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An important oversight in WHO diagnostic classification: chronic myeloid leukemia with *PML::RARA* fusion clone

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Running title: chronic myeloid leukemia with PML::RARA fusion clone

Conflict-of-interest disclosure

The authors declare no conflict of interest.

Author contributions

All authors were involved in designing the study, and WJ and XZ performed the

systematic review and wrote the paper. WS and SB and QL and CZ collected data. All authors assisted with the manuscript preparation. WJ and LF completed the revision of the manuscript, and the approval of final manuscript.

Founding

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Data availability

The data generated in this study are available upon request from the corresponding author.

The total number of words in the text is 1,495, as well as two figures and one supplemental table.

Letter to the editor:

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of t(9;22)(q34;q11), resulting in the BCR::ABL fusion gene. CML typically advances through three phases: chronic phase (CP), accelerated phase (AP), and blast phase (BP). Around 70% of cases display myeloid blasts, with 20–30% featuring lymphoid blasts during the transformation into BP.[1] However, a less recognized subtype of CML involves the presence of the PML::RARA fusion clone. This subtype can manifest as de novo CML with a minor PML::RARA fusion clone, de novo CML accompanied by acute promyelocytic leukemia (APL), and CML transforming into promyelocytic blastic crisis (PBC). In this context, we presented a case and conducted a literature review, addressing various aspects such as diagnosis, clinical features, treatment, and survival specific to this unique subtype of CML. This study was approved by the Institutional Review and Ethics Board of the First Affiliated Hospital of Nanchang University (IIT2024169 and IIT2014146).

A 58-year-old female CML-CP patient was admitted in September 2018. Cytogenetic analysis revealed the presence of t(9;22)(q34;q11.2) translocation in bone marrow (BM) cells. The international scale percent ratio (IS%) of the p210 BCR::ABL fusion transcript was 31%. The patient was initially treated 400 mg of imatinib daily. After 6 months, she achieved complete hematologic remission (CHR) and complete cytogenetic remission (CCyR). Unfortunately, CHR was lost 7 months into imatinib treatment, leading to a switch to dasatinib, and subsequently, another switch to nilotinib. At 10 months of tyrosine kinase inhibitor (TKI) treatment, the

patient experienced a transformation of CML to PBC. Detailed descriptions of BM morphology, karyotyping, quantification of fusion genes, next-generation gene sequencing, and ABL kinase mutations are provided in Figure 1A-E and Supplement Table 1.

The patient underwent induction therapy with all-trans-retinoic acid (ATRA), arsenic trioxide (ATO), idarubicin, cytarabine, and ponatinib, resulting in the achievement of complete remission (CR) of the BM. The BCR::ABL p210 IS% and PML::RARα transcript levels were reduced to 6.4% and 0, respectively. Karyotyping demonstrated 20 cells with 46, XX. The maintenance treatment included ponatinib, ATRA, and oral arsenic. However, at 34 months post CML-PBC, a routine blood test revealed a WBC of 14.95×10^9/L, hemoglobin (HGB) of 99 g/L, and platelets (PLT) of $1{,}897\times10^9$/L. BM morphology and quantification of fusion genes indicated a relapse of CML, with BCR::ABL IS% and PML::RARα by RT-PCR quantification reaching 92.34% and 0. An ABL kinase region mutation was identified as T315I. Despite the relapse, the patient chose not to pursue TKI treatment and opted for maintenance with hydroxyurea, oral arsenic, and ATRA. Unfortunately, she succumbed to a COVID-19 infection in May 2023 with an over survival of 56 months.

In contrast to common CML patients who typically exhibit favorable responses to TKI treatment and enjoy extended survival, this particular patient experienced a rapid disease progression, blastic transformation, and a relatively short survival. Upon reviewing the patient's BM specimen, we made a surprising observation of 0.3% PML::RARα chimeric mRNA at the time of initial diagnosis (Figure 2A-C).

Subsequently, a comprehensive review of 32 documented cases of CML-PBC was conducted (Supplement Table 1).[2-8] Among the 32 cases, five showed positive PML::RARα fusion gene. In two patients with concurrent CML and APL, one tested positive for both BCR::ABL and PML::RARA, while the other was diagnosed through the presence of abnormal promyelocytes and myeloblasts. Additionally, three cases retrospectively detected PML::RARα chimeric mRNA through previous samples. Notably, many CML patients are unaware of the presence of a PML::RARA clone in their bodies. Consequently, they do not undergo testing for PML::RARA fusion using either fluorescence in situ hybridization (FISH) or RT-PCR at the onset, nor do they monitor changes in PML::RARA fusion gene quantification until the disease worsens.

