

Complex variant of Philadelphia translocation involving chromosomes 1, 9, 12 and 22 in a case with chronic myeloid leukemia (CML)

Kompleksna varijantna translokacija Philadelphia kromosoma koja uključuje kromosome 1, 9, 12 i 22 u slučaju kronične mijeloične leukemije (KML)

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Abstract. Aim: Chronic myeloid leukemia (CML) is characterized by the Philadelphia (Ph) chromosome created by the reciprocal translocation t(9;22)(q34;q11), resulting in the hybrid gene breakpoint cluster region – Abelson (BCR-ABL1). Around 5–10% of CML cases develop complex variant Ph translocations involving one or more chromosomal regions besides 9 and 22. In this study we report a case of acute lymphoblastic leukemia (ALL) developed from CML displaying a novel four-way translocation. **Case report:** A complete blood analysis of a 46-year-old male patient showed an increase in white blood cells, lower levels of red blood cells and platelets. Bone-marrow sample was subjected to the conventional cytogenetic (Giemsa-banding) and fluorescence *in situ* hybridization methods and the four – way translocation was identified. Considering hepatosplenomegaly and breakpoint cluster region that was characterized as major (M-bcr) on the quantitative real-time polymerase chain reaction (RQ-PCR), ALL was diagnosed as a transformation from CML. **Conclusion:** This study reports a new four-way translocation ins(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11.2) in an ALL patient developed from the CML and was cross-checked in Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.

Key words: bone marrow; chronic myelogenous leukemia; cytogenetics; Philadelphia chromosome

Sažetak. Cilj: Kronična mijeloična leukemija (KML) karakterizirana je Philadelphia kromosomom (Ph) koji nastane recipročnom translokacijom t(9;22)(q34;q11.2) i rezultira fuzijskim genom BCR-ABL1. Kompleksna varijantna translokacija uključuje još jedan ili više kromosoma uz kromosome 9 i 22 te se javlja u 5 do 10 % svih slučajeva KML-a. U ovoj studiji prijavljujemo novi slučaj varijantne akutne limfoblastične leukemije (ALL) koja se transformirala iz KML-a. **Prikaz slučaja:** Kompletna krvna slika 46-godišnjeg pacijenta pokazala je povećane vrijednosti bijelih krvnih stanica, a snižene vrijednosti crvenih krvnih stanica i trombocita. Uzorak koštane srži (KS) poslan je na klasičnu citogenetičku (Giemsa-pruganje) i fluorescentnu *in situ* hibridizacijsku (FISH) analizu kojima je potvrđena nova varijantna translokacija između četiriju kromosoma. S obzirom na hepatosplenomegaliju i područje prijelomne točke koja je karakterizirana kao „major“ (M-bcr), korištenjem kvantitativne polimerazne lančane reakcije u stvarnom vremenu dijagnosticiran je ALL u transformaciji iz KML-a. **Zaključak:** Ova studija prijavljuje novu varijantnu translokaciju koja obuhvaća 4 kromosoma ins(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11.2) kod pacijenta s ALL-om transformiranim iz KML-a, na temelju provjere u bazama podataka *Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer*.

Ključne riječi: citogenetika; koštana srž; kronična mijeloična leukemija; Philadelphia kromosom

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome resulting from the reciprocal translocation $t(9;22)(q34;q11)$ ¹. This translocation between the long arms of chromosome 9 and 22 results in a shortened chromosome 22 (Ph) and found in over 90% of CML patients. The final product of this genetic rearrangement is a 210 kDa cytoplasmic fusion protein, p210^{BCR/ABL},

