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CASE REPORT

Insights from a rare myeloproliferative neoplasm with coexisting *BCR-ABL1* fusion gene, *CALR*, and *TET2* mutations treated with nilotinib and ruxolitinib

Li Huo[1] | **Jundan Xie**[1] | **Qian Wang**[1] | **Hongjie Shen**[1] | **Zixuan Ding**[1] | **Lijun Wen**[1] | **Zhao Zeng**[1] | **Yi Xu**[1] | **Changgeng Ruan**[1,2] | **Suning Chen**[1,2] | **Mengxing Xue**[1]

[1]Jiangsu Institute of Hematology, National Clinical Research Center for Hematologic Diseases, NHC Key Laboratory of Thrombosis and Hemostasis, The First Affiliated Hospital of Soochow University, Suzhou, China

[2]Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China

Correspondence
Mengxing Xue, Suning Chen, Jiangsu Institute of Hematology, National Clinical Research Center for Hematologic Diseases, NHC Key Laboratory of Thrombosis and Hemostasis, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, China.
Email: xuemengxing@suda.edu.cn and chensuning@suda.edu.cn

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Abstract

Myeloproliferative neoplasms (MPNs) with concurrent *BCR-ABL1* fusion gene and *CALR* mutation are especially rare. We report a patient with coexisting *BCR-ABL1* fusion gene, *CALR,* and *TET2* mutations who was treated with the combination of the second-generation TKI nilotinib and *JAK1/JAK2* inhibitor ruxolitinib.

KEYWORDS

BCR-ABL1 fusion gene, *CALR* mutation, myeloproliferative neoplasms, nilotinib, ruxolitinib

Li Huo, Jundan Xie, Qian Wang contributed equally.

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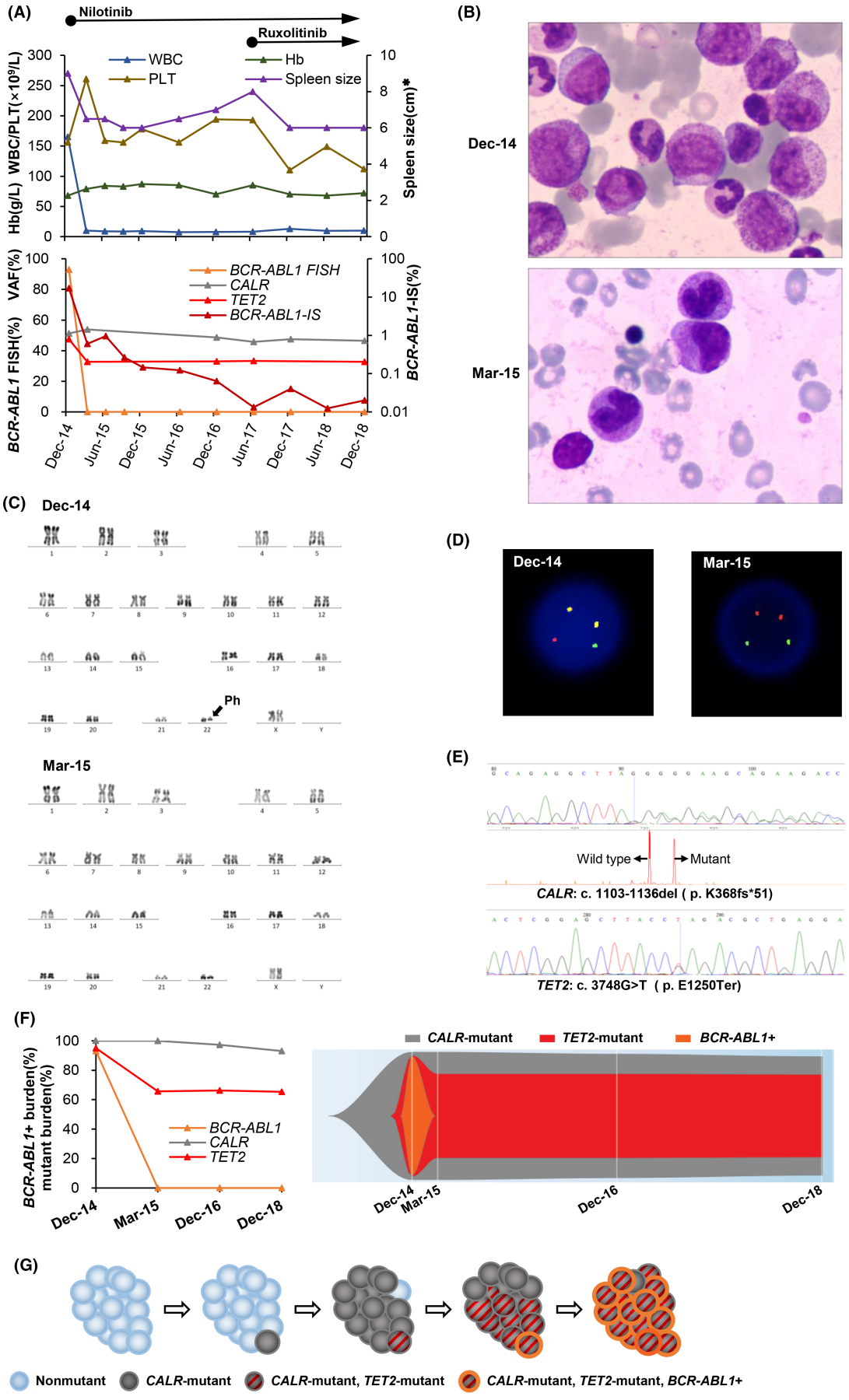
1 | INTRODUCTION