Notably, in retrospect, the diagnostic boundaries for this CML case with a minor PML::RARA fusion gene clone appear ambiguous. According to the diagnostic criteria outlined in the 5th edition of the World Health Organization Classification of CML-CP and APL, this case could be classified as either CML-CP or APL. It's worth noting that the WHO diagnostic criteria for APL do not define the proportion of promyelocytes and fusion gene quantification. Furthermore, the diagnosis of this case doesn't align with the criteria for inclusion in CML with BP, which are as follows: (1) ≥20% myeloid blasts in the blood or BM; or (2) the presence of an extramedullary proliferation of blasts; or (3) the presence of increased lymphoblasts in peripheral blood or BM. However, according to the international consensus classification of Myeloid Neoplasms and Acute Leukemias, the cutoff percentage for

t(15;17)(q24.1;q21.2)/PML::RARA for the diagnosis of APL is ≥ 10%. In this case, the percentage did not meet the criteria for APL and only conformed to the diagnosis of CML-CP.

The median interval time between CML diagnosis and blast crisis is documented as 24 months. Among the 22 CML-PBC patients, 11 patients experienced blast crisis during TKI therapy with the median interval time of 15 months, while the remaining 11 patients were in the pre-TKI era with 27 months. Interestingly, it appears that TKI therapy does not significantly delay the interval time to blast crisis.

Notably, during TKI therapy, the median WBC count at the onset of CML-PBC was 1.62×10^9/L, significantly lower than in the pre-TKI era. Lower WBC counts during TKI therapy may present challenges, potentially causing confusion with the adverse reactions of TKI and consequently delaying the diagnosis and therapy involving ATRA or ATO for CML-PBC. It is essential to be aware of fatigue and bleeding as noteworthy manifestations when the condition worsens.

The potential of combining TKIs with ATRA or ATO treatment for CML patients initially presenting with a PML::RARA fusion gene clone is an exciting prospect. It is noteworthy that patients with de novo CML and APL often do not require allo-SCT if they respond well to targeted therapies. Thus, timely recognition, initial treatment, and effective management are crucial for newly diagnosed CML patients with a PML::RARA fusion gene clone. However, for patients with CML-PBC, most cases currently adopt an APL induction regimen, with or without TKIs, followed by TKIs combined with ATRA and/or ATO as maintenance therapy. In some cases, HSCT is

considered.[9] Notably, outcomes for CML-PBC reported in the literature are significantly less favorable compared to de novo CML and APL patients.[2] The median survival time among 23 available patients was reported as 90 days after PBC. Among the 10 patients receiving ATRA, ATO, or a combination of these treatments with chemotherapy, the survival time at the 53.3% survival rate threshold was 118 days. In contrast, among the 10 patients who did not receive ATRA or ATO, the survival time was 30 days. The combination therapy of TKIs and ATO may be more effective than TKIs combined with retinoic acid.[10] Nevertheless, the sensitivity of CML-BP to ATRA, ATO, and new generation TKIs, as well as the necessity of allo-SCT, remain ambiguous.

The mechanisms leading to the transformation of CML into PBC remain unclear. Firstly, a small clone of the PML::RARα fusion gene is initially present in the CML patient's body, gradually evolving into dominant clones, thereby contributing to disease progression. Secondly, ABL kinase mutations, such as Y2253H, F359G, and T315I, may be associated with disease progression and recurrence. In cases of resistance, the uncontrolled activity of BCR::ABL leads to continued proliferation of leukemic cells, along with the development of secondary chromosomal or genetic defects, ultimately resulting in the evolution from CP to BP.[11] Thirdly, the PML protein plays a crucial role in maintaining the quiescence of leukemia-initiating cells (LICs), making them resistant to anti-leukemic agents.[12] The fusion of PML to RARα alters the intracellular distribution of the PML protein, contributing to the self-renewal capabilities of leukemic cells.[13] Additionally, the collaboration between PML::RARα

and BCR::ABL proteins at the CML stem cell level may induce excessive proliferation and TKI resistance.

