

SHORT REPORT

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Coexistence of recurrent chromosomal abnormalities and the Philadelphia chromosome in acute and chronic myeloid leukemias: report of five cases and review of literature

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Abstract

Progression of chronic myelogenous leukemia (CML) is frequently accompanied by cytogenetic evolution. Additional genetic abnormalities are seen in 10–20% of CML cases at the time of diagnosis, and in 60–80% of cases of advanced disease. Unbalanced chromosomal changes such as an extra copy of the Philadelphia chromosome (Ph), trisomy 8, and i(17)(q10) are common. Balanced chromosomal translocations, such as t(3;3), t(8;21), t(15;17), and inv(16) are typically found in acute myeloid leukemia, but rarely occur in CML. Translocations involving 11q23, t(8;21), and inv(16) are relatively common genetic abnormalities in acute leukemia, but are extremely rare in CML. In the literature to date, there are at least 76 Ph+ cases with t(3;21), 47 Ph+ cases with inv(16), 16 Ph+ cases with t(8;21), and 9 Ph+ cases with t(9;11). But most of what has been published is now over 30 years old, without the benefit of modern immunophenotyping to confirm diagnosis, and before the introduction of treatment regimes such as TKI. In this study, we explored the rare concomitant occurrence of coexistence current chromosomal translocation and t(9;22) in CML or acute myeloid leukemia (AML).

Keywords: Chronic myelogenous leukemia, Acute myeloid leukemia, Balanced chromosomal abnormalities, Clonal evolution, t(9;22)

Introduction

The prognostic significance of clonal evolution in CML is variable and depends on many factors, including the type of cytogenetic changes, the time and phase of emergence of clonal evolution, and other chromosomal abnormalities

[1–3]. Although cytogenetic evidence of clonal evolution in CML is common as the disease progresses to accelerated or blast phase, its impact is dependent on the specific chromosomal anomalies [4]. The coexistence of t(9;22)(q34;q11.2) and recurrent chromosomal abnormalities is extremely uncommon, especially t(9;11) and t(3;21). Few such cases have been reported. To date, coexistence of t(9;11) and t(9;22) has not been reported in China. Here, we have characterized 5 cases of hematologic malignancy exhibiting coexistence of t(9;22) and recurrent chromosomal abnormalities, including two cases of t(9;11) and one case each of t(3;21), t(8;21) and inv(16).

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Materials and methods

Patients

A retrospective study was conducted on 1382 patients with hematologic malignancies displaying t(9;22) translocations who were treated at the outpatient department or inpatient ward of the Blood Diseases Hospital (Institute of Hematology), Chinese Academy of Medical Sciences between January 2016 and December 2018. These patients included 834 males and 548 females, giving a male-to-female ratio of 3:2. Five patients (0.36%) had additional rare recurrent genetic abnormalities: 2 patients had t(9;11), 1 patient had t(3;21), 1 patient had t(8;21), and 1 patient had inv(16). The clinical and laboratory data from these 5 patients are described in detail. This study (registration no. NI2020002-EC-1) was approved by the institutional review board of the Institute of Hematology and Blood Diseases Hospital Chinese Academy of Medical Sciences and informed consent was obtained from all subjects.

Cytogenetic analysis

Chromosomal analyses were performed by examining short-term cultures of bone marrow specimens according to standard conventional cytogenetic protocols. Fresh bone marrow was collected from each patient and cultured for 24 h (in RPMI 1640 medium, 20% calf serum) without addition of any growth factors. A methanol–glacial acetic acid fixation method was used for obtaining metaphase cells, and R-banding was performed. Analysis was performed using an Ikaros automated scanning system (Metasystems, Germany). At least 20 cells in metaphase were analyzed in each case. Karyotype descriptions are based on the International System for Human Cytogenomic Nomenclature (ISCN 2016).

Fluorescent in situ hybridization analysis

FISH of interphase cells

Fluorescence in situ hybridization (FISH) was performed on interphase nuclei of samples. FISH analyses were performed to confirm Ph translocation and the presence of t(8;21). Dual color-dual fusion labeled LSI AML1/ETO and LSI BCR/ABL1 probes (Abbott Diagnostics, IL, USA), designed for their respective purposes. The slides were pretreated with 2× sodium saline citrate, pH 7.0, for 30 min at 37 °C, followed dehydrated in 70, 85, and 100% ethanol solutions for 2 min. Probes were denatured and hybridized according to the manufacturer's instructions. Samples were rapidly washed, 10 µl of DAPI/antifade reagent (Abbott Diagnostics, IL, USA) was added to each sample, and slides were covered. Analysis was performed using an Isis system (Metasystems, Germany). Five hundred interphase cells were analyzed per specimen.