Myeloproliferative neoplasms (MPNs) are divided into two categories: chronic myeloid leukemia (CML) characterized by the presence of the *BCR-ABL1* fusion gene and *BCR-ABL1*-negative MPNs. Somatic mutations in Janus kinase 2 (*JAK2*)-V617F, exon 10 of thrombopoietin receptor/myeloproliferative leukemia (*MPL*) or exon 9 of calreticulin (*CALR*), are found in >90% of patients with classical *BCR-ABL1*-negative MPNs.[1] *BCR-ABL1* fusion gene and *JAK2/MPL/CALR* mutations are usually considered to be mutually exclusive.[2] However, several recent case reports have described patients with coexisting *BCR-ABL1* fusion gene and *JAK2/MPL/CALR* mutations.[3–5] Among these isolated cases, patients with concurrent *BCR-ABL1* fusion gene and *CALR* mutation are especially rare. The clonal relationship between two disorders and other genetic events that might contribute to the cooccurrence of more than one type of MPN in a patient are uncertain. Moreover, the safety and efficacy of the combination of tyrosine kinase inhibitor (TKI) and *JAK1/JAK2* inhibitor for these patients are still unclear. Here, we report a patient with coexisting *BCR-ABL1* fusion gene, *CALR* and ten-eleven translocation oncogene family member 2 (*TET2*) mutations who was treated with the combination of the second-generation TKI nilotinib and *JAK1/JAK2* inhibitor ruxolitinib.

2 | CASE REPORT

In December 2014, a 42-year-old woman presented to our hospital with massive splenomegaly (9 cm below the costal margin). The initial complete blood count analysis showed a white blood cell count of 165×10^9/L, a hemoglobin (Hb) level of 68 g/L, and a platelet count of 156×10^9/L. Bone marrow aspiration showed marked granulocytic proliferation with an increased myeloid: erythroid ratio. Cytogenetic analysis revealed t(9;22)(q34;q11) in 10 out of 10 metaphase cells, and fluorescence in situ hybridization (FISH) for *BCR-ABL1* was also positive (93%). Reverse transcriptase polymerase chain reaction (RT-PCR) confirmed a *BCR-ABL1* fusion gene, and the *BCR-ABL1* transcript level, which was shown as the ratio of *BCR-ABL1* to *ABL1* on an international scale (*BCR-ABL1*^{IS}), was 17.035% by quantitative