Hence, current reports indicate that newly diagnosed CML with the coexistence of PML::RARα or CML progressing to acute PBC exhibit distinctive molecular profiles, leading to different clinical outcomes compared to de novo APL, CML, and advanced CML. Moreover, there is no consensus on the diagnosis, treatment, monitoring, and survival for this particular patient subtype. Notably, neither the 5th edition of the WHO Classification of Haematolymphoid Tumors nor the new International Consensus Classification (ICC) mentions this specific subtype of CML.[14,15] In our opinion, for newly diagnosed CML patients, quantitative detection of the PML::RARα fusion gene is necessary. Patients with CML or APL usually have a very favorable prognosis and even achieve a cure. However, if no intervention targets the PML::RARA clone in the early stages, the patient's survival time will be significantly shortened if the disease progresses to CML-PBC. From a risk-benefit perspective, patients should undergo PML::RARA fusion gene clone detection. If positive, patients should be simultaneously recommended TKI and ARTA therapies, with subsequent monitoring of BCR::ABL and PML::RARα fusion gene quantitation during treatment. If future research confirms the importance of our discoveries and the clinical interest in defining this new subtype of CML, it would make sense to include this subtype in the upcoming classifications by the WHO.

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Figure legends

Figure 1. The presentation of bone marrow (BM) morphology, FISH, karyotyping, quantification of fusion genes in this case**.** Figure 1A, B. BM aspirate displayed marked myeloid hyperplasia with 32% myeloblasts and 22.5% promyelocytes. Figure 1C, D. FISH was positive for the *BCR*::*ABL1* (Figure 1C) and *PML*::*RARα* (Figure 1D) fusions in 94% and 76% of interphase nuclei, respectively. Figure 1E. Cytogenetic analysis of BM cells with G-banding showed 46,XX,t(9;22)(q34;q11),t(15;17)(q22;q12) in all 20 cells examined. The red arrows respectively represent the abnormalities of BM morphology (Figure 1A, B), FISH (Figure 1C, D) and karyotyping (Figure 1E).

Figure 2. The levels of BCR::ABL and PML::RARα transcript detection by Reverse transcription polymerase chain reaction (RT-PCR) in this case. Figure 2A, B. The RT-PCR showed positive (0.3%) *PML*::*RARα* transcript at the period of new diagnosis via retrospective BM samples, whereas negative in control sample. Figure 2C. The dynamic quantification levels of *BCR::ABL* and *PML::RARα* transcript after Chronic myeloid leukemia (CML) transformation to blast phase (BP).