FISH of metaphase cells

FISH analyses of metaphase cells were performed to confirm Ph translocation and the presence of t(9;11), t(3;21), t(5;7) or inv(16). FISH analysis was performed on a R-banded slide for metaphase mapping to detect *BCR/ABL1* fusion gene and *MLL/AF9*, *EVII*, *CBFβ*, and *EGR1/D5S721*. After karyotype analysis, a well-dispersed metaphase mitosis figure with clear band and chromosomal abnormalities was identified, photographs were taken, and coordinates were recorded. Metaphase FISH was performed on the same mitotic figure twice. Metaphase FISH was performed using a *BCR/ABL1* and *MLL/AF9* dual-fusion probe; an *EVII* and *CBFβ* dual-color separation probe, and an *EGR1/D5S721* dual-color deletion probe. *BCR/ABL1*, *CBFβ*, and *EGR1/D5S721* probes were from Abbott Diagnostics (Vysis, IL); *MLL/AF9* and *EVII* probes were from Cytocell (Cambridge, UK). Sample slides were deparaffinized in xylene for 10 min, destained in methanol for 10 min, fixed in fresh fixative solution for 10 min, dehydrated through a graded series of ethanol solutions, and air-dried overnight. Probes were denatured and hybridized according to the manufacturer's instructions. Since samples had been treated with a hot salt solution, the denaturation temperature was increased to 75–78 °C, and the denaturation time was extended to 5 min. Samples were rapidly washed, 10 µl of DAPI/antifade reagent was added to each sample, and slides were covered. Analysis was performed using an Isis system (Metasystems, Germany).

Real-time quantitative RT-PCR assay

A real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) assay was performed for the detection of *BCR/ABL1*, *MLL/AF9*, *AML/ETO*, *CBFβ/MYH11* and *AML1-MDS/EVII* fusion gene transcripts. RNA was extracted from bone marrow samples using Trizol reagent (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's instructions. Reverse transcription was performed on 1 µg of total RNA using random hexamers and superscript II reverse transcriptase (Gibco-BRL) as described previously. The resulting complementary DNA was subjected to PCR to amplify fusion transcripts in an ABI 7500 real-time quantitative PCR instrument. Reaction systems using primers and conditions were as described previously [5, 6]. These results were then used to calculate the copy number of each target gene and its internal reference gene *ABL1* based on a standard curve. Target gene mRNA level (%) = (copy number of target gene / copy number of *ABL1*) × 100%.

Flow cytometric immunophenotyping

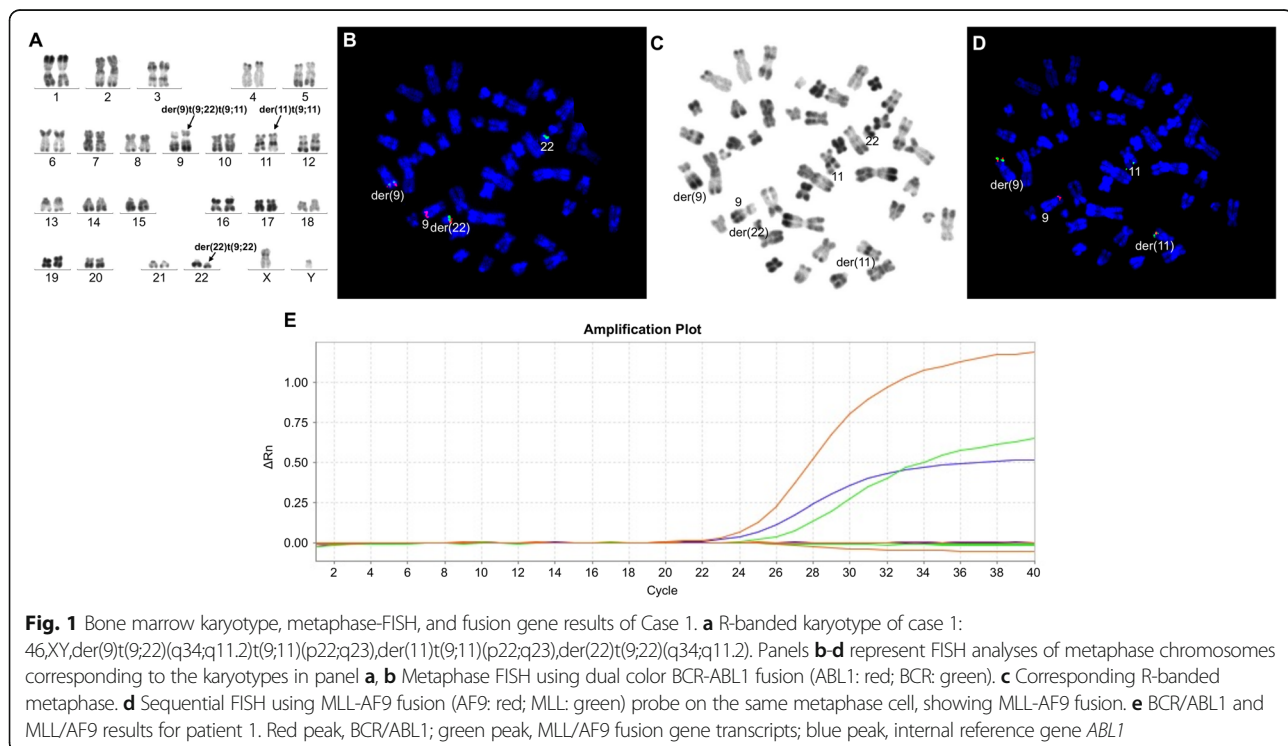
Eight-color multiparametric flow cytometry (MFC) was performed on peripheral specimens or bone marrow specimens using BD FACSCanto II instruments (BD Biosciences) according to the manufacturer's

