ANTICANCER RESEARCH *39*: 431-436 (2019)
doi:10.21873/anticanres.13130

B-ALL Relapses After Autologous Stem Cell Transplantation Associated With a Shift from e1a2 to e14a2 *BCR-ABL* Transcripts: A Case Report

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Abstract. *Background/Aim: The Philadelphia chromosome is found in 30% of acute lymphoblastic leukemia (ALL) patients, a distinct ALL subgroup where the BCR-ABL fusion gene is associated with poor prognosis. Treatment with tyrosine kinase inhibitors (TKIs) often induces complete remission and these patients subsequently undergo an autologous stem cell transplantation (ASCT). However, 20% of subjects experience a relapse associated with the selection of point-mutations in the BCR-ABL kinase domain. We report the clinical evolution of a Philadelphia-positive ALL patient co-expressing the e1a2 and e14a2 BCR-ABL transcript at diagnosis. Materials and Methods: Multiplex reverse transcriptase (RT)-PCR was used to detect BCR-ABL transcripts and their levels were measured by quantitative Real Time PCR. Clonal sequencing and next-generation sequencing (NGS) were used to identify mutations. Results: Although the patient underwent ASCT following treatment with multiple TKIs, he relapsed twice. The first time he exhibited the e1a2 transcript and the second time he presented only the e14a2 variant. Mutation analysis, performed by clonal sequencing and NGS, detected two alterations after the first relapse and a single mutation at the time of the second relapse. Conclusion: The observed shift from the e1a2 to the e14a2 variant and the selection of TKI-resistant clones heavily contributed to the fatal evolution of the disease.*

The Philadelphia (Ph) chromosome, resulting from the reciprocal translocation of the *ABL* gene at 9q34 with the *BCR* gene at 22q11, is the most frequent cytogenetic abnormality in both chronic myelogenous leukemia (CML) and adult acute lymphoblastic leukemia (ALL) (1-3). At the molecular level, the t(9,22) translocation generates the *BCR-ABL* chimeric oncogene that may encode for a 210-kD or a 190-kD protein both displaying constitutive tyrosine kinase activity and favoring cell-cycle progression (4-7). The fusion gene is found in more than 95% of patients with CML (8), and in 20-30% of adult ALL patients, with the incidence rising to more than 50% in patients aged >50 years where it is associated with an inferior prognosis (9). Several reports have found the e1a2 transcript to coexist with e13a2 or e14a2 isoform in patients with Ph-positive ALL (10, 11).

In the pre-tyrosine kinase inhibitor (TKIs) era, Allogeneic Stem Cell Transplant (allo-SCT) in first complete remission was the only potentially curative option (12) for the disease. Currently, the standard of care for patients with Ph-ALL is a combination of chemotherapy and a TKI followed by allo-SCT. However, 60-70% of Ph-ALL individuals are ineligible for allo-SCT due to their age, lack of an HLA-matched sibling, lack of a matched-unrelated donor or a suitable cord blood unit. These patients can then be considered for autologous stem cell transplantation (ASCT), although this procedure is associated with a higher incidence of relapse (13). Hence, TKIs can be used for the *in vivo* eradication of the malignant clone before ASCT, followed by maintenance therapy after transplantation.

In the present report, we describe a Ph-positive ALL patient who displayed both the e1a2 and e14a2 *BCR-ABL*

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Key Words: e1a2, e14a2, ALL, BCR-ABL, ASCT, TKIs.

mRNAs at diagnosis developing two relapses each expressing a different single oncogenic transcript.

Case Report

In December 2014, a 53-year-old man presented with a 3-week history of fever, fatigue and abnormal blood cell counts; his hemoglobin (Hgb) was 6.5 g/dl with 18.000 white blood cells (WBC) and 13.000 platelets (PLT). Flow cytometry analysis identified 53% of HLA-DR$^+$, CD19$^+$, CD20$^+$, CD10$^+$ and CD34$^+$ leukemic blasts while conventional cytogenetics detected the Philadelphia chromosome in all examined metaphases with no additional cytogenetic abnormalities (karyotype 46, XY, t(9;22)(q34;q11)). Multiplex reverse transcriptase (RT)-PCR revealed the presence of the e14a2 BCR-ABL transcript with *BCR-ABL/ABL* levels of 95.25% measured by Real Time (Q-PCR) (14). However, the patient also displayed the e1a2 *BCR-ABL* variant with 10.13% *BCR-ABL/ABL* transcripts that were barely noticeable at the multiplex RT-PCR amplification (Figure 1A).

