3 expression on APL, its clinical significance, and hence of the T-lineage program in APL, is not yet known. Diagnosis of MPAL, in contrast, may have profound implications for management. Several retrospective studies have consistently suggested that patients with MPAL have superior outcomes with acute lymphoblastic leukemia (ALL)-type regimens compared to AML-type regimens [27, 28], and successful induction chemotherapy is often followed by allogeneic stem cell transplantation [29, 30]. Although it is too simplistic to conclude that all patients with MPAL should receive ALL-type therapy and it is impossible to determine whether intensive chemotherapy would result in the same successful outcome for our two patients, ATRA and ATO-based regimens are highly effective for patients with *t*(15;17) and likely less toxic than intensive chemotherapy [13, 31, 32]. Paietta and colleagues previously reported on a patient with clinical and morphologic features of APL with co-expression of TdT, T6 (CD1a), and T3 (CD3) demonstrated by double-staining microscopy as well as FACS-analysis. Interestingly, karyotype was +8 at presentation and *t*(15;17) was seen only at relapse [33]. The authors report that following culture in the presence of GCT-conditioned medium, while the morphologic picture remained dominated by hypergranulated promyelocytes with strong staining for myeloperoxidase, there was no evidence of myeloid maturation, but the proportion of cells expressing T cell markers T11 (CD2) and T3 (CD3) increased, indicating differentiation along this pathway. Exposure of the patient's cells to retinoic acid in vitro resulted in marked increase in the expression of T3 along with morphological and immunological evidence of myeloid maturations, analogous to the clinical response seen in our two patients.

The identification of the specific PML-RARA transcript at diagnosis in patients with APL is essential for subsequent MRD analysis and disease monitoring by qRT-PCR. Whereas the breakpoint on the *RARA* gene is always in intron 2, breakpoints on the *PML* gene can vary: intron 6 for *bcr1* (55%), variable points on exon 6 for *bcr2* (5%), and intron 3 for *bcr3* (40%). The dual color, dual-fusion probe sets for FISH can detect all three variants of the *PML-RARA* translocation in 98% of cases [34]. The rare cases with complex translocations or cryptic translocations from submicroscopic insertions of the *PML* gene into *RARA* may be undetectable by FISH but can be identified RT-PCR [35–38]. It is exceedingly rare for RT-PCR to be negative in cases of APL that are identified by conventional cytogenetics or FISH, and this is usually the result of suboptimal sample quality or complex genomic fusion sequences that include a third gene or alternative splicing of *RARA* exon 2 [39]. Uncommonly, this may be due to mutations in either derivative chromosome that may affect primer binding sites [40]. Additional sequencing studies of the *PML/RARA* fusion gene are required to resolve these rare

cases with a discordant result by RT-PCR. For patient no. 2 in this report, a deletion in the exon 5 of the *PML* gene abrogated the internal forward primer binding site, precluding amplification of the fusion transcript and limiting our ability for MRD testing.

In conclusion, the unique pathological features of the two patients presented in this report add to our understanding of stem cell plasticity, clarify the dilemma of classifying acute leukemia when encountering the unusual T/Myeloid immunophenotype with t(15;17), and increase awareness of interpreting the rare situation with discordant FISH (positive) and RT-PCR (negative) results. Our report highlights the importance of integrating multiple modalities of laboratory investigation for disease classification and careful consideration of disease biology prior to initiation of chemotherapy. Based on our experience, t(15;17) APL with aberrant expression of CD3 behaves more similar to APL than MPAL, and the T/myeloid phenotype does not abrogate the good prognosis conferred by t(15;17) when an ATRA and ATO-based regimen is utilized.

Author contributions Z.N.S. performed analysis of pathology of bone marrow aspirates and biopsies and developed the first draft of the manuscript; R.K. and S.S. provided an independent review and confirmation of the diagnoses; Y.Z. performed cytogenetic analysis; L.T. and N.A. performed genetic analysis on patient 2 sample; V.H.D. assisted in the writing and editing of the manuscript; M.R.B. helped edit the manuscript; F.E.C. and A.E. formulated the treatment plan, followed patients in the study, and wrote the manuscript. All authors provided critical revisions, gave final approval, and agreed to be accountable for the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