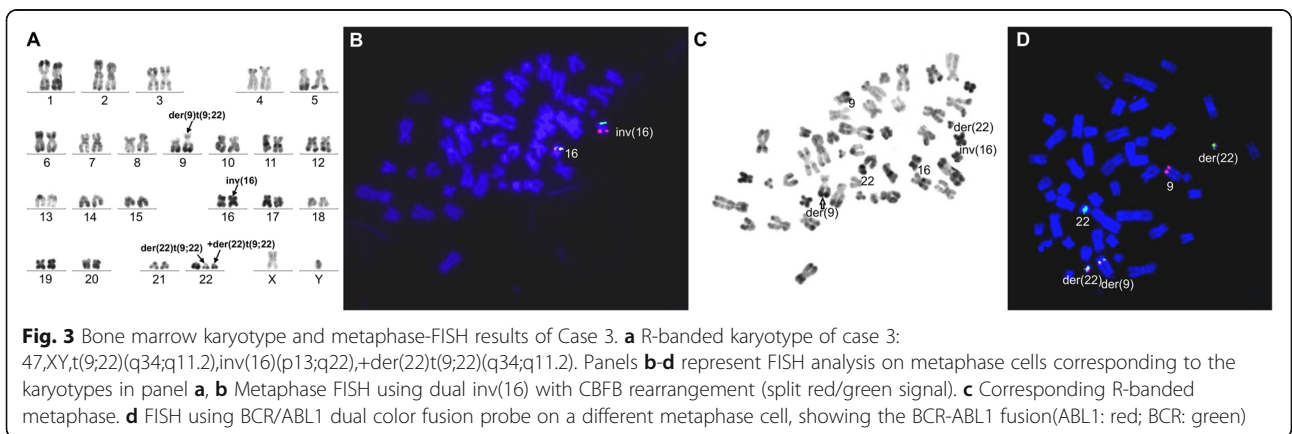
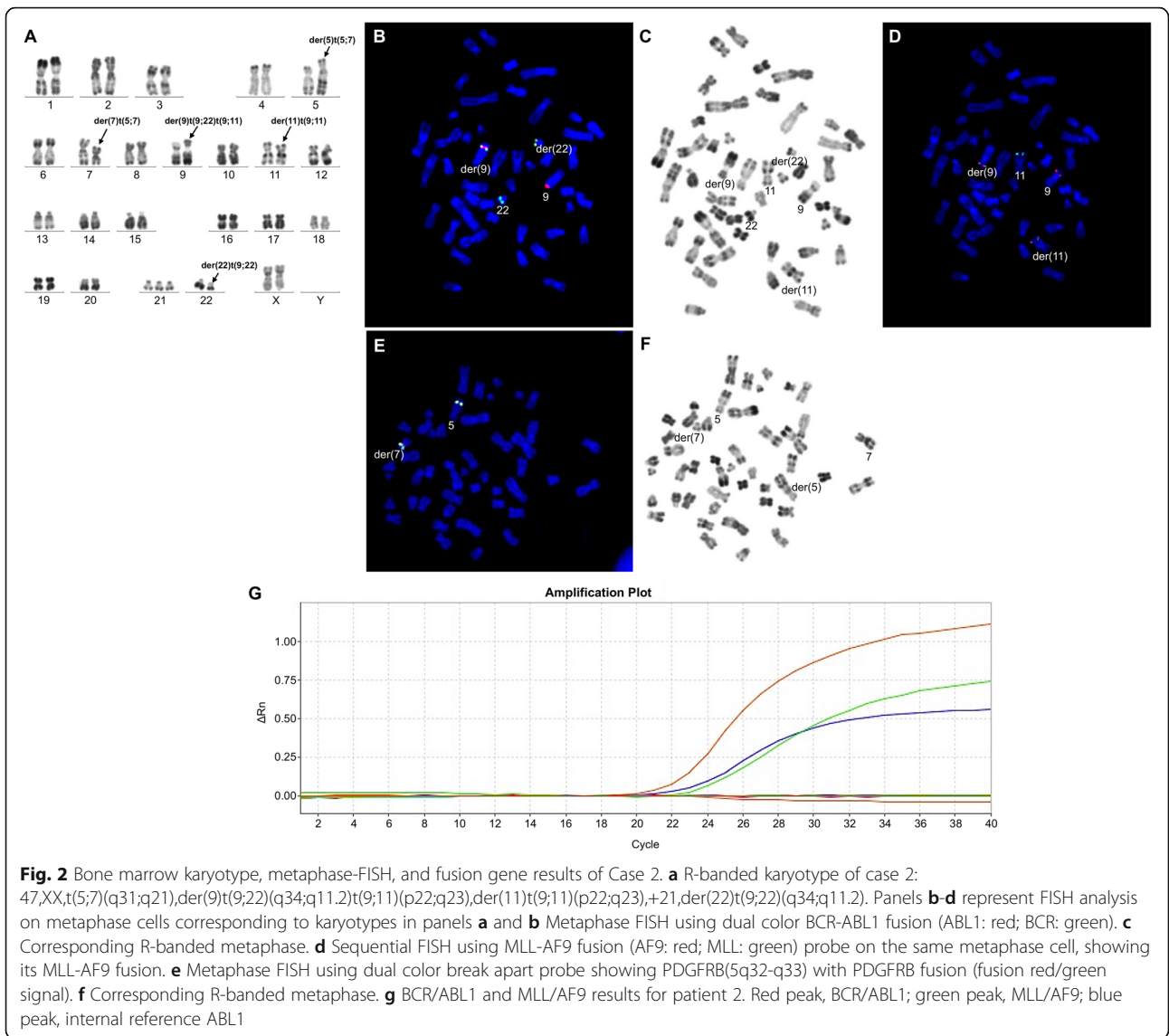
instructions. Incubation of cells with monoclonal antibodies at 4 °C was followed by RBC lysis with NH_4Cl for 10 min and washing with phosphate-buffered saline solution. Cells were resuspended and fixed with 1% formaldehyde. A screening panel consisting of 4 tubes was performed: (1) CD64/CD117/CD34/CD33/CD7/HLA-DR/CD38/CD45; (2) CD15/CD13/CD34/CD123/CD56/CD16/CD11b/CD45; (3) CD36/CD10/CD5/CD20/CD4/CD14/CD19/CD45; (4) TdT/MPO/CD9/CD2/cCD79a/mCD3/cCD3/CD45. Data analysis was performed using FCS Express 5 software (De Novo Software). A total of 10,000 events were acquired in each case. Major cell populations were defined by CD45/SSC (side-scatter) characteristics. All populations were further refined by forward and side-scatter gates to exclude nonspecific binding. A positive flow cytometry result was defined as the presence of circulating myeloblasts, lymphoblasts, or monocytes with aberrant antigen expression or lymphoproliferative disorder. Criteria for judging results: For each antibody, appropriate negative levels were determined by comparison with an isotype-matched control sample. Leukemia cell membrane surface antigen > 20% was judged to be positive, and intracellular antigen > 10% was judged to be positive [7].

