Case Reports in Oncology

Case Rep Oncol 2023;16:1443–1450
DOI: 10.1159/000534862
Received: October 6, 2023
Accepted: October 25, 2023
Published online: November 23, 2023

Case Report

An Acute Promyelocytic Leukemia Resistant to All-Trans Retinoic Acid: A Case Report of the *ZBTB16::RARa* Variant and Review of the Literature

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Keywords
Case report · Acute promyelocytic leukemia · *ZBTB16::RARa* · All-trans retinoic acid resistance · Integrated diagnostics · t(11;17)(q23;q21) · Cytomorphology

Abstract
Introduction: Acute promyelocytic leukemia (APL) is characterized by the *PML::RARa* gene fusion and treatment consists of all-trans retinoic acid (ATRA). Rarely, genetic APL variants have been described which are insensitive to ATRA treatment and are therefore associated with a worse prognosis. Rapid identification of the APL variant is essential to start the correct treatment. ***Case Presentation:*** Here, we present a case of a 66-year-old male patient with weight loss and arthralgia. Laboratory results showed an anemia and mild leukocytosis with predominantly monocytes. Bone marrow investigation unexpectedly revealed a t(11;17)(q23;q21). This raised suspicion of an ATRA-resistant APL. By demonstrating the *ZBTB16::RARa* gene fusion, the diagnosis was confirmed. ***Conclusion:*** This case study emphasizes the importance of integrated diagnostics and provides guidance to recognize the *ZBTB16::RARa* APL, which is the most prevalent ATRA-resistant APL. Furthermore, an overview of other genetic APL variants is presented and how to treat these uncommon diseases in clinical practice.

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DOI: 10.1159/000534862

Introduction

Acute promyelocytic leukemia (APL) constitutes 5–8% of all cases of acute myeloid leukemia (AML) and is characterized by a maturation arrest at the promyelocytic stage. There are two morphological variants: a hypergranular variant (75%) in which multiple Auer rods or faggot cells are present, and the hypogranular variant (25%) with characteristically bi-lobed, butterfly-shaped nuclei. APL is commonly associated with a t(15;17), causing a fusion of the genes PML and RARa found on chromosomes 15q24 and 17q21, respectively. A patient with APL generally has a good prognosis if treatment is started immediately. In the diagnostic phase, diffuse intravascular coagulation and hyperfibrinolysis can be present and can cause life-threatening complications. Therefore, prompt treatment with all-trans retinoic acid (ATRA) is essential. ATRA induces differentiation of the promyelocytes and forms the cornerstone of APL treatment. Rarely, genetic APL variants have been described which are resistant to ATRA therapy. In this case report, a patient is presented with an APL in which ATRA is not effective. The clinical course was unusually indolent. Our aim was to describe the diagnostic difficulties and to provide clues for clinicians to rapidly recognize this disease in the future. Furthermore, this case illustrates the significance of integrated diagnostics to detect a rare disease that can present ambiguously.

Case Presentation

A 66-year-old male patient was first seen in the outpatient department of a different hospital with complaints of generalized arthralgia and progressive weight loss of 8 kg in the previous 2 months, and a single episode of fever (39°C) 2 weeks ago. Formerly a fit patient, he was now bedbound. During physical examination, no abnormalities were found. The laboratory findings at the first consultation are summarized in Table 1 (t = 1). Autoimmune serology was negative and repeated blood cultures remained negative. A PET-CT scan did not show abnormalities suggestive for vasculitis, arthritis or solid malignancy, but it did show increased fluorodeoxyglucose-uptake in the bone marrow. Because of the peripheral blood monocytosis a bone marrow aspirate was performed to investigate the possibility of a chronic myelomonocytic leukemia (CMML). Cytomorphology showed increased cellularity with a left shift of the myeloid lineage. No increase in blast count was observed. Reactive changes or a CMML-1 were considered. Flow cytometry of peripheral blood, however, did not support a CMML diagnosis as the percentage of classical monocytes was approximately 55% (in patient with CMML: >94% classical monocytes). Additional laboratory analysis revealed a ferritin level of 7,390 μg/L and based on the working diagnosis adult-onset Still's disease, treatment with steroids was initiated. Unfortunately, our patient did not show any clinical improvement, upon which steroids were tapered and discontinued.

