An important oversight in the World Health Organization diagnostic classification: chronic myeloid leukemia with the *PML::RARA* fusion clone

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, 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 present a case and the findings of 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 patient with CML-CP was admitted to hospital in September 2018. Cytogenetic analysis revealed the presence of a t(9;22)(q34;q11.2) translocation in bone marrow cells. The International Scale percent ratio (IS%) of the p210 *BCR::ABL* fusion transcript was 31%. The patient was initially treated with 400 mg of imatinib daily. After 6 months, she achieved complete hematologic remission and complete cytogenetic remission. Unfortunately, the complete hematologic remission was lost 7 months into imatinib treatment, leading to a switch to dasatinib, and subsequently, another switch to nilotinib. At 10 months of treatment with tyrosine kinase inhibitors, the patient experienced a transformation of CML to PBC. Detailed descriptions of bone marrow morphology, karyotyping, quantification of fusion genes, next-generation gene sequencing, and ABL kinase mutations are provided in Figure 1A-E and *Online Supplementary Table S1*.

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 of the bone marrow. The *BCR::ABL* p210 IS% and *PML::RARA* 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 after CML-PBC, a routine blood test revealed a white blood cell count of 14.95×10^9/L, hemoglobin of 99 g/L, and platelet count of $1,897\times10^9$/L. Bone marrow morphology and quantification of fusion genes indicated a relapse of CML, with *BCR::ABL* IS% and *PML::RARA* by reverse transcription polymerase chain reaction (RT-PCR) quantification being 92.34% and 0, respectively. 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 coronavirus disease 2019 infection in May 2023 with an overall survival of 56 months.

In contrast to patients with common CML, who typically exhibit favorable responses to TKI treatment and enjoy extended survival, this particular patient experienced rapid disease progression, blastic transformation, and a relatively short survival. Upon reviewing the patient's bone marrow specimen, we made a surprising observation of 0.3% *PML::RARA* chimeric mRNA at the time of initial diagnosis (Figure 2A, B). Subsequently, we conducted a comprehensive review of 32 documented cases of CML-PBC (*Online Supplementary Table S1*).[2-8] Among the 32 cases, five had been found to be positive for the *PML::RARA* 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. In three cases *PML::RARA* chimeric mRNA was retrospectively detected in 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 the *PML::RARA* fusion using either fluorescence *in situ* hybridization or RT-PCR at the onset, nor are changes in *PML::RARA* fusion gene quantification monitored until the disease worsens.

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 (WHO) Classification of CML-CP and APL, this case could be classified as either CML-CP or APL. It is worth noting that the WHO diagnostic criteria for APL do not define the proportion of promyelocytes or the quantity of fusion gene. Furthermore, the diagnosis of this case does not align with the criteria for inclusion in CML with BP, which are as follows: (i) ≥20% myeloid blasts in the blood or bone marrow; or (ii) the presence of an extramedullary proliferation of blasts; or (iii) the presence of increased lymphoblasts in peripheral blood or bone marrow. However, according to the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias, the cutoff percentage for t(15;17)(q24.1;q21.2)/