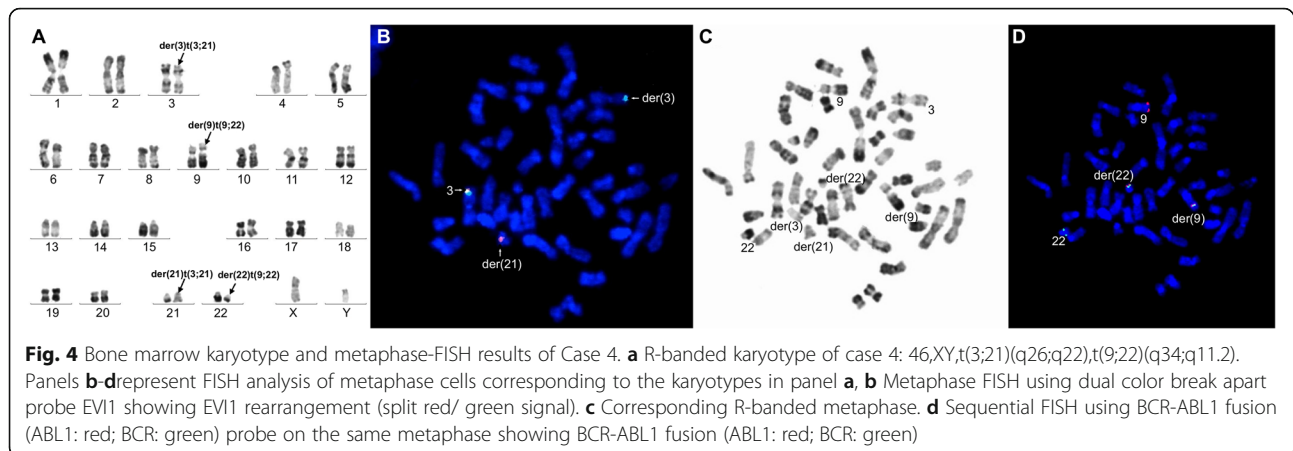
Results

We retrospectively studied 1382 patients with hematologic malignancies accompanied by a t(9;22) translocation. Among these, 5 patients (0.36%) had additional rare recurrent genetic abnormalities in addition to t(9;22). In addition to t(9;22)(q34;q11), 2 of these 5 patients displayed

the t(9;11)(p22;q23) abnormality. These 2 patients were confirmed as having both t(9;22) and t(9;11) in the same metaphase mitotic chromosome figure through karyotype analysis and BCR/ABL1 and MLL/AF9 metaphase FISH. In addition, the two abnormalities t(9;22) and t(9;11) existed in the same chromosome 9, namely der(9)t(9;22)t(9;11). Of which one case, was accompanied by t(5;7)(q31;q21) in addition to der(9)t(9;22)t(9;11). Metaphase FISH using the PDGFRB (5q32-q33) dual color break apart probe showed a PDGFRB (5q32-q33) gene on chromosome 7, confirming the t(5;7) translocation; karyotype and FISH results are shown in Figs. 1a and 2f. Quantitative PCR detection of fusion genes was also performed. The peak profile results for the BCR/ABL1 and MLL/AF9 fusion genes are shown in (Fig. 1e, Fig. 2g). In the other 3 patients, in addition to the t(9;22) translocation, one patient also had t(3;21)(q26;q22), one patient also had t(8;21)(q22;q22), and one patient also had inv(16)(p13;q22). Interphase FISH was performed for the patients with t(8;21). Metaphase FISH was performed for the remaining 2 patients to confirm karyotype results. Patient 3, with inv(16)(p13;q22) was tested by FISH for BCR/ABL1 and CBF β , and gave a positive result for both genes; karyotype and metaphase FISH results are shown in Fig. 3a-d. Patient 4 with t(3;21) was positive for the BCR/ABL1 fusion gene, and in the same mitotic figure was also positive for the EVI1 break apart probe. The breakpoint was located on chromosome 21. Molecular techniques also showed that the BCR/ABL1 and AML1-MDS/EVI1 fusion genes were







positive; karyotype and metaphase FISH results are shown in Fig. 4a-d. Patient 5 had positive interphase FISH results for BCR/ABL1 and RUNX1-RUNX1T1. Karyotype and FISH results are shown in Fig. 5a-c. Three of the five patients were newly diagnosed with chronic myelogenous leukemia, and all relapsed after imatinib treatment. Of the 5 patients, the two relapsed patients with t(9;11) were AML-M2, and the patient with t(8;21) had accelerated phase CML. The other two cases had AML-M5 at the time of initial diagnosis, and no history of CML. The prognosis of these 5 patients was very poor, and all died within 1 week to 8 months. Their laboratory data results are shown in Table 1. The 3 patients who relapsed after imatinib treatment were all initially diagnosed with typical CML, with only the t(9;22) translocation and only the *BCR/ABL1* fusion gene abnormality present. Additional chromosomal abnormalities appeared after relapse, and the morphology and disease course also changed. The morphological and flow cytometric results of all 5 patients are shown in Table 2.

Discussion

Chronic myelogenous leukemia progression is often accompanied by cytogenetic evolution, its impact on prognosis is dependent on the specific chromosomal anomalies that occur. The most common forms of which are translocations that produce a Philadelphia chromosome (Ph), + 8, or i(17)(q10) [8]. The coexistence of the Ph and recurrent abnormalities are extremely rare in AML and CML [9]. We describe 5 cases of a rare type of Ph+ leukemia with coexistent t(9;11), t(3;21), t(8;21) and inv(16), as assessed by cytogenetic and molecular analysis. In our study, we found that only 0.36% of patients displayed recurrent genetic abnormalities, and even fewer cases presented with t(9;22) accompanied by t(9;11) and t(3;21). To date, no cases of t(9;22) accompanied by t(9;11) have been reported in China. One case of t(9;22) accompanied by t(3;21) was reported in 2006 [10].