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which is essential and sufficient for the malignant transformation of CML, and also responsible for the phenotypic abnormalities of chronic phase CML². At the molecular level reciprocal exchange involves Abelson oncogene (ABL1) located on chromosome 9 and the breakpoint cluster region (BCR) gene on chromosome 22 forming a BCR-ABL1 hybrid gene³. In 5-10% of newly diagnosed CML cases, one or more additional chromosomes were involved in the translocation, which are termed variant or complex translocation⁴. The treatment of CML was revolutionized by the introduction of imatinib mesylate (IM), a BCR-ABL1 tyrosine kinase inhibitor (TKI)⁵. It blocks the BCR-ABL1 tyrosine kinase activity and subsequently induces apoptosis followed by the reduction in the proliferation of BCR-ABL1-expressing cells in both CML and acute lymphocytic leukemia (ALL). The treatment of CML patients with IM significantly increased the survival and improved the quality of life⁶. The second-generation BCR-ABL1 TKIs, nilotinib and dasatinib, showed significant activity in clinical trials in patients intolerant or resistant to IM therapy⁵. To the best of our knowledge, this case report is the first to study four-way Ph translocation 46, XY, $ins(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11.2)$ in a CML patient with a new complex rearrangement.

CASE REPORT

A 46-year-old male patient was diagnosed with ALL in 2016. The hematological analysis revealed a white blood cell (WBC) count of $70.8 \times 10^9/L$ (normal range, $3.4-9.7 \times 10^9/L$), eosinophils 2% (normal range, 0-7%), basophils 2% (normal range, 0-1%), immature neutrophil value 9% (normal range, 0-2%), a mature neutrophil value of 40% (normal range, 44-72%), a lymphocyte value of 5% (20-46%), a metamyelocyte value of 1% (normal range, 0-0%), a myelocyte value of 2% (normal range, 0-0%), a promyelocyte value 1% (normal range, 0-0%), a blast cells 38% (normal range, 0-0%), a red blood cell (RBC) count of $2.87 \times 10^{12}/L$, (normal range, $4.34-5.72 \times 10^{12}/L$), a hemoglobin (Hb) level of 85 g/L (normal range, 138-175 g/L), a hematocrit (Htc) of 0.261 L/L (normal range, 0.415-0.530 L/L), a mean corpuscular volume (MCV) of 91.0 fL (normal range, 83.0-97.2 fL), a mean corpuscular hemoglobin (MCH) level of 29.5 pg (normal range, 27.4-33.9 pg), a mean corpuscular hemoglobin concentration (MCHC) of 325 g/L (normal range, 320-345 g/L), a platelet count of $5 \times 10^9/L$ (normal range, $158-424 \times 10^9/L$), and an erythrocyte sedimentation rate of 14mm/h (normal range, 2-13mm/h).

Complete blood count laboratory test

Complete blood count (CBC) was performed using an Automatic Hematological Analyzer (Sysmex xe 5000, America). The hematological analyzer that was used for the complete blood count measured the level of WBCs, RBCs, platelets and Hb, the MCV, MCH, MCHC. The patient's peripheral blood (PB) sample was taken in a test tube with tripotassium ethylenediaminetetraacetic acid (K_3 -EDTA) anticoagulant. An increase in WBCs, lower levels of RBCs and platelets, and the appearance of blast cells showed suspicion of hematological disorder, and the patient was considered for further examination.

RQ-PCR

Reverse transcription real time quantitative polymerase chain reaction (RQ-PCR) is routinely used to quantify levels of BCR-ABL1 mRNA transcripts in PB and bone marrow (BM) samples⁷. The fusion protein encoded by BCR-ABL1

varies in size, depending on the breakpoint in the BCR gene. Three breakpoint cluster regions have been characterized to date: major (M-bcr), minor (m-bcr) and micro (mu-bcr). The vast majority of CML patients have a p210 BCR-ABL1 gene (M-bcr), and the smallest of the fusion proteins, p190 BCR-ABL1, (m-bcr breakpoint) is principally associated with Ph-positive ALL. CML resulting from a p230 BCR-ABL1 gene (mu-bcr breakpoint) is also rare, and has been associated with the chronic neutrophilic leukemia variant and/or with marked thrombocytosis⁸. In this case, the M-bcr transcript from the BM sample was confirmed.

Cytogenetic analysis

BM specimen for RQ-PCR, conventional cytogenetic analysis, and fluorescence *in situ* hybridisa-

tion (FISH) analysis was collected before treatment. Conventional cytogenetic analysis was performed on unstimulated 24-hour culture of a BM specimen using a standard technique⁹. In total, 15 Giemsa banding (G-banding) BM metaphases were analyzed. Fifteen metaphases were captured and analyzed on fluorescence light microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and Ikaros software (Metasystem, Heidelberg Germany), and all had the present variant translocation. The karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2016)¹⁰. The conventional cytogenetic analysis identified the presence of a complex, four-way Ph chromosome translocation $\text{ins}(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11)$ (Figure 1).