As the clinical condition of our patient worsened, analysis of the bone marrow cytogenetics revealed a t(11;17)(q23;q21) in 9 of 20 analyzed cells. Additional fluorescence in-situ hybridization (FISH) showed the involvement of the RARa gene, located in 17q21, and not *KMT2A*, located in 11q23. This made us consider a genetic APL variant, so our patient was directly admitted to the emergency department. The complete blood count had deteriorated rapidly (shown in Table 1; t = 2). A severe anemia and thrombocytopenia were found, together with a strongly increased leukocyte count. At this point in time, an elevated promyelocyte count was observed. The coagulation tests were normal. Already in 1993, it was first reported that a t(11;17)(q23;q21) can lead to a fusion of the genes *ZBTB16* (previously known as *promyelocytic leukemia zinc finger, PLZF*) and *RARa* [1]. The fusion protein that arises from this translocation causes an APL, but this disease is insensitive to treatment with ATRA.

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Table 1. Laboratory values after the first consultation (t = 1) and at diagnosis (t = 2)

Laboratory test	Result t = 1	Result t = 2	Reference range	Unit
Hemoglobin	6.6	4.5	8.5–11.0	mmol/L
MCV	102	96	80–100	fl
Thrombocytes	262	20	150–400	$\times 10^9$/L
Leukocytes	11.1	47.4	4.0–10.0	$\times 10^9$/L
Eosinophils	0.1	0.0	0.0–0.5	$\times 10^9$/L
Basophils	0.1	0.0	0.0–0.2	$\times 10^9$/L
Blasts	0.1	0.0	0.0	$\times 10^9$/L
Promyelocytes	0.1	13.27	0.0	$\times 10^9$/L
Myelocytes	0.3	12.32	0.0	$\times 10^9$/L
Metamyelocytes	0.2	2.37	0.0	$\times 10^9$/L
Band neutrophils	0.6	4.74	0.0–0.6	$\times 10^9$/L
Segmented neutrophils	2.6	8.06	1.5–6.8	$\times 10^9$/L
Lymphocytes	2.8	1.42	1.0–3.5	$\times 10^9$/L
Monocytes	4.2	5.21	0.1–1.0	$\times 10^9$/L
Creatinine	75	227	62–134	μmol/L
Lactate dehydrogenase	421	512	<282	U/L
Uric acid		437	200–450	μmol/L
PT		12.4	9.7–11.9	s
APTT		25	22–29	s
Fibrinogen		2.6	1.5–4.0	g/L
D-dimer		>80.00	<0.50	mg/L
C-reactive protein	305	149	<8	mg/L

Therefore, the preferred treatment strategy for our patient was a conventional AML remission-induction cycle containing cytarabine and daunorubicin. Prior to chemotherapy administration, the bone marrow puncture was repeated. In contrast to the previous examination, cytomorphology now showed predominantly promyelocytes with relatively round nuclei and absence of Auer rods (shown in Fig. 1). The cytoplasm of the promyelocytes was hypogranular, but contrary to the well-known hypogranular APL, no bilobed nuclei were seen. Peripheral blood smear demonstrated similar promyelocyte characteristics. Flow cytometry of the bone marrow showed a strong increase of promyelocytes but did not reveal expression of aberrant markers. Cytogenetics again demonstrated the t(11;17)(q23;q21), now in 20 of 20 analyzed cells (shown in Fig. 2). FISH analysis confirmed the gene fusion of *ZBTB16* and *RARa* in our patient (shown in Fig. 3). An extensive myeloid leukemia molecular mutation panel did not reveal any additional gene mutations. Using the next-generation sequencing (NGS) platform Archer-Fusionplex the t(11;17)/*ZBTB16::RARa* gene fusion was also demonstrated and thus confirmed in our patient.