11q23 rearrangements in chronic myelogenous leukemia are extremely rare, accounting for less than 1% of cases reported in the literature to date [11]. CML with the t(9;22) translocation rarely incurs the additional t(9;

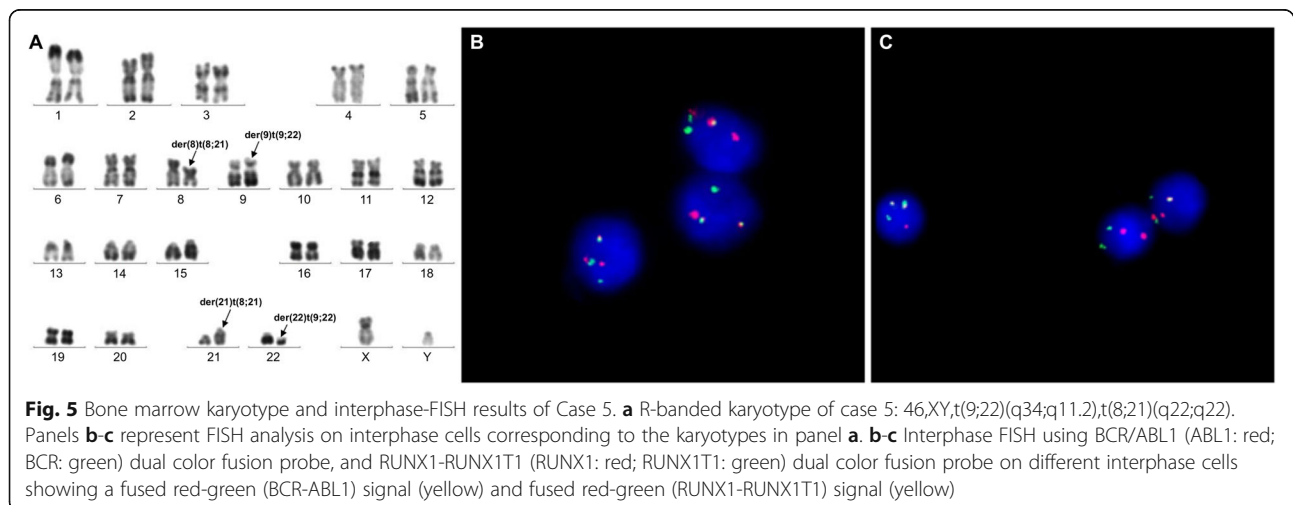


Table 1 Laboratory data

| Case | Sex | Age | WBC | HGB | PLT | Disease stage | Clinical diagnosis | Outcome | Karyotype | RT-PCR | FISH |
|------|-----|-----|--------|-----|-----|-------------------|--------------------------|---|--|--|--|
| 1 | M | 47 | 298.3 | 83 | 603 | Initial diagnosis | CML | Recurrence at 9 months after CML diagnosis and imatinib treatment | 46,XY,t(9;22)(q34;q11.2)[15] | BCR/ABL positive, MLL/AF9 negative | Interphase: BCR/ABL positive, MLL/AF9 undetermined |
| | | | | | | Post-recurrence | CML blastic phase AML-M2 | Died 4 months after recurrence of CML as AML-M2 and chemotherapy | 46,XY,der(9)t(9;22)(q34;q11.2)t(9;11)(p22;q23),der(11)t(9;11)(p22;q23),der(22)t(9;22)(q34;q11.2)[20] | BCR/ABL positive, MLL/AF9 positive | BCR/ABL positive, MLL/AF9 positive |
| 2 | F | 25 | 414.7 | 75 | 297 | Initial diagnosis | CML | Recurrence at 1 year after CML diagnosis and imatinib treatment | 46,XX,t(9;22)(q34;q11.2)[20] | BCR/ABL positive, MLL/AF9 negative | Interphase: BCR/ABL positive, MLL/AF9 undetermined |
| | | | | | | Post-recurrence | CML blastic phase AML-M2 | Died 6 months after recurrence of CML as AML-M2 and chemotherapy | 47,XX,t(5;7)(q33;q21),der(9)t(9;22)(q34;q11.2)t(9;11)(p22;q23),der(11)t(9;11)(p22;q23),+21,der(22)t(9;22)(q34;q11.2)[20] | BCR/ABL positive, MLL/AF9 positive | BCR/ABL positive, MLL/AF9 positive |
| 3 | M | 41 | 150.02 | 123 | 82 | Initial diagnosis | AML-M5 | Died 6 months after AML diagnosis and treatment | 46,XY,t(9;22)(q34;q11.2),inv(16)(p13;q22)[17]/47,idem,+der(22)t(9;22)(q34;q11.2)[3] | BCR/ABL positive, CBFβ/MYH11 positive | BCR/ABL positive, CBFβ positive |
| 4 | M | 58 | 46 | 64 | 34 | Initial diagnosis | AML | Died 8 months after AML diagnosis and treatment | 46,XY,t(3;21)(q26;q22),t(9;22)(q34;q11.2)[20] | BCR/ABL positive, AML1-MDS/EVI1 positive | BCR/ABL positive, EVI1 positive |
| 5 | M | 46 | 113.52 | 88 | 554 | Initial diagnosis | CML | Recurrence at 4 months after CML diagnosis and oral imatinib treatment (treatment voluntarily discontinued at times) | 46,XY,t(9;22)(q34;q11.2)[15] | BCR/ABL positive, RUNX1-RUNX1T1 negative | Interphase: BCR/ABL positive, RUNX1-RUNX1T1 undetermined |
| | | | | | | Post-recurrence | CML | Post-recurrence morphology and flow cytometry results indicated accelerated phase of CML. Died of internal organ bleeding 1 week after recurrence | 46,XY,t(9;22)(q34;q11.2)[10]/46,idem,t(8;21)(q22;q22)[10] | BCR/ABL positive, RUNX1-RUNX1T1 positive | BCR/ABL positive, RUNX1-RUNX1T1 positive |