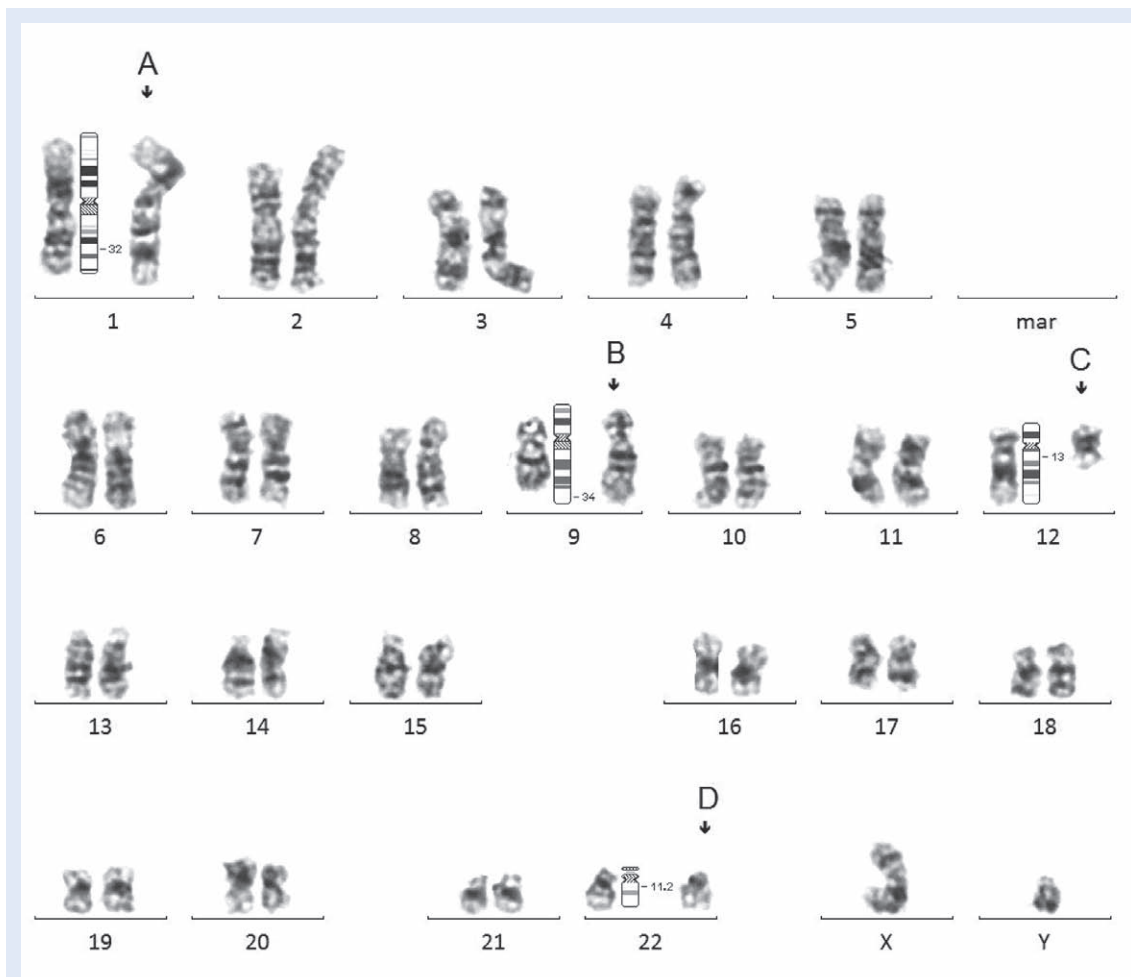


Figure 1. The karyotype of four-way translocation: $\text{ins}(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11.2)$. All derivative chromosomes are highlighted by arrow heads. Chromosome 22 was not shortened as in classical Ph translocation ($22q11.2$, D). Part of chromosome 12 ($12q13$, C) was translocated to chromosome 1 ($1q32$, A). Derivative chromosome 9 ($9q34$, B).