In conclusion, our patient was diagnosed with an ATRA resistant APL. At first consultation, no increase in promyelocytes was observed, but after disease progression, the promyelocytes were evidently present. He was treated with two AML remission-induction cycles. Subsequently, no measurable residual disease was detected as determined by flow cytometry. As a consequence of the poor prognosis of an ATRA insensitive APL, consolidation with an allogeneic stem cell transplantation was performed after extensive patient counseling. In absence of a

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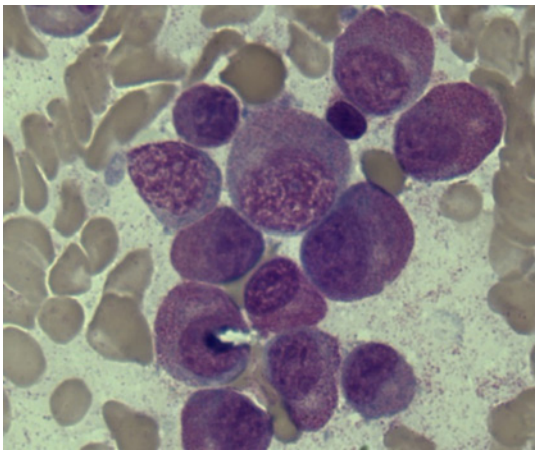

Fig. 1. Bone marrow cytomorphology of a genetic APL variant with t(11;17)(q23;q21). Visible are promyelocytes with relatively round nuclei and absence of Auer rods.