- Dong HY, Kung JX, Bhardwaj V, McGill J (2011) Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. *Am J Clin Pathol* 135:76–84
- Paietta E, Andersen J, Gallagher R et al (1994) The immunophenotype of acute promyelocytic leukemia (APL): an ECOG study. *Leukemia* 8:1108–1112
- Paietta E (2003) Expression of cell-surface antigens in acute promyelocytic leukaemia. *Best Pract Res Clin Haematol* 16: 369–385
- Orfao A, Chillon MC, Bortoluci AM et al (1999) The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARalpha gene rearrangements. *Haematologica* 84:405–412
- Khan AA, Saraf A, Bhargava M, Kumar V (2013) Role of flow cytometry in the diagnosis of acute promyelocytic leukemia. *Am J Clin Pathol* 139:829
- Lo Coco F, Avvisati G, Diverio D et al (1991) Rearrangements of the RAR-alpha gene in acute promyelocytic leukaemia: correlations with morphology and immunophenotype. *Br J Haematol* 78:494–499
- Guglielmi C, Martelli MP, Diverio D et al (1998) Immunophenotype of adult and childhood acute promyelocytic leukaemia: correlation with morphology, type of PML gene breakpoint and clinical outcome. A cooperative Italian study on 196 cases. *Br J Haematol* 102:1035–1041
- Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 127:2391–2405
- Bene MC, Castoldi G, Knapp W et al (1995) Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia* 9:1783–1786
- Chapiro E, Delabesse E, Asnafi V et al (2006) Expression of T-lineage-affiliated transcripts and TCR rearrangements in acute promyelocytic leukemia: implications for the cellular target of t(15;17). *Blood* 108:3484–3493
- Gabert J, Beillard E, van der Velden VH et al (2003) Standardization and quality control studies of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. *Leukemia* 17:2318–2357
- Biondi A, Rambaldi A, Pandolfi PP et al (1992) Molecular monitoring of the myl/retinoic acid receptor-alpha fusion gene in acute promyelocytic leukemia by polymerase chain reaction. *Blood* 80(2):492–497
- Iland HJ, Bradstock K, Supple SG, Catalano A, Collins M, Hertzberg M, Browett P, Grigg A, Firkin F, Hugman A, Reynolds J, di Iulio J, Tiley C, Taylor K, Filshie R, Seldon M, Taper J, Szer J, Moore J, Bashford J, Seymour JF, for the Australasian Leukaemia and Lymphoma Group (2012) All-trans-retinoic acid, idarubicin, and IV arsenic trioxide as initial therapy in acute promyelocytic leukemia (APML4). *Blood* 120:1570–1580
- Wouters BJ, Jorda MA, Keeshan K, Louwers I, Erpelinck-Verschueren CAJ, Tieleman D, Langerak AW, He Y, Yashiro-Ohtani Y, Zhang P, Hetherington CJ, Verhaak RGW, Valk PJM, Lowenberg B, Tenen DG, Pear WS, Delwel R (2007) Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. *Blood* 110: 3706–3714
- Yan L, Ping N, Zhu M, Sun A, Xue Y, Ruan C, Drexler HG, MacLeod RAF, Wu D, Chen S (2012) Clinical, immunophenotypic, cytogenetic, and molecular genetic features in 117 adult patients with mixed-phenotype acute leukemia defined by WHO-2008 classification. *Haematologica* 97:1708–1712
- Eckstein OS, Wang L, Punia JN, Kornblau SM, Andreeff M, Wheeler DA, Goodell MA, Rau RE (2016) Mixed-phenotype acute leukemia (MPAL) exhibits frequent mutations in DNMT3A and activated signaling genes. *Exp Hematol* 44:740–744
- Lucena-Araujo AR, Kim HT, Jacomo RH et al (2014) Internal tandem duplication of the FLT3 gene confers poor overall survival in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline-based chemotherapy: an International Consortium on Acute Promyelocytic Leukemia study. *Ann Hematol* 93:2001–2010
- Souza Melo CP, Campos CB, Dutra AP, Neto JCA, Fenelon AJS, Neto AH, Carbone EK, Pianovski MAD, Ferreira ACS, Assumpção JG (2015) Correlation between FLT3-ITD status and clinical, cellular and molecular profiles in promyelocytic acute leukemias. *Leuk Res* 39:131–137
- Noguera NI, Breccia M, Divona M, Diverio D, Costa V, de Santis S, Avvisati G, Pinazzi MB, Petti MC, Mandelli F, Lo Coco F (2002) Alterations of the FLT3 gene in acute promyelocytic leukemia: association with diagnostic characteristics and analysis of clinical