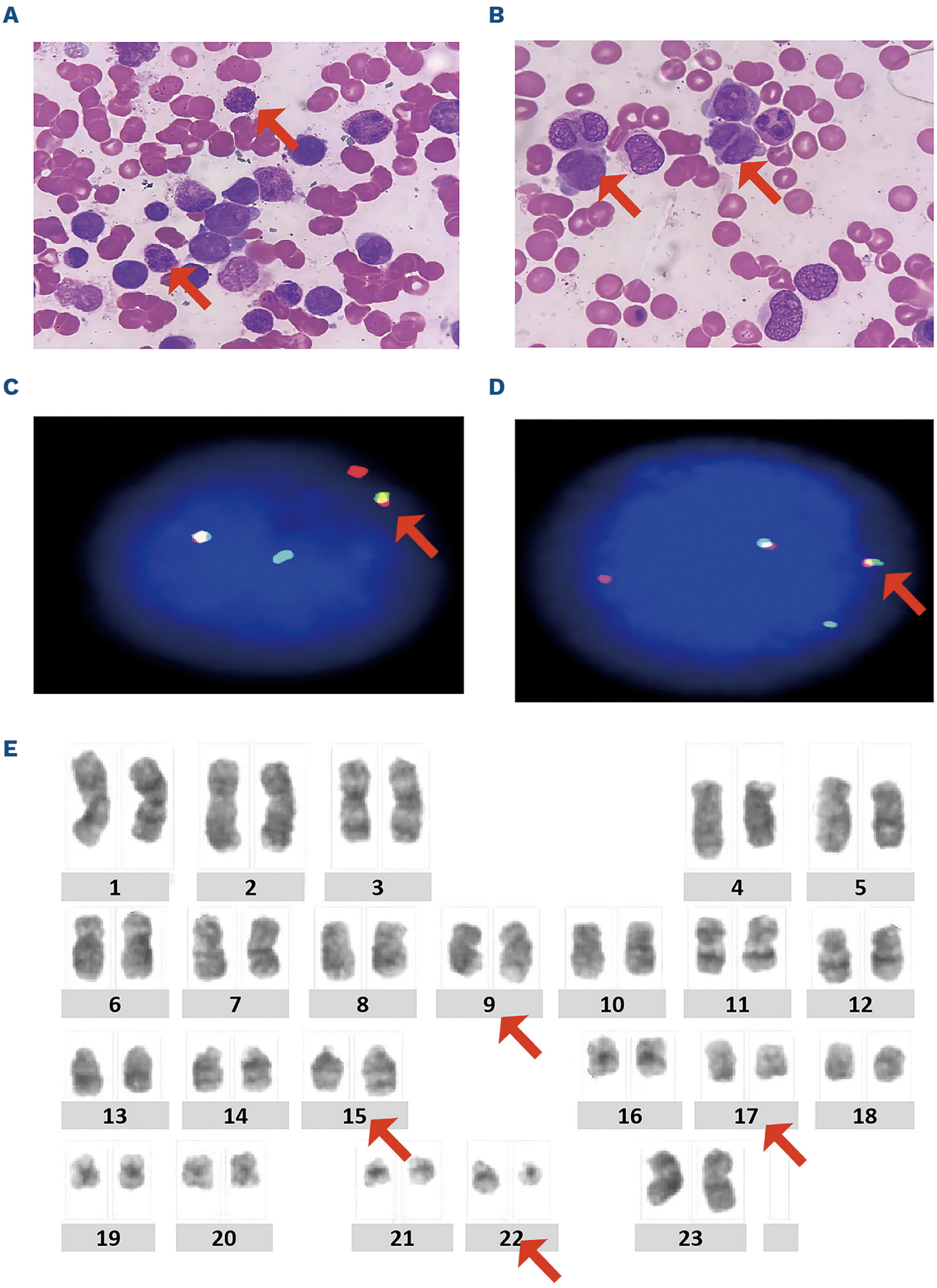

Figure 1. The presentation of bone marrow morphology, fluorescence *in situ* hybridization findings, karyotyping, and quantification of fusion genes in this case. (A, B) Bone marrow aspirate displayed marked myeloid hyperplasia with 32% myeloblasts and 22.5% promyelocytes. (C, D) Fluorescence *in situ* hybridization (FISH) was positive for the *BCR::ABL1* (C) and *PML::RARA* (D) fusions in 94% and 76% of interphase nuclei, respectively. (E) Cytogenetic analysis of bone marrow 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 represent the abnormalities of bone marrow morphology (A, B), FISH results (C, D) and karyotyping (E).

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 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 at a median time of 15 months, while the remaining 11 patients were managed in the pre-TKI era and the median interval between diagnosis and blast crisis in these patients was 27 months. Interestingly, it appears that TKI therapy does not significantly delay the time to blast crisis.

Notably, during TKI therapy, the median white blood cell count at the onset of CML-PBC was 1.62×10^9/L, which is significantly lower than in the pre-TKI era. Lower white cell 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

