J Clin Exp Hematop
Vol. 56, No. 1, June 2016

Case Study

A Rare Case of Acute Myeloid Leukemia with a t(2;3) Chromosomal Translocation Characterized by Thrombophilia and Chemoresistance

Cecilia Bozzetti,[1)] Seval Türkmen,[2)] Ulrich Richter,[1)] Lars Fransecky,[3)] Gürkan Bal,[4)] Carsten-Oliver Schulz,[1)] Philipp Hemmati,[5)] Renate Arnold,[5)] Hanno Riess,[1,5)] and Philipp le Coutre[1,5)]

We hereby report a case of acute myeloid leukemia with translocation t(2;3) and involvement of the *ectopic virus integration site-1 (EVI1)* gene. Like most other 3q26-related disorders reported thus far, we describe a phenotype with elevated platelet counts and dysmegakaryopoesis. The clinical course of our patient was complicated by symptomatic thrombophilia and chemoresistance. In addition, our case exhibited *FLT3* (Fms-related tyrosine kinase 3) internal tandem duplication. Although anagrelide was successful in controlling elevated platelet counts, allogeneic stem cell transplantation failed to overcome chemoresistance due to simultaneous graft-versus-host-disease and relapse of acute myeloid leukemia. Given the dismal outcome of our case and previously reported cases, we propagate the implementation of targeted therapies to newly diagnosed patients with acute myeloid leukemia t(2;3). Preclinical models indicate drugs that plausibly target the *EVI1*-related molecular vulnerability as candidates for basket trials. Anagrelide exhibited a hopeful signal of activity in 3q26-related thrombocytosis and should be evaluated for implementation as supportive care. 〔*J Clin Exp Hematop 56(1):64-68, 2016*〕

Keywords: acute myeloid leukemia, translocation (2;3), 3q26, *EVI1*, anagrelide

INTRODUCTION

Diagnosis of acute myeloid leukemia (AML) is currently made by bone marrow cytology and is significantly augmented by immunophenotypic and molecular genetic studies. Pre-therapeutic disease classification mostly depends on cytogenetic analyses: many recurrent cytogenetic abnormalities harbor not only prognostic but also predictive value.[1,2] The WHO classification of AML currently includes seven recurrent cytogenetic abnormalities. One of those is rare and involves the chromosome 3 characterized by inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) and constitutes a subgroup of AML with dismal outcome.

We hereby report on a patient with *de-novo* AML presenting a rare, but recurrent cytogenetic abnormality: t(2;3)(p21;q26) with involvement of the *ectopic virus integration site-1 (EVI1)*. To our knowledge, there are only 17 cases of AML with t(2;3) reported to this date (Table 1),[3-5] which resemble some properties of inv(3) and t(3;3) AML. This case report should expand our understanding of biology, risk and prognosis of AML with t(2;3).

CASE REPORT

Here we report on a 48-year-old male without a history of prior malignancy or chronic illness who was admitted to our hospital with recent onset of skeletal discomfort. The paraclinical work-up included leukocytosis (40/nL), thrombocytosis (1,174/nL) and elevated lactate dehydrogenase (540 U/L). The morphological assessment of the peripheral blood revealed 25% myeloblasts (Fig. 1A) and a bone marrow

Received: December 3, 2015
Revised: January 22, 2016
Accepted: February 2, 2016

[1)]Charité Campus Mitte, Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, Schumannstr. 20/21, 10117 Berlin, Germany
[2)]Institute für Humangenetik, Labor Berlin – Charité Vivantes GmbH, Sylter Straße 2, 13353 Berlin, Germany
[3)]Charité Campus Benjamin Franklin, Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, Hindenburgdamm 30, 12203 Berlin, Germany
[4)]Charité Campus Virchow Klinikum, Institut für Transfusionsmedizin, Augustenburger Platz 1, 13353 Berlin, Germany
[5)]Charité Campus Virchow Klinikum, Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, Augustenburger Platz 1, 13353 Berlin, Germany
Corresponding author: Dr. Cecilia Bozzetti, Charité Campus Mitte, Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, Schumannstr. 20/21, 10117 Berlin. Germany
E-mail: cecilia.bozzetti@charite.de

Table 1. Summary of clinical data of patients with t(2;3) acute myeloid leukemia (AML)