Figure 1

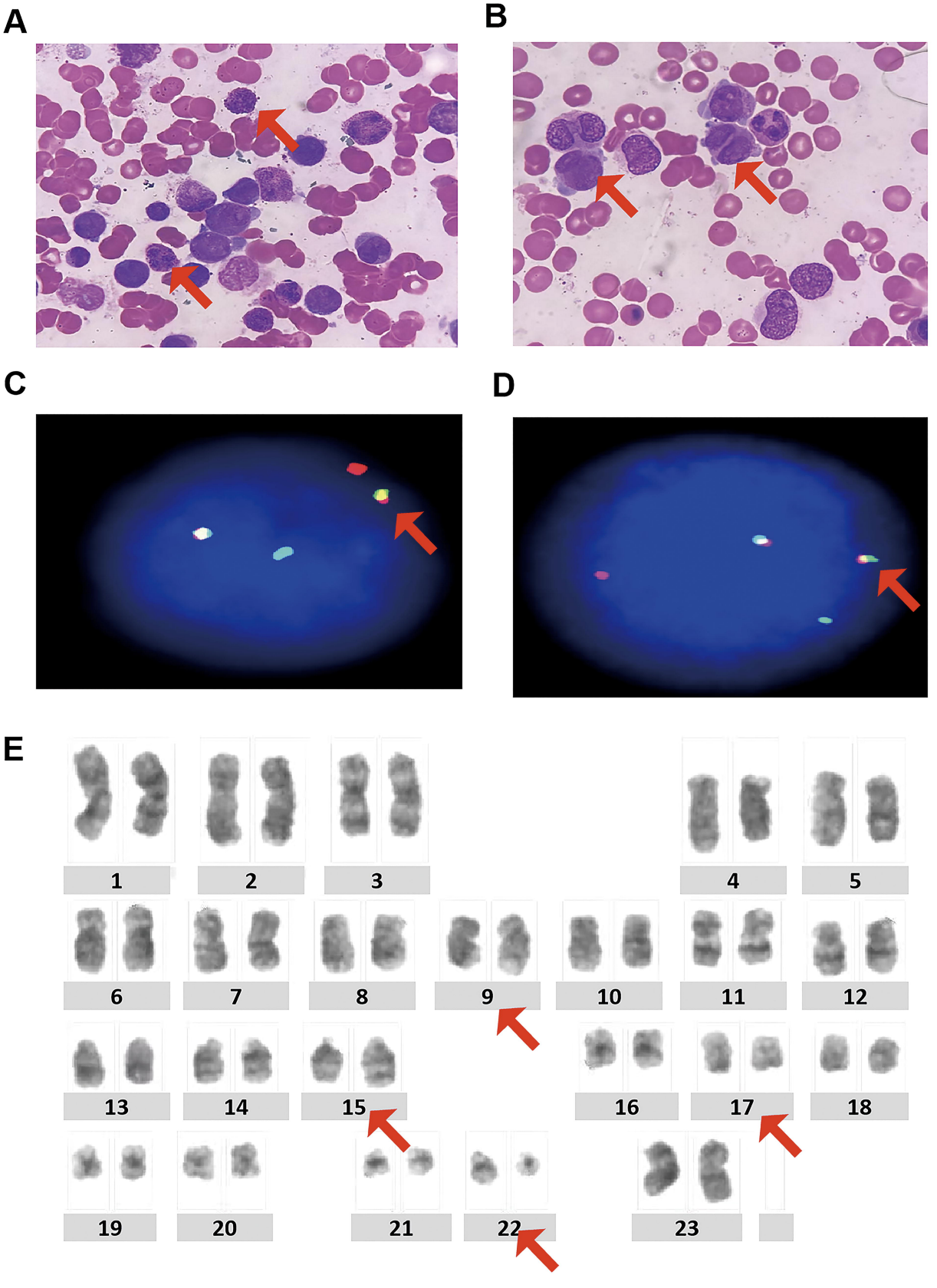
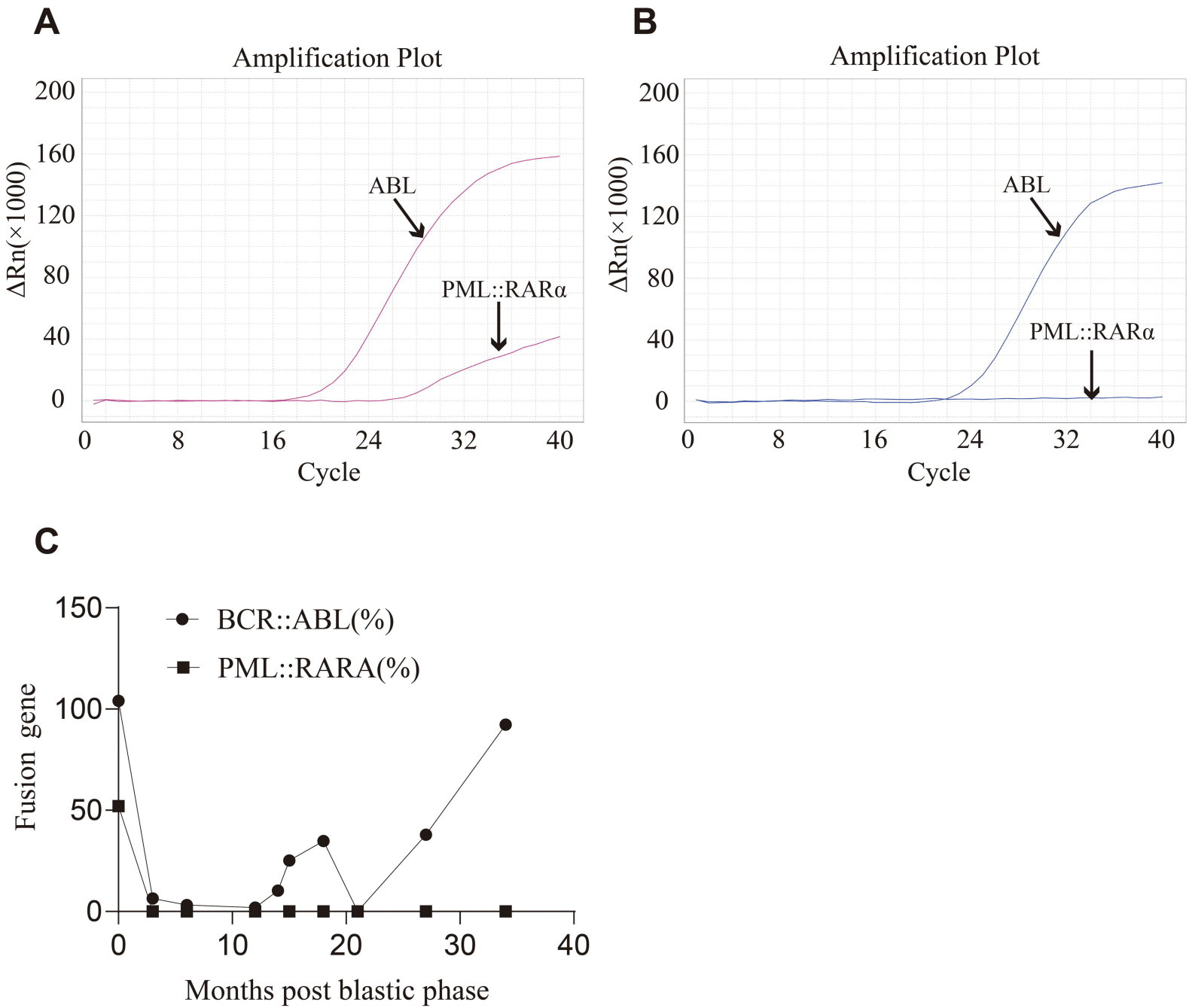