Fluorescence *in situ* hybridisation (FISH) analysis

FISH procedure was performed on fixed cell suspension obtained with conventional cytogenetic method on microscope slides according to the manufacturer's instructions. The process of denaturation and hybridization was performed on the automated machine Hybrante (HYBrite™, Vysis GmbH, Stuttgart-Fasanenhof, Germany). As a probe was used Vysis LSI BCR-ABL1 Dual color Dual fusion Translocation Probe kit (Abbott Labo-

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ratories, Illinois, U.S.A.). Two hundred interphase cells of patient were analyzed on fluorescence microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany) under 1000× magnification. Associated optical filters were green, orange, and triplet – a filter that can see aqua, red, and green signals at the same time. Image capturing and processing were performed using an Isis imaging system (Isis software, Altusheim, Germany). The result was expressed as a percentage of Ph positive interphase cells.

A probe used for FISH is a mixture of the LSI BCR probe labeled with SpectrumGreen and the LSI ABL1 probe labeled with SpectrumOrange. The BCR probe is labeled with SpectrumGreen that spans a genomic distance of about 1.5 megabases (Mb). The ABL1 probe labeled with SpectrumOrange includes 650 kb, and it extends from an area centromeric of the argininosuccinate synthetase gene (ASS) to telomeric of the last ABL1 exon¹¹.

To confirm translocation, Whole Chromosome Probes (WCP) (KREATECH Diagnostics, Amsterdam, The Netherlands) was also used. WCP are optimized to detect unique sequences spanning the length of individual entire chromosomes. The analysis carried out with these probes was performed on cultured cells on metaphase chromosomes¹².

In general, FISH analysis of interphase nuclei of the ABL1 (9q34) gene was identified by fluores-

cent red signal, and BCR (22q11.2) gene by green signal. Negative cells without translocation include two red and two green signals (2R2G). With the dual color, dual fusion BCR-ABL1 probe, the positive reciprocal t(9;22) signal pattern revealed two fusions (derivative chromosome 9 and derivative chromosome 22), one red (normal chromosome 9) and one green (normal chromosome 22) (2F1R1G). Cases with variant translocation t(9;22) appears with one fusion signal (derivative chromosome 22), two red (normal and derivative chromosome 9) and two green signals (normal chromosome 22 and third involved chromosome) (1F2R2G). For present study, BCR-ABL1 translocation was detected by FISH analysis in 91% of the 200 interphase nuclei counted, and with 1F2R2G signals. (Figure 2). The remaining 9% of the cells were Ph negative with 2R2G signals.

Treatment

The patient was treated initially with standard induction therapy for ALL which include HyperC-VAD-HD-MA and IM at daily dose of 600 mg. On IM the patient developed an allergy in the form of a generalized rash and urticaria. For this reason, the patient was considered intolerant to IM, and a second-generation TKI, nilotinib, 200 mg 2×2 was started orally. After remission was achieved, the treatment was continued with transplantation of allogenic HLA related PB stem cells. Three months after the transplant, there is a relapse of the disease and the return of the primary clone of the disease. It is necessary to reintroduce the nilotinib that the patient took before transplantation to keep the disease under control until the onset of lymphocyte action. The dose was 600 mg daily. One month after, patient received first of total two donor lymphocyte infusion from original donor. Since then, the disease is in remission, and the patient has no sign of graft versus host disease (GVHD).