FIGURE 1 (A) Spleen size, hematological, cytogenetic, and molecular data acquired during the follow-up of this patient with myeloproliferative neoplasm with coexisting *BCR-ABL1* fusion gene, *CALR,* and *TET2* mutations. *BCR-ABL1* transcript levels are shown as the ratio of *BCR-ABL1* to *ABL1*, expressed as a percentage on the international scale (*BCR-ABL1*-IS). The *CALR* and *TET2* mutations were detected by next-generation sequencing and confirmed by Sanger sequencing. *CALR* mutation monitoring was performed using polymerase chain reaction followed by capillary electrophoresis. The variant allele frequency (VAF) of the *CALR* mutation was calculated by dividing the mutant peak area by the sum of the mutant and wild-type peak areas. *TET2* mutation monitoring was performed using next-generation sequencing. (B) Bone marrow aspirate smears of this patient at the first diagnosis of CML and 3 months after treatment. The bone marrow aspirate smear at the first diagnosis of CML showed expansion of the neutrophil lineage and increased basophiles. (C) Chromosome karyotypes of this patient at the first diagnosis of CML and 3 months after treatment. At the first diagnosis of CML, the patient had the characteristic t(9;22)(q34;q11) reciprocal translocation which resulted in Ph chromosome, while the cytogenetic analysis revealed the normal karyotype after 3 months of treatment. (D) Fluorescence in situ hybridization (FISH) with dual-color and dual-fusion translocation probes for *ABL1* (red) and *BCR* (green) of this patient at the first diagnosis of CML and 3 months after treatment. *BCR-ABL1*-positive cells at the first diagnosis of CML showed two fusion (red/green, yellow), one red and one green signals (2F1R1G), while the normal cells showed two red and two green signals (2R2G) after 3 months of treatment. (E) Sanger sequencing of *CALR* confirmed a frameshift mutation (c.1103_1136del, p.K368fs*51). Fragment length analysis by capillary electrophoresis showed concurrent amplification of the wild-type allele and a mutant allele. Sanger sequencing of *TET2* confirmed a nonsense mutation (c.3748G > T, p.E1259Ter). (F) Clonal evolution from the first diagnosis of CML to the last follow-up. Mutant burdens were estimated according to the percentage of *BCR-ABL1*-positive cells by FISH and the VAF of *CALR/TET2* mutations. Fish plot[15] showed that the *CALR*-mutant, *TET2*-mutant, and *BCR-ABL1*-positive subclone disappeared immediately after nilotinib treatment, while the *CALR*-mutant, *TET2*-mutant, *BCR-ABL1*-negative subclone and the initial dominant *CALR*-mutant, *TET2*-wild, *BCR-ABL1*-negative clone persisted even after treatment with a combination of nilotinib and ruxolitinib. (G) Schematic diagram of speculative clonal architecture and the order of alteration acquisition in this MPN patient. WBC, white blood cell; Hb, hemoglobin; PLT, platelet; *, below the costal margin. December 14, first diagnosis of CML; March 15, 3 months of nilotinib treatment; December 16, diagnosis of primary myelofibrosis; December 18, 18 months of combination therapy with nilotinib and ruxolitinib