Diagnosis	Age	Sex	Survival	Platelets ($x10^9$/L)	Dysmega-karyopoesis	SCT	Karyotype
AML[a]	51	M	5	211	+	-	46,XY,t(2;3)(p21-23;q26-27),idic(6)(q12)
AML[a]	42	M	6	196	-	-	46,XY,t(2;3)(p21-23;q26-27),del(5q),del(7q)
t/AML[a]	68	M	9	44	+	-	46,XY,t(2;3)(p21-23;q26-27)
AML[a]	30	F	26	166	+	-	46,XY,t(2;3)(p21-23;q26-27),del(5)(q31)
AML[a]	45	F	10	368	+	-	46,XY,t(2;3)(p21-23;q26-27)
AML[a]	12	M	15	115	+	-	46,XY,t(2;3)(p22;q26)
AML[a]	48	F	5	NA	+	-	45,XY,t(2;3)(p16;q26),-7
t/AML[a]	63	F	22	60	+	-	48,X,-X,t(2;3)(p13;q28),+4,del(5)(q14q34),-7,+8,-13,-17,add(17)(p13),+3-4mar[cp4]
AML[a]	53	F	12	72	-	-	45,XX,t(2;3)(p15-21;q26-27),del(7)(q11q32),del(12)(p12),-21[cp29]
AML[a]	68	F	8	7	+	-	43-45,XX,t(2;3)(p15-21;q26-27),-5,-7,-15,del(17)(q?),-22,+1-4mar[cp13]
AML[b]	36	M	4 to 14	130	-	#	46,XY,t(2;3)(p21;q27),45,XY,idem,-7,46,XY
AML[b]	36	F	4 to 14	54	+	#	46,XX,t(2;3)(p21;q26),48,XX,idem,+15,+22
AML[b]	50	F	4 to 14	171	+	#	46,XX,t(2;3)(p15;q27)
AML[b]	55	M	4 to 14	8	NA	#	47,XY,t(2;3)(p22;q27),+14
AML[b]	36	M	4 to 14	137	NA	#	46,XY,t(2;3)(p21;q27)
AML[b]	59	M	4 to 14	104	NA	#	46,XY,t(2;3)(p21;q26)
AML[c]	56	M	NA	2,191	+	-	46,XY,t(2;3)(p22;q26.2)
AML[*]	48	M	4	1,117	+	+	46,XY,t(2;3)(p2?1;q26)

Clinical and paraclinical characteristics of patients diagnosed with AML with t(2;3) as previously reported by [a]Stevens-Kroef, *et al*.,[3] [b]Trubia, *et al*.[5] and [c]Yamamoto, *et al*.[4] including the hereby reported case[*]. Survival in months from the time point of diagnosis. SCT, stem cell transplantation; #, SCT was performed in 5 of 6 cases reported by Turbia *et al*.

aspiration was performed that resulted in the diagnosis of AML (Fig. 1B) with an unremarkable immunophenotype ($CD13^+$, $CD33^+$, $CD34^+$, $CD117^+$, myeloloperoxidase$^+$). Conventional cytogenetics analysis (Fig. 1C) revealed the presence of a translocation 46,XY,t(2;3)(p21;q26)[20]/46,XY[2] including the *EVI1* locus as detected by fluorescence *in situ* hybridization (Fig. 1D). Moreover, we identified an internal tandem duplication of FLT3 (Fms-related tyrosine kinase 3) (*FLT3*-ITD [FLT3–internal tandem duplication]$^+$) but no other molecular marker typically present in AML (mixed lineage leukemia$^-$, nucleophosmin 1$^-$, CEBPA [CCAAT/enhancer binding protein (C/EBP), α]$^-$). Intensive induction chemotherapy with a classical DA 7+3 regimen (i.e. cytarabine: 100 mg/m^2, days 1-7, daunorubicine: 60 mg/m^2, days 3-5) was started including prophylactic anticoagulation due to the elevated platelet count. Chemotherapy associated complications included neutropenic sepsis with pneumonia of the right upper lobe. Disease related complications were catheter-associated deep venous thrombosis of the right internal jugular vein and bilateral pulmonary embolism (Fig. 2B). Clinical resolution of all treatment-related complications was achieved by conservative measures and platelet counts decreased to 25/nL on day 13 of first induction. The sharp increase of platelet counts thereafter was reflected by a suboptimal response as assessed by bone marrow aspiration on day 16 exhibiting an aplastic marrow with a significant population of residual myeloblasts.