Figure 2


A
Amplification Plot
ΔRn(×1000)
200
160
120
80
40
0
ABL
PML::RARα
0
8
16
24
32
40
Cycle
B
Amplification Plot
ΔRn(×1000)
200
160
120
80
40
0
ABL
PML::RARα
0
8
16
24
32
40
Cycle
C
150
100
50
0
BCR::ABL(%)
PML::RARA(%)
Fusion gene
0
10
20
30
40
Months post blastic phase

Supplementary Table1: Summary of CML patients with PML::RARA fusion gene.

case	Age (y*)	sex	Fusion gene (First visit)	Fusion gene quantification		Interval between CML diagnosis and blast crisis (months)	Mutations in ABL kinase region	Treatment before PBP	WBC count of PBC $\times 10^9$/L	Treatment during PBP	Sensitive to ARTA or ATO	Other gene mutations	Survival # /Outcome	Reference
				First visit	blast crisis									
1	22	M	BCR/ABL, PML/RARA	ND	ND	concurrent	ND	ND	ND	ATRA, chemotherapy, allogeneic SCT	ND	ND	118 days	2
2	3	M	BCR/ABL	ND	ND	42	ND	Busulfan, alpha-2a interferon, hydroxyurea, cytarabine	80	Chemotherapy	ND	ND	2 months	2
3	60	M	BCR/ABL	ND	ND	36	ND	ND	80	Cytarabine, mitoxantrone, etoposide, idarubicine, 6-thioguanine	ND	ND	3 weeks	2
4	52	F	BCR/ABL	ND	ND	36	ND	hydroxyurea	183	Mitoxantrone, etoposide	ND	ND	6 weeks	2
5	55	M	BCR/ABL	ND	ND	24	ND	hydroxyurea	681	ATRA, mitoxantrone, cytosine arabinoside, etoposide	ND	ND	ND	2
6	50	M	BCR/ABL	ND	ND	36	ND	ND	2.4	ND	ND	ND	ND	2
7	32	F	BCR/ABL	ND	ND	96	ND	Busulfan	ND	Doxorubicin	ND	ND	1 months	2