DISCUSSION

There are three distinct clinical stages of CML: chronic phase (CP), accelerated phase (AP) and blast phase (BP). The CP lasts several years and is characterized by accumulation of myeloid precur-

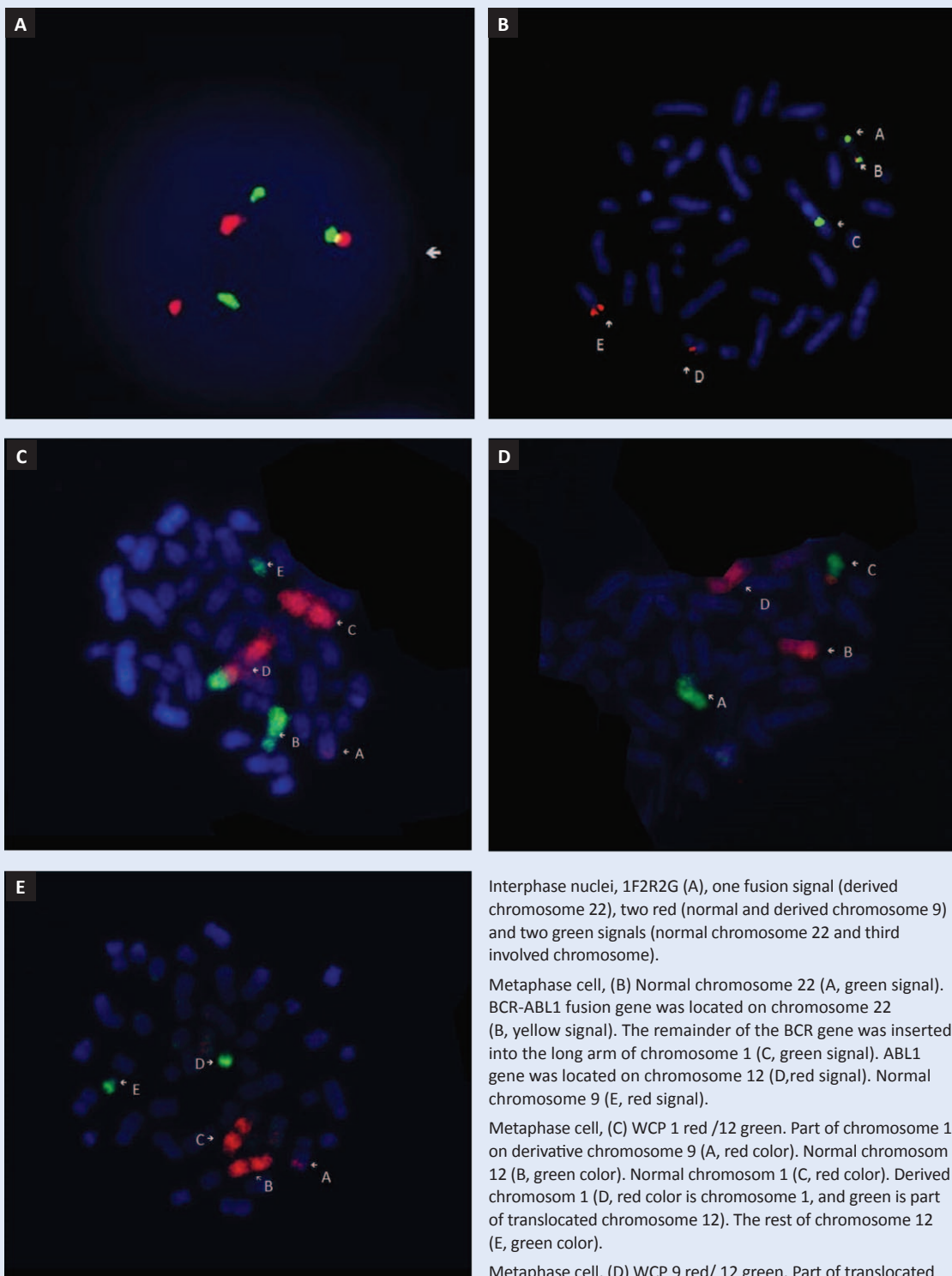


Figure 2. Representative FISH signal patterns using LSI BCR-ABL1 Dual color Dual fusion Translocation Probe Kit and Whole Chromosome Probes (WCP)

Interphase nuclei, 1F2R2G (A), one fusion signal (derived chromosome 22), two red (normal and derived chromosome 9) and two green signals (normal chromosome 22 and third involved chromosome).