Consequently, we initiated a salvage chemotherapy with FLAG-Ida (i.e. fludarabine: 30 mg/m^2, days 1-5; idarubicine: 10 mg/m^2, days 1-3; cytarabine 2,000 mg/m^2 days 1-5) and interpreted declining platelet counts during this course as an indication for disease control. While tolerability was acceptable, blood counts failed to reconstitute and remission assessment by bone marrow biopsy confirmed refractory disease with a subtotal infiltration of the bone marrow by myeloblasts.

We fast-tracked allogenic stem cell transplantation in aplasia from a matched related donor and successfully bridged to transplant with a third induction cycle in analogy to the MICE protocol (i.e. mitoxantrone: 7 mg/m^2, days 1, 3, 5; etoposide 100 mg/m^2, days 1-3; cytarabine 100 mg/m^2, days 1-7).

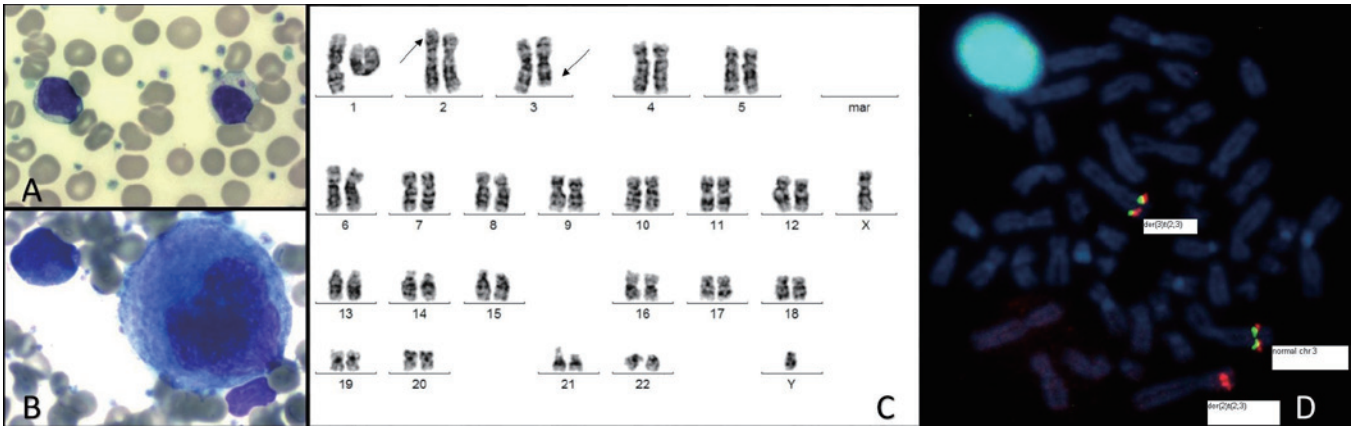

Fig. 1. Cytomorphological and cytogenetic assessment of acute myeloid leukemia. (***1A***) The blood smear revealed peripheral myeloblasts and excess of platelets (Pappenheim stain, x50). (***1B***) Dysmegakaryopoesis with excess of immature megakaryocytes in the bone marrow (Pappenheim stain, x50). (***1C***) Karyotyping at first diagnosis exhibiting t(2;3)(p21;q26) (indicated by *arrows*). (***1D***) Fluorescence *in situ* hybridization analyses confirmed translocation t(2;3)(p21;q26) and implicated the *ectopic virus integration site-1* (EVI1) locus by detecting breakpoints at t(2;3)(p21;q26).