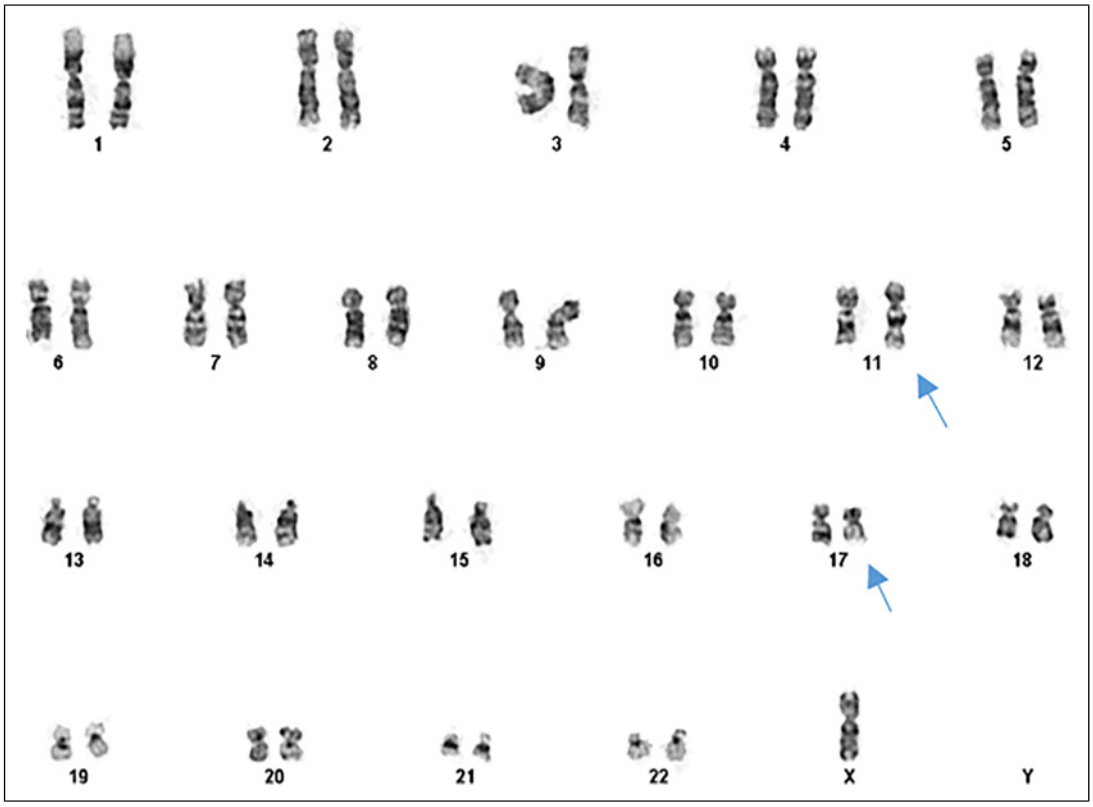

Fig. 2. Representative karyogram of a genetic APL variant with a t(11;17)(q23;q21).

human leukocyte antigen-identical donor, a haplo-identical stem cell donor was selected, and our patient was conditioned with busulfan and fludarabine. Graft-versus-host prophylaxis consisted of posttransplant cyclophosphamide, tacrolimus, and mycophenolate mofetil. The allogeneic stem cell transplantation was performed almost without complications and our patient is currently 1.5 years after transplantation without signs of disease relapse. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000534862).

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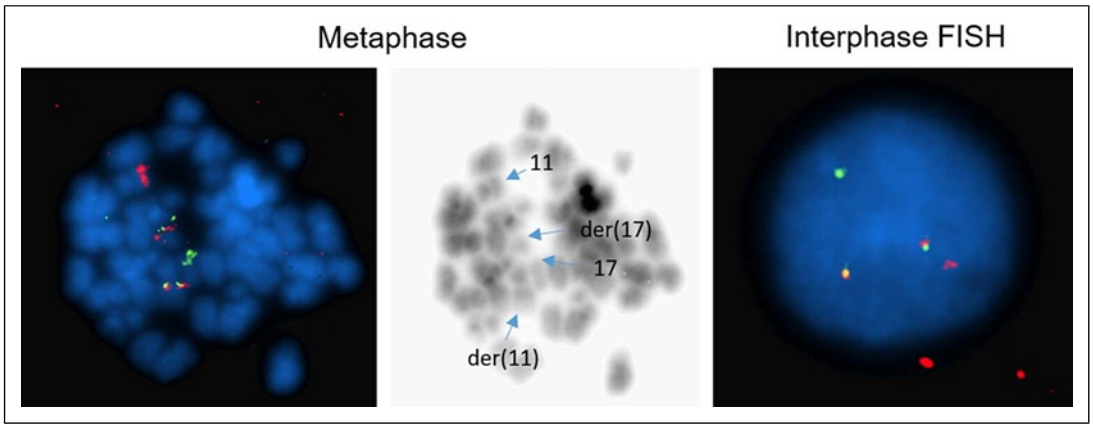

Fig. 3. Metaphase and interphase fluorescence in-situ-hybridization (FISH) using the ZBTB16-RARA Dual Fusion/Translocation FISH Probe (CytoTest, Rockville, USA). Three metaphases were investigated. Left (metaphase): DAPI-stain (blue), the probe on 11q23 (red), the probe on 17q21 (green). Middle (metaphase): inverted DAPI-stain with the normal chromosomes 11 and 17 (blue arrows) and the aberrant derivative chromosomes caused by the t(11;17) (blue arrows). Right (interphase): fusion of ZBTB16 and RARa (yellow). FISH result according to the ISCN2020 nomenclature: ish t(11;17)(q23;q21) (ZBTB16+,RARA+;RARA+,ZBTB16+) nuc ish ZBTB16,RARA)x3)(ZTBT16 con RARAx2).