WBC Reference range: 4.0–10.0 × 10⁹/L; HGB Reference range: Males: 120–160 g/L, Females: 110–150 g/L; PLT Reference range: 100–300 × 10⁹/L

11) during disease progression. In the last twenty years, no more than 5 cases have been reported in the literature [12–14], and none have been reported in China. Our study found that the proportion of patients with t(9;22) accompanied by the t(9;11) translocation was even lower 0.14% (2/1382). Both cases were patients initially diagnosed with CML who relapsed after imatinib treatment, and died within 6 months of relapse. When initially diagnosed with CML, the only genetic abnormality in each patient was t(9;22). The t(9;11) translocation appeared with disease progression to AML. One of these 2 patients had the t(5;7) translocation in addition to t(9;11), but the survival periods of the two patients did not differ significantly, so t(9;11) may have a greater effect on CML progression. The coexistence of t(9;22) and t(9;11) in the same clone may affect differentiation and development of pluripotent stem cells. The 11q23/MLL rearrangement may be a gene activated by pluripotent stem cells, and t(9;11) may induce loss of progenitor cells during differentiation. Therefore, the appearance of t(9;11) in

cancer patients with the t(9;22) translocation enhances the malignancy of tumor cells, accelerates the disease process, and has a greater impact on prognosis.

Since 1992, a total of 5 patients worldwide have been reported to have both the t(8;21) and t(9;22) translocations at the same time. Most of these were CML patients that progressed to AML [15–18]. The fifth case was published in the Chinese Journal of Medical Genetics in 2019 [19], so it will not be described in detail here. Patient 1 had both t(9;22) and t(3;21), and patient 4 had both t(9;22) and inv(16). Both were initially diagnosed with AML-M5. Neither had a history of CML, and both died after 6–8 months of treatment. The t(3;21)(q26;q22) translocation forms the fusion gene AML1-MDS/EVI1, which can be detected by reverse transcription polymerase chain reaction (RT-PCR) or EVI1 FISH. It is common in MDS and AML [20], and very rarely appears at the same time as t(9;22)(q34;q11.2). This was the second case found in China. Liu et al [10]. described the case of a CML patient with t(3;21)(q26;q22) during the acceleration process. The