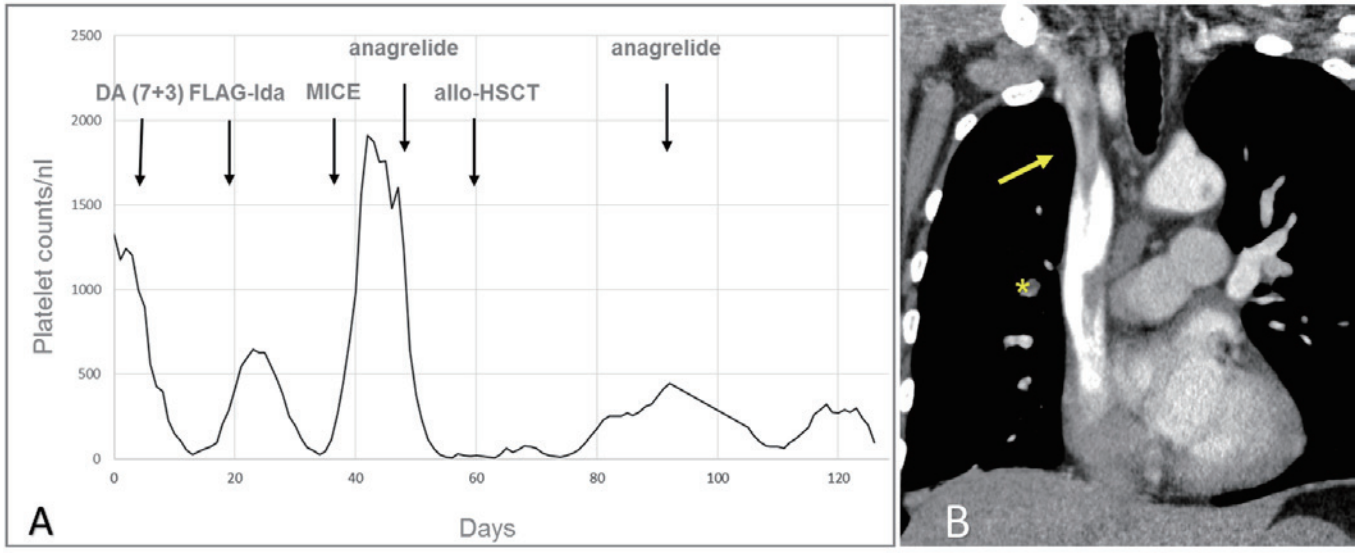

Fig. 2. Clinical characteristics. (**2A**) Platelet counts from first diagnosis (d0) to d126 indicated clinical activity of acute myeloid leukemia. (**2B**) Computed tomography scan revealed thrombosis of the superior vena cava (indicated by *arrow*) and right pulmonary embolism (indicated by*).

At this point, we successfully controlled platelet counts until transplantation with anagrelide (1 mg/day, bidaily)(Fig. 2A), which is regularly used for the treatment of patients with essential thrombophilia. Notably, anagrelide was effective, even though we detected a thrombopoietin serum concentration only marginally above normal.[6]

Allogenic hematopoietic stem cell transplantation from his HLA-identical brother (10/10-Ag) was performed 60 days after diagnosis using FLAMI/RIC conditioning (fludarabine 30 mg/m^2, days 1-4, cytarabine 2,000 mg/m^2, days 1-4, mitoxantrone 10 mg/m^2, days 1-4, total body irradiation 4 Gy, day 8, cyclophosphamide 60 mg/m^2, days 9-10). Therapy associated complications included neutropenic fever, BK-virus associated cystitis and a severe mucositis (common toxicity criteria [CTC] grade III/IV). On day 22 after transplantation, the patient developed an acute cutaneous graft-versus-host disease (CTC grade I) which responded rapidly to glucocorticoids. On day 14 after transplantation, blood counts reconstituted and the bone marrow biopsy performed at day 28 after transplantation exhibited complete remission.

However, only a couple of days after discharge on day 42 post allogeneic stem cell transplantation, the patient was admitted due to deteriorating liver function tests and exanthema. Moreover, platelet counts were again on the rise and

Table 2. Detailed description of paraclinical characteristics at baseline

Whole blood count	
Leu cocyte [nL]	40
Promyelocytes [%]	1
Matamyelocytes [%]	3
Myelocytes [%]	0
Neutrophilic bandform granulocytes [%]	12
Neutrophilic hypersegmented granulocytes [%]	52
Lymphocytes [%]	3
Reactive lymphatic cells [%]	3
Atypical lymphocytes [%]	0
Prolymphocytes [%]	0
Plasma cells [%]	0
Monocytes [%]	1
Eosinophils [%]	0
Basophiles [%]	0
Erythroblasts [%]	2.9
Fragmentocytes [%]	0.6
Myeloblasts [%]	25
Reticulocytes [%]	1.73
Immature platelets [%]	5.1
Heboglobin [g/dl]	13.1
Mean corpuscular volume [fL]	97
Platelet [nL]	1,117
Cytochemistry	POX^+, $Esterase^-$