(A)
Nilotinib
Ruxolitinib
WBC
PLT
Hb
Spleen size
WBC/PLT(×10⁹/L)
Hb(g/L)
Spleen size(cm)*
VAF(%)
BCR-ABL1 FISH(%)
BCR-ABL1-IS(%)
BCR-ABL1 FISH
CALR
TET2
BCR-ABL1-IS
Dec-14
Jun-15
Dec-15
Jun-16
Dec-16
Jun-17
Dec-17
Jun-18
Dec-18
(B)
Dec-14
Mar-15
(C)
Dec-14
Ph
Mar-15
(D)
Dec-14
Mar-15
(E)
Wild type
Mutant
CALR: c. 1103-1136del (p. K368fs*51)
TET2: c. 3748G>T (p. E1250Ter)
(F)
BCR-ABL1+ burden(%)
mutant burden(%)
BCR-ABL1
CALR
TET2
CALR-mutant
TET2-mutant
BCR-ABL1+
Dec-14
Mar-15
Dec-16
Dec-18
(G)
Nonmutant
CALR-mutant
CALR-mutant, TET2-mutant
CALR-mutant, TET2-mutant, BCR-ABL1+

RT-PCR (qRT-PCR). Thus, the patient was diagnosed with chronic-phase CML and was initially treated with hydroxyurea, which caused a rapid reduction in the leucocyte count to a normal level. A week later, hydroxyurea was substituted with nilotinib at a dosage of 600 mg/day. Although splenomegaly and anemia persisted (6 cm below the costal margin and Hb: 87 g/L after 1 year of nilotinib treatment), the patient achieved a complete cytogenetic response (CCyR) after 3 months of nilotinib treatment and achieved a major molecular response 12 months later (Figure 1A–D).

In December 2016, the patient arrived at our hospital with appetite loss, fatigue, and increased splenomegaly (7 cm below the costal margin). Cytogenetic analysis demonstrated a CCyR, while *BCR-ABL*^{IS} was 0.06% by qRT-PCR. The bone marrow biopsy showed granulocytic and erythrocytic hyperplasia accompanied by reticulin fibrosis grade 3. Molecular studies detected a 34-bp deletion in *CALR* exon 9 (c.1103-1136del, K368fs*51; Figure 1E), which was also observed in a previous study,[1] with a variant allele frequency (VAF) of 51.3% in the absence of *JAK2* V617F and *MPL* mutations. Retrospective analysis of historical samples showed that the *CALR* mutation had been present since the initial diagnosis of CML with a stable allele burden regardless of the decrease in *BCR-ABL1*^{IS} (Figure 1A). To investigate other genetic mutations that might contribute to this rare event of the concurrent *BCR-ABL1* fusion gene and *CALR* mutation, we also retrospectively studied the mutational status of 51 genes (Table S1) that are usually mutated in hematologic malignancies by next-generation sequencing. A mutation in *TET2* (c. 3748G > T, p. E1250Ter; Figure 1E) was detected in all historical samples analyzed, and the VAFs were slightly lower than that of the *CALR* mutation (Figure 1A). Ruxolitinib was administered at a dosage of 10 mg twice daily with the continuation of nilotinib, when the patient's constitutional symptoms worsened with progressive splenomegaly (8 cm below the costal margin) in June 2017. After 6 months of combination therapy, which the patient tolerated well, there was significant improvement in the constitutional symptoms and reduction in splenomegaly (6 cm below the costal margin). At the last follow-up at our hospital in December 2018, the patient continued the combination treatment with nilotinib and ruxolitinib and maintained the original doses. Her constitutional symptoms had disappeared, while the spleen size remained 6 cm below the costal margin. Within 18 months of combination therapy, the VAF of *CALR* mutation fluctuated from 45.7% to 46.5%, and *BCR-ABL1*^{IS} decreased to 0.01% (Figure 1A). Since 2019, this patient returned to the local hospital for treatment. As the telephone follow-up in October 2020, she continued the combination treatment, splenomegaly and anemia still existed, *BCR-ABL1*^{IS} was lower than 0.01%, and *CALR* mutation and *TET2* mutation were not analyzed.