Metaphase cell, (B) Normal chromosome 22 (A, green signal). BCR-ABL1 fusion gene was located on chromosome 22 (B, yellow signal). The remainder of the BCR gene was inserted into the long arm of chromosome 1 (C, green signal). ABL1 gene was located on chromosome 12 (D, red signal). Normal chromosome 9 (E, red signal).

Metaphase cell, (C) WCP 1 red /12 green. Part of chromosome 1 on derivative chromosome 9 (A, red color). Normal chromosome 12 (B, green color). Normal chromosome 1 (C, red color). Derived chromosome 1 (D, red color is chromosome 1, and green is part of translocated chromosome 12). The rest of chromosome 12 (E, green color).

Metaphase cell, (D) WCP 9 red/ 12 green. Part of translocated chromosome 12 on derivative chromosome 1 (A, green color). Normal chromosome 9 (B, red color). The rest of chromosome 12 (C, green color) with part of the ABL1 gene from the derivative chromosome 9 (C, red color). Derivative chromosome 9 (D, red color).

Metaphase cell, (E) WCP 1 red/ 22 green. Part of chromosome 1 on derivative chromosome 9 (A, red color). Normal chromosome 1 (B, red color). Derivative chromosome 1 (C, red color). Normal chromosome 22 (D, green color). Derivative Ph chromosome 22 (E, green color).

sors and mature cells in BM, PB and extramedullary sites. The AP lasts 4 to 6 months and is characterized by an increase in disease burden and in the frequency of progenitor/precursor cells rather than terminally differentiated cells. The BP lasts only a few months and is characterized by the rapid expansion of a population of myeloid or lymphoid differentiation-arrested blast cells¹³. In present case, the patient was administered in BP of ALL, but considered hepatosplenomegaly and breakpoint cluster region that was characterized as major (M-bcr) on RQ-PCR, it was diagnosed as a transformation from CML. CML is primarily caused due to balanced translocation between the long arms of 9 and 22 chromosomes and secondarily by the variant and complex translocation patients. In such cases, the third, fourth, or even fifth chromosome was involved and is termed as four-, five-, or six-way translocation⁶. The mechanisms of the generation of the variant translocations are not fully understood, but some authors have suggested 2 different mechanisms: a 1-step mechanism in which chromosome breakage occurs simultaneously on 3 or 4 different chromosomes in 3-way or 4-way translocation, respectively, and a 2-step mechanism involving 2 sequential translocations in which a standard t(9;22) translocation is followed by a second translocation involving additional chromosomes¹.

Genomic instability is probably responsible for two major problems in CML: TKI resistance and disease progression¹⁴. The majority of CML-BP patients not previously treated with TKIs do initially respond to treatment with these agents, either alone or in combination with conventional chemotherapeutic drugs, but most still relapse within a few months of achieving a seemingly complete hematologic or even cytogenetic response. Therefore, any CML-BP patient who does respond to modern therapy should proceed, if possible, to allogeneic stem cell transplant prior to relapse. To date, there is strong evidence supporting the idea that the level of BCR-ABL1 kinase activity plays a pivotal role in almost all CML patients undergoing progression and that BCR-ABL1-induced genetic/chromosomal abnormalities can predispose to transformation and/or

markedly influence the aggressiveness of the blast crisis progenitor cell clone¹⁵.

Since the conventional cytogenetic analysis and the FISH method can not determine which exact genes other than BCR-ABL1 are involved, we can not predict the effect of other translocation or BCR insertion on patient prognosis or response to therapy. Also, in the literature, we did not find a description of the aforementioned translocation and insertion in leukemia. Numerous studies have deal with the topic of variant translocation in CML and response to TKI, but the correlation between poorer TKI response in variant translocation patients has not been demonstrated^{1,16}.

Up to now, patient showed a good response to nilotinib treatment and transplantation of allogeneic HLA. At the time this article was written, 315 days has elapsed since transplantation, and the disease is in remission.

CONCLUSION

In conclusion, this study reports new four-way translocation 46, XY, ins(22;1)(q11.2;q31q32)t(1;9;12;22)(q32;q34;q13;q11.2) in an ALL patient developed from the CML for the first time and was cross-checked in Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.

Conflicts of interest statement: the authors report no conflicts of interest.

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