Clinical chemistry
Lactate dehydrogenase [U/L] 537
Cytology
Bone marrow: 90% cellularity, 60% myeloblasts, Dysplastic granulopoiesis and erythropoiesis, Dysmegakaryopoiesis with micromegakaryocytes
Immunophenotype
$CD13^+CD33^+CD34^+CD117^+MPO^+HLA\text{-}DR^{+/-}$
Molecular genetics
FLT3-ITD^+; FLT3-TKD^-; MLL^-; $NMP1^-$; $CEBPA^-$
Cytogenetics
46,XY,t(2;3)(p21;q26)[20]/46,XY[2]

a bone marrow workup consecutively confirmed relapse with myeloblast infiltration, mixed donor chimerism (85%), and re-emerging translocation 46,XY,t(2;3)(p21;q26)[2]/46,XY[1] (only three metaphases could be analysed). Once more, anagrelide was used to control platelet counts. We clinically diagnosed exanthema and decreasing liver function tests as acute hepatic and cutaneous graft versus host disease (GvHD) (both CTC grade III). Challenged by simultaneous relapse and GvHD, we increased immunosuppression. Unfortunately, we detected replication of cytomegalovirus (CMV) in the peripheral blood (CMV status donor: IgM negative, IgG positive, CMV status recipient: IgM negative, IgG positive) and *pneumocystis jirovecii* pneumonia. Shortly after, the patient succumbed with hepatic and respiratory failure secondary to acute hepatic GvHD and infectious complication with underlying smoldering relapse of AML.

DISCUSSION

The identification of prognostic and ultimately predictive markers to decide upon treatment intensity has become a standard procedure in heterogeneous malignancies like AML. While some recurrent cytogenetic aberrations have been proven to justify treatment intensification by e.g. up-front allogenic stem cell transplantation, the impact of less common genetic abnormalities often remain unclear.

Our patient exhibited a rare, but recurrent translocation of the short arm of chromosome 2 and 3q26, the distal part of chromosome 3. Implicated in the more common t(3;3)(q21;q26) and inv(3)(q21q26), 3q26-related disorders share paraclinical features such as dysmegakaryopoesis and elevated platelet counts;[7] a finding that also holds true for our patient. Therefore, it is tempting to assume a common pathobiological mechanism related to *EVI1*, the gene mapping to 3q26. Indeed, t(3;3) and inv(3) were found to rearrange an oncogenic distal *GATA2* enhancer on 3q21 resulting in ectopic *EVI1* activation and concomitant *GATA2* haploinsufficiency.[7] Accordingly, in AML with t(2;3), ectopic expression of *EVI1* was observed in most cases,[3,5] albeit involvement of the oncogenic *GATA2* enhancer seems unlikely to contribute to EVI1 overexpression in this entity as assessed by breakpoint analyses of 3q26. However, breakpoint analyses of the short arm of chromosome 2 have failed to identify a common pathobiological mechanism as of yet.[3,5]

Our report reinforces the notion made by previous reports, that AML with t(2;3)(p21;q26) including the *EVI1* locus is associated with symptomatic thrombophilia, chemoresistance and adverse risk.[4]

On a clinical level, we conclude that the occurrence of AML with t(2;3)(p21;q26) warrants up-front treatment intensification as induction chemotherapy alone failed to induce complete remission. To improve success rates of allogeneic transplantation, we believe new therapeutic approaches should be implemented in AML with high *EVI1* expression. In preclinical models, all-trans retinoic acid (ATRA),[8] the CD52 antibody[9] and the BET bromodomain inhibitor JQ1[7,10,11] have shown promising results in *EVI1* positive

AML. We believe that the combination of different treatment approaches, such as ATRA and induction chemotherapy, might be a valuable option. Moreover, anagrelide was safe and effective for the treatment of thrombocytosis.

This case study demonstrates once again, how molecularly informed therapies may challenge randomized controlled trials due to ever smaller patient cohorts. To improve the dismal outcome of patients with rare subtypes of cancer, new forms of clinical trials (e.g. basket trials, umbrella trials, n-of-1 trials) are required.

INFORMED CONSENT

The patient gave his consent for publication of this case report and any accompanying images prior to his death.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

UR, COS, PH, RA and HR participated in the patient's treatment and collected clinical data. ST carried out cytogenetic and fluorescence *in situ* hybridization analyses. GB carried out the analysis of thrombopoietin serum concentration. LF provided images of cytological analysis and critically reviewed the manuscript. CB and PL designed and carried out the patient's treatment and wrote the manuscript. All authors have read and approved the final manuscript.

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