3 | RESULTS AND DISCUSSION

To the best of our knowledge, there have been 21 patients with coexisting *BCR-ABL1* fusion gene and the *CALR* mutation reported in the literature, including our patient (Table 1). Only two of these patients had both the *BCR-ABL1* fusion gene and *CALR* mutation identified simultaneously at initial diagnosis (Table 1). Five of the seven patients with the initial diagnosis of *BCR-ABL1*-negative MPN had no detectable *BCR-ABL1* fusion gene and/or Ph + chromosome at initial diagnosis and then acquired this molecular alteration later with a median time to the second diagnosis of 48 months (range, 30–336 months); the other two patients had no available data (Table 1). Otherwise, 9/12 patients with the initial diagnosis of *BCR-ABL1*-positive CML had not only the *BCR-ABL1* fusion gene but also the *CALR* mutation by retrospective analysis of initial samples at the first diagnosis, and three patients did not undergo retrospective analysis of the *CALR* mutation (Table 1). These isolated cases suggested that the *CALR* mutation usually occurred earlier than the *BCR-ABL1* fusion gene. As observed in other case reports,[2,6,7] in our patient, the mutant *CALR* retained a high VAF of 45.7%–53.8%, while the *BCR-ABL1*^{IS} decreased to 0.01% after nilotinib treatment. Therefore, we speculate that the *CALR*-mutant, *BCR-ABL1*-positive subclone sensitive to nilotinib, arose from the initial dominant *CALR*-mutant, *BCR-ABL1*-negative clone, which was resistant to nilotinib (Figure 1F,G). Additionally, we also detected the *TET2* mutation in our patient, which was found in 10%–20% of all MPN subtype.[8] At the initial diagnosis, the VAF of *TET2* mutation was 47.5%; together with the VAF of the *CALR* mutation (51.3%) and the *BCR-ABL1*-positive cells determined by FISH (93%), this suggests that the *CALR* mutation, *TET2* mutation, and *BCR-ABL1* fusion gene coexisted in nearly all cells at this time. As the *BCR-ABL1*^{IS} decreased to 0.597% after 3 months of nilotinib treatment, the VAF of the *TET2* mutation also decreased to 32.8% and was maintained at a similar level, which was lower than that of the *CALR* mutation (Figure 1A). This result suggested that the *BCR-ABL1*-positive subclone arose from the *CALR*-mutant, *TET2*-mutant clone, and the *TET2* mutation might have occurred after *CALR* mutation (Figure 1F,G). A previous study,[9] which found that patients in whom the *JAK2* mutation was acquired first presented at a younger age than patients in whom the *TET2* mutation was acquired first (60.71 years vs. 71.17 years), also supported our

TABLE 1 Characteristics of reported patients of cooccurrence of *BCR-ABL1* fusion gene and *CALR* mutation