Table 2 Morphology and flow cytometry results

| Case | Morphology | Flow cytometry |
|------|--|---|
| 1 | Significantly active bone marrow hyperplasia. Progenitor and immature cells accounted for 82.5%. Cell bodies were non-uniform in size and round or nearly round in shape, with few azurophilic granules and visible Auer rods. Nuclei were irregular with indentations, folds, kidney shapes, and so on. Nuclear chromatin appeared as uniformly distributed fine granules, with 2–5 nucleoli. | 91.81% of cells were abnormal; (+): CD33, CD13, CD117, CD123; Partial expression: HLA-DR; dim: MPO, CD64;(-): CD34, CD38, CD7, CD5, CD11b, CD56, CD19, CD20, CD10, CD4, CD14, CD36, cCD3, cCD79a |
| 2 | Significantly active bone marrow hyperplasia with increased proportion of granulocytes. The majority of cells were granulocyte progenitors, accounting for 70.5%. Cell bodies were non-uniform in size, round or nearly round in shape, had deep margins, and few azurophilic granules. Nuclei were irregular with indentations and folds. Nuclear chromatin appeared as uniformly distributed fine granules, with 2–4 nucleoli. | 83.5% of cells were abnormal; (+): CD33, CD13, CD117, CD123; Partial expression: HLA-DR,CD34; dim: MPO, CD64; (-): CD38, CD7, CD5, CD11b, CD56, CD19, CD20, CD10, CD4, CD14, CD36, cCD3, cCD79a |
| 3 | Extremely active bone marrow hyperplasia. Progenitor and immature cells accounted for 84%. Cell bodies were non-uniform in size and round or nearly round in shape, with few azurophilic granules. Nuclei were irregular with indentations, folds, and so on. Nuclear chromatin appeared as uniformly distributed fine granules, with 2–4 nucleoli. | 73.91% of cells were abnormal (+): CD33, CD13, CD123; Partial expression: CD34, CD117, HLA-DR dim: MPO, CD38; (-): CD7, CD5, CD11b, CD56, CD19, CD20, CD64, CD10, CD4, CD14, CD36, cCD3, cCD79a |
| 4 | Significantly active bone marrow hyperplasia. Progenitor and immature cells accounted for 46%. Cell bodies were non-uniform in size and round or nearly round in shape, and had small amounts of cytoplasm. Purple-red granules were visible in some cells. Most nuclei were distorted and folded, with 1–4 nucleoli, some of which were unclear. | 28.8% of cells were abnormal; (+): CD117,HLA-DR, CD13, CD33, CD34, CD38, CD15, CD64, CD11c, MPO; (-): CD7, CD5, CD56, CD19, CD20, CD10, CD4, CD14, CD36, cCD3, cCD79a |
| 5 | Significantly active bone marrow hyperplasia with increased proportion of granulocytes. Granulocyte progenitors accounted for 4%, of which the majority were early, middle, late, and rod-shaped. Lobulated neutrophils and eosinophils were occasionally found. Basophils were not found. | Low proportion of myeloid progenitor cells on flow cytometry, accounting for 1.57% of nucleated cells. CD117 expression was lost in some cells. Abnormal expression of CD56 and CD13/CD11b differentiation antigens in some granulocytes. Eosinophils were found. |

patient in the present study had both t(3;21) and t(9;22), and had AML at the time of onset. In previous studies [21–24], t(3;21)(q26;q22) has been more common in patients with CML that progressed to AML, or patients with treatment-related MDS or AML. This additional chromosomal abnormality was considered an acquired abnormality during leukemia development and progression.

Inv(16) is more common in AML (especially the AML-M4EO subtype) where it is accompanied by an increased number of eosinophils [25, 26]. AML with Inv(16) and t(9;22) is extremely rare. Clonal evolution appearing in the process of CML acceleration or AML progression has been more frequently reported in the literature [27–32]. The case in the present study did not have a history of CML, and was initially diagnosed with AML-M5. No increase was observed in eosinophils or basophils by morphology or flow cytometry. ABL kinase mutations were negative at initial diagnosis, but positive during treatment. Patient prognosis was very poor, and the patient died after 6 months.

Tumor cell evolution and disease phenotype maybe associated with chromosomal changes. Additional chromosomal changes appearing in the context of CML are one of the most important hallmarks of disease progression. Clonal evolution in CML is a prognostic factor, and associates types of chromosome changes with disease stage and progression. For example, trisomy 8, i(17q), and

complex karyotypes are genetic changes associated with poor prognosis. However, our study shows that patients with recurrent genetic abnormalities have an even worse prognosis, illustrated by 5 patients that all died within 4–9 months. Due to the extremely rare incidence and small case numbers, more cases need to be assessed in the future in to study their prognostic characteristics.

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Authors' contributions

Jin-Ying Gong designed the study. Zhen-Hao Zhang drafted the manuscript. Xiaofang Feng for providing technical support in flow cytometric immunophenotyping and calculation of the results, Huijun Wang for analyzing the results of flow cytometric immunophenotyping, Wei Zhang for the skillful technical assistance in cytogenetics and fluorescence in situ hybridization investigations for data management and the preparation of figures. Ji Zhou for providing support in molecular biology work (reverse transcription polymerase chain reaction), analysis of the results, and Guoqing Zhu for providing professional guidance with this manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Ethics approval and consent to participate

This study (registration no. NI2020002-EC-1) was approved by the institutional review board of the Institute of Hematology and Blood Diseases Hospital Chinese Academy of Medical Sciences.

Consent for publication

Informed consent was obtained from all subjects.

Competing interests

The authors declare no conflicts of interest.

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