8	82	F	BCR/ABL	ND	BCR/ABL:0.6% PML/RARA: ND	24	ND	Imatinib	0.56	ND	ND	ND	ND	2
9	26	M	BCR/ABL	ND	ND	concurrent	ND	NA	63	Cytarabine, arabinoside, daunorubicin, dasatinib, allogeneic SCT	ND	ND	ND	2
10	38	M	BCR/ABL	ND	ND	25	ND	Allopurinol, busulfan	ND	ND	ND	ND	47 days	2
11	48	M	BCR/ABL	ND	ND	72	ND	ND	ND	ATRA	ND	ND	＞87 days	2
12	27	M	BCR/ABL	ND	ND	48	ND	Natural interferon-α	ND	ND	ND	ND	ND	2
13	85	F	BCR/ABL	ND	ND	10	ND	ND	37	ND	ND	ND	2 days	2
14	38	M	BCR/ABL	ND	ND	23	ND	ND	120	Chemotherapy	ND	ND	34 months	3
15	51	M	BCR/ABL	ND	ND	20	ND	ND	160	Chemotherapy	ND	ND	31 months	3
16	58	F	BCR/ABL	ND	ND	71	ND	ND	105	Chemotherapy	ND	ND	85 months	3
17	31	M	BCR/ABL	ND	ND	27	ND	Cytosine arabinoside, 6-thioguanine	ND	ND	ND	ND	3 months	2
18	30	M	BCR/ABL	ND	ND	10	ND	Busulfan	18	N^4-Behenoyl-1-β-Darabinofuranosyl-cytosine, daunorubicin, 6-mercaptopurine, prednisone	ND	ND	5 months	2

19	38	M	BCR/ABL	ND	ND	25	ND	ND	32.9	Aziridinyl-benzoquinone	ND	ND	1 month	2
20	61	M	BCR/ABL	ND	ND	30	ND	Busulfan	21	Busulphan and hydroxyurea	ND	ND	6 days	4
21	37	M	BCR/ABL	ND	ND	10	ND	Misulban	218	Daunorubicin, cytosine arabinoside	ND	ND	ND	2
22	15	M	BCR/ABL	ND	ND	9	ND	Busulfan	134	6-thioguanine	ND	ND	10 days	5
23	66	F	BCR/ABL	ND	ND	9	ND	Imatinib	0.3	IA, ATRA	ND	ND	＞24 months	6
24	58	M	BCR/ABL	ND	ND	24	ND	Bosutinib	ND	ATRA, ATO, gemtuzumab	ND	ASXL1, IKZF1, WT1	＞1 months	7
25	40	M	BCR/ABL, PML/RARA	BCR/ABL: 93.869% PML/RARA: ND	BCR/ABL:49.667% PML/RARA:29.09%	17	ND	Imatinib	2	ATRA, ATO, dasatinib	ND	ND	＞26 months	2
26	78	M	BCR/ABL	ND	BCR/ABL:48.25% PML/RARA:46.44%	84	ND	Dasatinib	1.03	ATRA, ATO	ND	ASXL1	2 months	2
27	32	M	BCR/ABL, PML/RARA	BCR/ABL: 1.59% PML/RARA: 0.0321%	BCR/ABL:2.21% PML/RARA:0.81%	6	ND	Imatinib	4.9	Imatinib, ATRA	ND	ND	ND	2
28	31	M	BCR/ABL	ND	BCR/ABL:88.36% PML/RARA:25.4%	4	ND	Hydroxycarbamide, imatinib	1.23	ATRA, ATO, dasatinib, idarubicin, cytarabine, allogeneic SCT	ND	ND	＞20 months	2

29	69	F	BCR/ABL	ND	ND	13	ND	Imatinib	1.1	ATRA, idarubicin, Ara-C, imatinib, ATO	ND	ND	＞12 months	2
30	27	M	BCR/ABL, PML/RARA	BCR/ABL: 26% PML/RARA: 5%	BCR/ABL: ND PML/RARA:51.92%	0.6	ND	Imatinib	30.79	ATRA, ATO, Imatinib	ND	ND	ND	8
31	72	F	BCR/ABL	ND	BCR/ABL:99.73% PML/RARA:13.28%	ND	ND	Imatinib	17.8	ATRA and ATO	ND	ND	2 months	6
32	35	M	BCR/ABL	BCR/ABL: 30.02%	BCR/ABL:53.22% PML/RARA: ND	26	ND	Dasatinib	45.67	Idarubicin, ATRA, dasatinib, allo-HSCT	ND	ND	ND	6
33	58	F	BCR/ABL, PML/RARA	BCR/ABL: 31% PML/RARA: 0.3%	BCR/ABL: 94% PML/RARA:76%	concurrent	Y2253H, F359G, and T315I	Nilotinib	155.79	IA, ATRA, ATO, ponatinib	ND	CBL, CUX1, BCOR	56 months	This case

PBP, promyelocytic blast phase. PBC, promyelocytic blastic crisis. ND, no data. ATRA, all-trans retinoic acid. ATO, arsenic trioxide. HSCT, hematopoietic stem-cell transplantation. F, female. M, male, y*, years. #, represents survival (after blast Crisis onset).