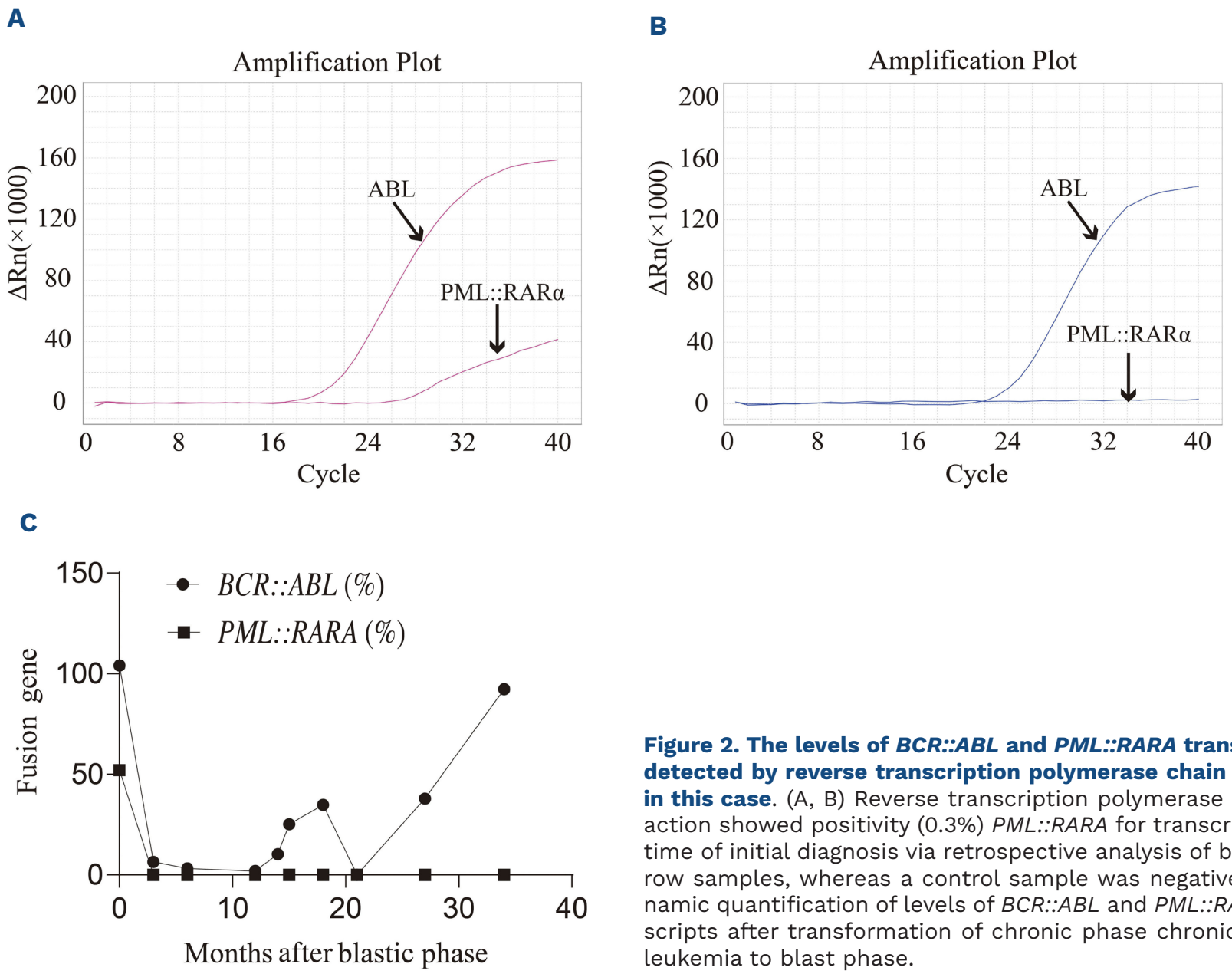

Figure 2. The levels of *BCR::ABL* and *PML::RARA* transcript, as detected by reverse transcription polymerase chain reaction, in this case. (A, B) Reverse transcription polymerase chain reaction showed positivity (0.3%) *PML::RARA* for transcript at the time of initial diagnosis via retrospective analysis of bone marrow samples, whereas a control sample was negative. (C) Dynamic quantification of levels of *BCR::ABL* and *PML::RARA* transcripts after transformation of chronic phase chronic myeloid leukemia to blast phase.

aware of fatigue and bleeding as noteworthy manifestations when the condition worsens.

The potential of combining TKI 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 allogeneic stem cell transplantation 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 receive an APL induction regimen, with or without TKI, followed by TKI combined with ATRA and/or ATO as maintenance therapy. In some cases, hematopoietic stem cell transplantation is considered.[9] Notably, outcomes for CML-PBC reported in the literature are significantly less favorable compared to those of *de novo* CML and APL patients.[2] The median survival among 23 available patients was reported as 90 days after PBC. Among the ten patients who received 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 ten patients who did not receive ATRA or ATO, the survival time was 30 days. Combination therapy with TKI and ATO may be more effective than the combination of TKI with retinoic acid.[10] Nevertheless, the sensitivity of CML-BP to ATRA, ATO, and new generation TKI, as well as the necessity of allogeneic stem cell transplantation remain unclear.

The mechanisms leading to the transformation of CML into PBC are poorly understood. Firstly, a small clone of the *PML::RARA* fusion gene is initially present in the CML patient's body and gradually evolves into the dominant clone, 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, 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 resistance to TKI. Hence, current reports indicate that newly diagnosed CML with the coexistence of PML::RARα and CML progressing to acute PBC exhibit distinctive molecular profiles, leading to different clinical outcomes compared to *de novo* APL, CML, and advanced CML. There is no consensus on the diagnosis, treatment, monitoring, and survival of patients with this particular subtype of CML. Notably, neither the 5th edition of the WHO Classification of Haematolymphoid Tumors nor the new International Consensus Classification mentions this specific subtype of CML.[14,15] In our opinion, quantitative detection of the *PML::RARA* fusion gene is necessary for newly diagnosed CML patients. Patients with CML or APL usually have a very favorable prognosis and can even be cured. However, if no intervention targets the *PML::RARA* clone in the early stages, the patient's survival 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 recommended simultaneous therapy with TKI and ATRA, with subsequent monitoring of the amounts of *BCR::ABL* and *PML::RARA* fusion genes 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 upcoming classifications by the WHO.

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Contributions

All authors were involved in designing the study. JieW and ZX performed the systematic review and wrote the paper. SW, BS, LQ and ZC collected data. All authors assisted with the manuscript preparation. JiaW and FL completed the revision of the manuscript, and approved the final manuscript.

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Data-sharing statement

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

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