Discussion

A genetic variant of APL which does not involve the *PML::RARa* gene fusion is very rare and constitutes <2% of all APL cases. Currently, 17 distinct gene fusion partners of *RARa* have been described that resulted in the development of an APL or APL-like disease [2, 3]. Because all genetic APL variants involve the *RARa* gene a clinician should consider this diagnosis if a patient suspected of an APL has a *RARa* rearrangement, but no involvement of the *PML* gene. Most gene fusion partners of *RARa* have only been described once [4]. The genetic APL variants that have been reported more frequently are summarized in Table 2. Furthermore, the susceptibility of the APL variant for ATRA is mentioned. Commonly, these genetic APL variants do not have an indolent disease course like in our patient, so early recognition is important.

Within the group of genetic APL variants, an APL caused by the *ZBTB16::RARa* gene fusion is the most prevalent, accounting for approximately 1% of all APL cases [5, 6]. This genetic APL variant is resistant to treatment with ATRA. The ZBTB16 protein, previously known as PLZF, functions in the nucleus as a suppressor of different transcription factors involved in myeloid differentiation [7]. Among others, ZBTB16 activates certain histone deacetylases, inhibiting transcription of promoters involved in differentiation. Especially in *ZBTB16::RARa* APL, the function of the lineage-determining transcription factor *CCAAT/enhancer-binding protein alpha (CEBPa)* appears to be strongly impaired [8].

It is not clear why this genetic APL variant is resistant to ATRA therapy. In mice models harboring the *ZBTB16::RARa* gene fusion, ATRA did cause degradation of the *ZBTB16::RARa* protein but did not induce differentiation of the leukemic clone, suggesting other factors play a role in therapy resistance [9]. Guidez et al. [10] demonstrated that the reciprocal translocation product RARa-ZBTB16 leads to an increased expression of *cellular retinoic acid binding protein I* (*CRABPI*), a receptor involved in the catabolism of retinoids. This mechanism may explain the strongly reduced susceptibility for ATRA. It is unknown if addition of high-dose ATRA to chemotherapy is feasible, but based on the high ATRA resistance threshold in cell lines it seems more suitable to pursue other strategies. Recently, the epigenetic enhancer

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Table 2. Genetic APL variants and susceptibility to ATRA treatment

Gene fusion	Cytogenetics	ATRA susceptibility
ZBTB16-RARa	t(11;17)(q23;q21)	–
NPM1-RARa	t(5;17)(q35;q12-21)	+
STAT5b-RARa (interstitial deletion)	t(17;17)(q21;q21)	–
FIP1L1-RARa	t(4;17)(q12;q21)	+
NUMA-RARa	t(11;17)(q13;q21)	+
BCOR-RARa	t(X;17)(p11;q21)	+
PRKAR1a-RARa	del(17)(q21q24)	+
IRF2BP2-RARa	t(1;17)(q42;q21)	+

of zeste homolog 2 has been implicated in reducing ATRA susceptibility in *ZBTB16::RARa* APL, providing a possible future therapeutic target to overcome resistance [11].

The clinical presentation of a patient with a genetic APL variant is usually similar to a patient with a *PML::RARa* APL. Cytomorphology of the genetic APL variants is mostly indistinguishable from the classical APL, but the *ZBTB16::RARa* APL is characterized by a distinctive morphology. In literature, promyelocytes carrying this gene fusion are described to have relatively round nuclei, abundant cytoplasm, fine granules, and absence of Auer rods. Moreover, an increased amount of pseudo Pelger-Huët anomalies are seen together with hypogranulation [12, 13]. These morphological features were also clearly present in the second bone marrow aspirate from our patient (shown in Fig. 1). Remarkably, in the first aspirate no increase in promyelocytes was observed, possibly because of early-stage disease. Furthermore, in the first aspirate more maturation was observed compared to the second aspirate. In previous studies, a subset of patients with *ZBTB16::RARa* APL was described to express a morphologic spectrum intermediate between AML with maturation and APL [12]. This could also explain the relative lack of promyelocytes recognized in the first aspirate.