Pt.	Age at 1st Dx, y	Sex	1st Dx	2nd Dx	Interval between Dx, m	Phase of CML at Dx	Driver mutations at 1st Dx		Driver mutations at 2nd Dx		Type of *CALR* mutation	Type of *BCR-ABL1* transcript	TKIs	RUX	Other therapy	Best response to TKIs	Status	OS from 1st Dx, m	REF
							CALR	*BCR-ABL1*	*CALR*	*BCR-ABL1*									
1	78	F	CML + MPN, NOS	—	concurrent	BP	pos	pos	—	—	52-bp deletion (L367fs)	e1a2	ND	ND	ND	ND	ND	ND	[16]
2	65	M	CML + MF	—	concurrent	CP	pos	pos	—	—	52-bp deletion (L367fs)	e13a2	Dasat	N	N	CMR	alive	27+	[4]
3	26	M	ET	CML	43	CP	pos	neg	pos	pos	5-bp insertion (K385fs)	e14a2	Nilot	N	IFN	CHR	alive	48+	[6]
4	70	M	Post-ET MF	CML	48	ND	ND	ND	pos	pos	E364fs	ND	IM/Dasat	Y	Anagr	CHR	alive	60+	[3]
5	63	F	PMF	CML	30	CP	ND	neg	pos	pos	ND	e14a2	IM	Y	Hu/ allo-SCT	ND	alive	60+	[4]
6	55	F	MPN, NOS	CML	336	CP	ND	neg	pos	pos	52-bp deletion (L367fs)	ND	IM/Nilot	N	Hu	CHR	alive	420+	[17]
7	46	F	ET	CML	216	CP	ND	neg	pos	pos	5-bp insertion (K385fs)	e13a2/ e14a2	IM	Y	Hu/Anagr	MMR	alive	240+	[5]
8	65	F	ET	CML	48	CP	ND	neg	pos	pos	52-bp deletion (L367fs)	e13a2/ e14a2	Dasat	N	Aspirin	ND	alive	68+	[18]
9	46	M	ET	CML	132	CP	ND	ND	pos	pos	c.1182_1215del (L368fs)	e13a2/ e14a2	IM	N	IFN	MMR	alive	156+	[19]
10	73	F	CML	MPN, NOS	90	CP	pos	pos	pos	pos	52-bp deletion (L367fs)	ND	IM/Dasat	N	Peg-IFN/Hu/ EPO	MMR	alive	91+	[2]
11	67	M	CML	PMF	7	CP	pos	pos	pos	pos	52-bp deletion (L367fs)	ND	Dasat	N	N	CCyR	alive	10+	[7]
12	50	F	CML	PMF	48	CP	pos	pos	pos	MR4.0	52-bp deletion (L367fs)	e14a2	IM	N	N	MR4.5	alive	60+	[20]
13	54	M	CML	MF	24	ND	ND	pos	pos	MMR	ND	e13a2/ e14a2	Dasat	N	N	MMR	alive	48+	[3]
14	61	F	CML	ET	165	CP	pos	pos	pos	MR4.5	52-bp deletion (L367fs)	ND	IM	N	Hu/Peg-IFN/ CML VAX	MR4.5	alive	181+	[21]
15	67	F	CML	ET	3	CP	pos	pos	pos	pos	52-bp deletion (L367fs)	ND	IM	N	N	ND	alive	21+	[22]
16	55	F	CML	MPN, NOS	120	AP	ND	pos	pos	MMR	c.1095_1140del (L367fs)	ND	IM/Nilot/ Dasat	N	Hu/IFN+Ara-C	MMR	alive	180+	[23]
17	54	M	CML	ET	120	CP	pos	pos	pos	pos	ND	ND	IM	N	Hu	CMR	alive	120+	[24]
18	73	F	CML	ET	3	CP	pos	pos	pos	pos	52-bp deletion (L367fs)	e13a2/ e14a2	IM	N	Hu/Anagr/ Peg-IFN	MR4.0	alive	26+	[25]
19	80	M	CML	MPN, NOS	3	CP	ND	pos	pos	pos	52-bp deletion (L367fs)	ND	IM/Bosut	N	Hu	MMR	alive	ND	[26]
20	33	F	CML	ET	51	CP	Pos	Pos	Pos	MR4.0	c.1099_1136delinsAGGT	ND	IM/Nilot	N	Hu	MR4.0	alive	51+	[27]
21	42	F	CML	PMF	24	CP	pos	pos	pos	MMR	34-bp deletion (K368fs)	e13a2/ e14a2	Nilot	Y	Hu	MR4.0	alive	70+	Our Pt.

Abbreviations: allo-SCT, allogeneic stem cell transplantation; Anagr, anagrelide; AP, accelerated phase; Ara-C, cytosine arabinoside; Bosut, bosutinib; BP, blast phase; CCyR, complete cytogenetic response; CHR, complete hematologic response; CML VAX, a peptide vaccination protocol; CML, chronic myeloid leukemia; CMR, complete molecular response; CP, chronic phase; Dasat, dasatinib; Dx, diagnosis; EPO, erythropoietin; ET, essential thrombocythemia; F, female; Hu, hydroxyurea; IFN, interferon; IM, imatinib; M, male; m, months; MF, myelofibrosis; MMR, major molecular response; MPN, NOS, Myeloproliferative neoplasm, not otherwise specified; MR, molecular response; N, no; ND, no data; neg, negative; Nilot, nilotinib; OS, over survival; Peg-IFN, peginterferon; PMF, primary myelofibrosis; pos, positive; Pt, patient; REF, reference; RUX, ruxolitinib; TKIs, tyrosine kinase inhibitors; y, years; Y, yes; —, not applicable.