- outcome in patients treated with the Italian AIDA protocol. *Leukemia* 16:2185–2189
20. Callens C, Chevret S, Cayuela JM et al (2005) Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. *Leukemia* 19:1153–1160
 21. Singh H, Werner L, Deangelo D et al (2010) Clinical outcome of patients with acute promyelocytic leukemia and FLT3 mutations. *Am J Hematol* 85:956–957
 22. Barragan E, Montesinos P, Camos M, Gonzalez M, Calasanz MJ, Roman-Gomez J, Gomez-Casares MT, Ayala R, Lopez J, Fuster O, Colomer D, Chillon C, Larrayoz MJ, Sanchez-Godoy P, Gonzalez-Campos J, Manso F, Amador ML, Vellenga E, Lowenberg B, Sanz MA, on behalf of the PETHEMA and HOVON Groups (2011) Prognostic value of FLT3 mutations in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monotherapy. *Haematologica* 96:1470–1477
 23. Poire X, Moser BK, Gallagher RE et al (2014) Arsenic trioxide in front-line therapy of acute promyelocytic leukemia (C9710): prognostic significance of FLT3 mutations and complex karyotype. *Leuk Lymphoma* 55:1523–1532
 24. Molica M, Breccia M (2015) FLT3-ITD in acute promyelocytic leukemia: clinical distinct profile but still controversial prognosis. *Leuk Res* 39:397–399
 25. Cicconi L, Divona M, Ciardi C, Ottone T, Ferrantini A, Lavorgna S, Alfonso V, Paoloni F, Piciocchi A, Avvisati G, Ferrara F, di Bona E, Albano F, Breccia M, Cerqui E, Sborgia M, Kropp MG, Santoro A, Levis A, Sica S, Amadori S, Voso MT, Mandelli F, Lo-Coco F (2016) PML-RAR α kinetics and impact of FLT3-ITD mutations in newly diagnosed acute promyelocytic leukaemia treated with ATRA and ATO or ATRA and chemotherapy. *Leukemia* 30:1987–1992
 26. Fehling HJ, Krotkova A, Saint-Ruf C, von Boehmer H (1995) Crucial role of the pre T-cell receptor alpha gene in the development of alpha beta but not gamma delta T cells. *Nature* 375:795–798
 27. Matutes E, Pickl WF, Van't Veer M et al (2011) Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. *Blood* 117:3163–3171
 28. Zhang Y, Wu D, Sun A, Qiu H, He G, Jin Z, Tang X, Miao M, Fu Z, Han Y (2011) Clinical characteristics, biological profile, and outcome of biphenotypic acute leukemia: a case series. *Acta Haematol* 125:210–218
 29. Wolach O, Stone RM (2015) How I treat mixed-phenotype acute leukemia. *Blood* 125:2477–2485
 30. Munker R, Brazauskas R, Wang HL, de Lima M, Khoury HJ, Gale RP, Maziarz RT, Sandmaier BM, Weisdorf D, Saber W (2016) Allogeneic hematopoietic cell transplantation for patients with mixed phenotype acute leukemia. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 22:1024–1029
 31. Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S, Ferrara F, Fazi P, Cicconi L, di Bona E, Specchia G, Sica S, Divona M, Levis A, Fiedler W, Cerqui E, Breccia M, Fioritoni G, Salih HR, Cazzola M, Melillo L, Carella AM, Brandts CH, Morra E, von Lilienfeld-Toal M, Hertenstein B, Wattad M, Lübbert M, Hänel M, Schmitz N, Link H, Kropp MG, Rambaldi A, la Nasa G, Luppi M, Ciceri F, Finizio O, Venditti A, Fabbiano F, Döhner K, Sauer M, Ganser A, Amadori S, Mandelli F, Döhner H, Ehninger G, Schlenk RF, Platzbecker U (2013) Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med* 369:111–121
 32. Burnett AK, Russell NH, Hills RK, Bowen D, Kell J, Knapper S, Morgan YG, Lok J, Grech A, Jones G, Khwaja A, Friis L, McMullin MF, Hunter A, Clark RE, Grimwade D (2015) Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. *Lancet Oncol* 16:1295–1305
 33. Paietta E, Dutcher JP, Wiemik PH (1985) Terminal transferase positive acute promyelocytic leukemia: in vitro differentiation of a T-lymphocytic/promyelocytic hybrid phenotype. *Blood* 65:107–114
 34. Brockman SR, Patemoster SF, Ketterling RP, Dewald GW (2003) New highly sensitive fluorescence in situ hybridization method to detect PML/RARA fusion in acute promyelocytic leukemia. *Cancer Genet Cytogenet* 145:144–151
 35. Blanco EM, Curry CV, Lu XY, Sarabia SF, Redell MS, Lopez-Terrada DH, Roy A (2014) Cytogenetically cryptic and FISH-negative PML/RARA rearrangement in acute promyelocytic leukemia detected only by PCR: an exceedingly rare phenomenon. *Cancer Genet* 207:48–49
 36. Han JY, Kim KE, Kim KH, Park JJ, Kim JS (2007) Identification of PML-RARA rearrangement by RT-PCR and sequencing in an acute promyelocytic leukemia without t(15;17) on G-banding and FISH. *Leuk Res* 31:1239–1243
 37. Kim MJ, Cho SY, Kim MH, Lee JJ, Kang SY, Cho EH, Huh J, Yoon HJ, Park TS, Lee WI, Marschalek R, Meyer C (2010) FISH-negative cryptic PML-RARA rearrangement detected by long-distance polymerase chain reaction and sequencing analyses: a case study and review of the literature. *Cancer Genet Cytogenet* 203:278–283
 38. Koshy J, Qian YW, Bhagwath G, Willis M, Kelley TW, Papenhausen P (2012) Microarray, gene sequencing, and reverse transcriptase-polymerase chain reaction analyses of a cryptic PML-RARA translocation. *Cancer Genet* 205:537–540
 39. Walz C, Grimwade D, Saussele S et al (2010) Atypical mRNA fusions in PML-RARA positive, RARA-PML negative acute promyelocytic leukemia. *Genes Chromosomes Cancer* 49:471–479
 40. Park TS, Kim JS, Song J, Lee KA, Yoon S, Suh B, Lee JH, Lee HJ, Kim JK, Choi JR (2009) Acute promyelocytic leukemia with insertion of PML exon 7a and partial deletion of exon 3 of RARA: a novel variant transcript related to aggressive course and not detected with real-time polymerase chain reaction analysis. *Cancer Genet Cytogenet* 188:103–107