Flow cytometry of the promyelocytes in a genetic APL variant is similar to that of a classical APL, e.g., $CD34^{-}$, $CD117^{+}$, human leukocyte antigen-DR^{--}, $CD33^{+}$, $CD13^{+}$, and $cytMPO^{+}$. However, expression of CD56 on malignant promyelocytes has strikingly been reported in 67% of *ZBTB16::RARa* positive APL cases [13]. Promyelocytes of our patient did not show expression of CD56. A recent paper described the incidence of molecular mutations in 7 patients with *ZBTB16::RARa* APL [14]. In 5/7 patients, a gene mutation was demonstrated in the malignant promyelocytes, with most common mutations in *ARID1A* (71%), *TET2* (57%), and *RUNX1* (29%). ARID1A functions as a chromatin modifier, inducing transcriptional activity of target genes.

Although only limited literature is available, most of the reported patients with an ATRA-resistant APL are considered to have a poor prognosis within the total group of AML patients. There are observations that these genetic APL variants, among which *ZBTB16::RARa*, are not only resistant to ATRA but also insensitive to arsenic trioxide (ATO) and have reduced susceptibility to anthracyclines [15, 16]. ATO normally functions as a modifier of the PML part of the PML-RARa protein, inducing degradation of this protein. This PML part is absent in genetic APL variants. For these reasons, our patient was not treated with ATRA and/or ATO, but with conventional AML remission-induction chemotherapy. Based on age, low hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score and decreased susceptibility to (chemo) therapy, in our patient a consolidation strategy with an allogeneic stem cell transplantation was preferred. However, not enough data are available to substantiate the type of consolidation in patients with a genetic APL variant.

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Because patients with a genetic APL variant, similar to patients with classical APL, have a high risk of developing a (life-threatening) disseminated intravascular coagulation, starting the correct therapy without delay is highly important. In general practice, if an APL is suspected, treatment with ATRA will be started immediately. However, as soon as the patient is diagnosed with an ATRA resistant APL variant, the treatment plan must be reconsidered and modified to remission-induction chemotherapy for AML. The *RARa* fusion can be detected by FISH, which is a relatively fast and easy method for APL (variant) diagnosis. Different FISH techniques are available such as break-apart probes or fusion probes. Break-apart probes target two areas of a specific gene sequence and if intact, show a fusion signal. If a break in the gene sequence occurs, two separate colors will be visible. For fusion probes, only cells that have an aberrantly fused gene sequence will show one color, also known as a fusion signal. These different types of probes can be used to quickly detect a *RARa* fusion if an APL (variant) is suspected.

In conclusion, APL frequently originates because of the *PML::RARa* gene fusion, but there are alternative gene fusions which can cause the disease. These genetic APL variants have variable susceptibility to ATRA treatment. The most prevalent APL variant resistant to ATRA is caused by the *ZBTB16::RARa* gene fusion, of which a case has been described in this paper. This case also stresses the importance of integrated diagnostics, as cytogenetics can indicate the presence of an APL insensitive to ATRA if morphology is not typical. Integrated diagnostics and increased awareness lead to faster recognition of APL variants and administration of the correct treatment.

Take-Home Messages

1. In case of a clinical suspicion of an acute (promyelocytic) leukemia and absence of a PML::RARa gene fusion, also consider the genetic APL variants. A RARa rearrangement found by FISH can be indicative.
2. Some genetic APL variants are resistant to ATRA and are treated with conventional AML remission-induction chemotherapy.
3. The most prevalent genetic APL variant is caused by a ZBTB16:RARa gene fusion as a result of t(11;17)(q23;q21) and is characterized by a distinctive cytomorphology and often expression of CD56.

Statement of Ethics

Ethical approval is not required for this study in accordance with local or national guidelines. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was not supported by any sponsor or funder.

Author Contributions

D.C. designed the study. D.C., G.S., B.V., P.P., and M.W. were all involved in data acquisition. D.C., G.S., P.P., and M.W. performed data analysis and data visualization. D.C. drafted the paper. G.S., B.V., P.P., and M.W. critically reviewed and edited the paper. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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