hypothesis, as our patient was only 42 years old at initial diagnosis. The clinical and morphologic appearance of our case at the initial presentation was more suggestive of a diagnosis of CML, despite the coexistence of *BCR-ABL1* and *CALR* mutation. With the decrease in *BCR-ABL1*^{IS} after nilotinib therapy, the patient showed constitutional symptoms, increased splenomegaly, and myelofibrosis, which suggested the presence of a *CALR* mutation and supported the diagnosis of primary myelofibrosis (MF). This finding suggested that the clinical and morphologic features of patients with coexisting *BCR-ABL1* and *CALR* mutation might be dominated by *BCR-ABL1*, which was also observed in previous reports.[6,7] Given the overlapping clinical and morphologic features of MPN, it is important to test all of the mutations in *JAK2/MPL/CALR* and the *BCR-ABL1* status in patients suspected of having MPNs.

As observed in CML patients[10] and patients with coexistent *BCR-ABL1* and *JAK2* mutation,[11] combination treatment with TKI and ruxolitinib in our patient was safe and effective, which was also confirmed by three previous patients with coexistent *BCR-ABL1* and *CALR* mutation, one treated with imatinib/dasatinib and ruxolitinib[3] and the others with imatinib and ruxolitinib[4,5] Moreover, recent studies found that the combination of ruxolitinib and nilotinib had a synergistic effect against both CML stem cells[12] and MF cells,[13] so our combination of nilotinib and ruxolitinib may have been better for this patient. However, the *CALR* mutation allele burden was not decreased and the splenomegaly persisted after 18 months of ruxolitinib therapy. Other choices should be considered for this patient. Interferon-α was reported to induce molecular responses in *CALR*-mutated essential thrombocythemia, but not for the patients with additional nondriver mutations (including *TET2* mutation).[14] Allogeneic stem cell transplant, if eligible for this procedure, may be a superior option. Summarizing such rare cases had important clinical significance. Firstly, with the continuous development of sequencing technology, more and more patients were reported, but its pathogenesis and clonal evolution were still inconclusive, which were under further research. Secondly, as for treatment, in addition to the traditional treatment, TKIs combined with ruxolitinib had considerable efficacy and safety, which provided more possibilities for the treatment of such patients. However, due to the scarcity of cases, the optimal treatment modality for patients with coexisting *BCR-ABL1* and *JAK2/MPL/CALR* mutations still remains uncertain, and a multicenter study with a large patient population may help address this unknown issue.

AUTHOR CONTRIBUTIONS

Li Huo: Data curation; formal analysis; visualization; writing – original draft. **Jundan Xie:** Formal analysis; methodology; visualization. **Qian Wang:** Formal analysis; visualization; writing – review and editing. **Hongjie Shen:** Methodology. **Zixuan Ding:** Methodology. **Lijun Wen:** Methodology. **Zhao Zeng:** Methodology. **Yi Xu:** Methodology. **Changgeng Ruan:** Supervision; writing – review and editing. **Suning Chen:** Conceptualization; data curation; funding acquisition; supervision; writing – review and editing. **Mengxing Xue:** Conceptualization; data curation; formal analysis; funding acquisition; visualization; writing – original draft.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

This study was approved by the Ethics committee of the First Affiliated Hospital of Soochow University.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Suning Chen https://orcid.org/0000-0001-6294-972X
Mengxing Xue https://orcid.org/0000-0003-4306-308X

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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