The patient received chemotherapy according to the HyperCVAD protocol (15), but after a single course of treatment all drugs were temporarily discontinued because of grade IV gastrointestinal and cutaneous toxicities. Upon reinstating the HyperCVAD regimen, he achieved a deep molecular response with *BCR-ABL/ABL* levels of 0.0029% (e14a2) and no e1a2 transcript detected. He subsequently received Dasatinib 140 mg/day (Figure 1B). Since an HLA-matched donor could not be found, in the presence of a deep molecular response (undetectable e14a2 transcript and 0,0061% e1a2 *BCR-ABL/ABL*), the patient underwent peripheral blood stem cell mobilization and collection for an ASCT that was performed in July 2015.

In September 2015, he maintained a deep molecular response for both the e14a2 and the e1a2 transcripts. However, three months later e1a2 *BCR-ABL/ABL* levels began to increase (0.00266%) (Figure 1B). He was, therefore, placed on imatinib 400 mg/day as it was impossible to restart dasatinib because of regulatory constraints of the Italian Agency for Antineoplastic Drugs.

Nevertheless, in April 2016 the patient experienced his first disease relapse. At this time, his leukocyte count rose to 69,000/mm^3 with 70% blasts bearing an immunophenotype indicative of B-ALL. The e1a2 *BCR-ABL/ABL* levels increased to 145.13%, with e14a2 transcripts still compatible with a deep molecular response. Mutational analysis performed both by clonal sequencing (to discriminate between compound and/or polyclonal mutations) and next generation sequencing (NGS, to detect variants below the 1% threshold) identified a T315I mutation in all Ph-positive clones. NGS also detected a 35-nucleotide insertion from ABL intron 8 (at the junction between exons 8 and 9) in 3.8% of the leukemic blasts (Figure 1B). He, therefore, received 4 cycles of chemotherapy with vincristine (2 mg/week) and doxorubicin (36 mg/m^2) once weekly as debulking therapy achieving a complete hematological remission (blast cells <5 % on bone marrow smear and undetectable e1a2 *BCR-ABL/ABL*) and in May 2016 ponatinib (45 mg/die) was started maintaining the attained response (Figure 1B).

However, in November 2016 a second relapse occurred (40% lymphoid blasts in the bone marrow smear), with conventional cytogenetic analysis detecting novel chromosomal abnormalities: 46, XY, del (20)(q13) (2 metaphases/20); 45, XY, -3, der(7)t(3;7)(q11;p11), t(9;22)(q34; q11)del(9)(p12), del (11)(q15), del (20), q13) (6 /20); 46, XY (12/20). Molecular analysis displayed an increase only in e14a2 *BCR-ABL* transcripts (56.9%) while the e1a2 variant remained undetectable. Furthermore, mutational analysis performed by NGS showed the E279K substitution in 35% of the Ph-positive clones. This mutation has been associated with moderate sensitivity to ponatinib (16, 17).

In January 2017 the patient received one course of inotuzumab ozogamicin (IO) which was administered according to the schedule 0.8 mg/m^2 *i.v.* on day +1 and 0.5 mg/m^2 iv on days +8 and +15 (the drug was obtained through a compassionate use program) as described by Pirosa *et al.* (18) (Figure 1B). A bone marrow biopsy performed after IO showed disease remission (e14a2 *BCR-ABL/ABL* 0.01071% with undetectable e1a2). In February 2017, the patient also received one course of chemotherapy with cyclophosphamide 1,000 mg/m^2 day +1 and cytarabine 75 mg/m^2 *i.v.* days +2, +3, +4, +5, as a maintenance regimen. Complete remission was sustained until May 2017 (as evidenced by bone marrow morphology, immuno-phenotyping and PCR with an e14a2 *BCR-ABL/ABL* level of 0.00785% in the absence of detectable e1a2 transcripts) (Figure 1B).

Central nervous system (CNS) surveillance with periodical rachicenteses performed in March and May 2017 was negative for leukemia relapse. In June 2017, he lamented frequent headaches with emotional disturbance. A lumbar puncture confirmed leukemic spread to the CNS. The clinical picture evolved rapidly to uncontrollable epilepsy shortly thereafter followed by death.

Materials and Methods

Total cellular RNA was extracted from peripheral blood or bone marrow leucocytes, using the RNeasy mini kit (Qiagen, KJ Venlo, The Netherlands), according to the manufacturer's protocol. cDNA synthesis was carried out using random hexamers (Invitrogen, Paisley, UK). Both e1a2 and e14a2 *BCR-ABL* fusion transcripts were screened by Q-PCR according to the suggested recommendations (19) and molecular analysis was performed as previously reported (14). Cytogenetic analysis was performed using conventional techniques (20). Mutation analysis of the ABL kinase domain by clonal sequencing or NGS was performed as previously described (21, 22).

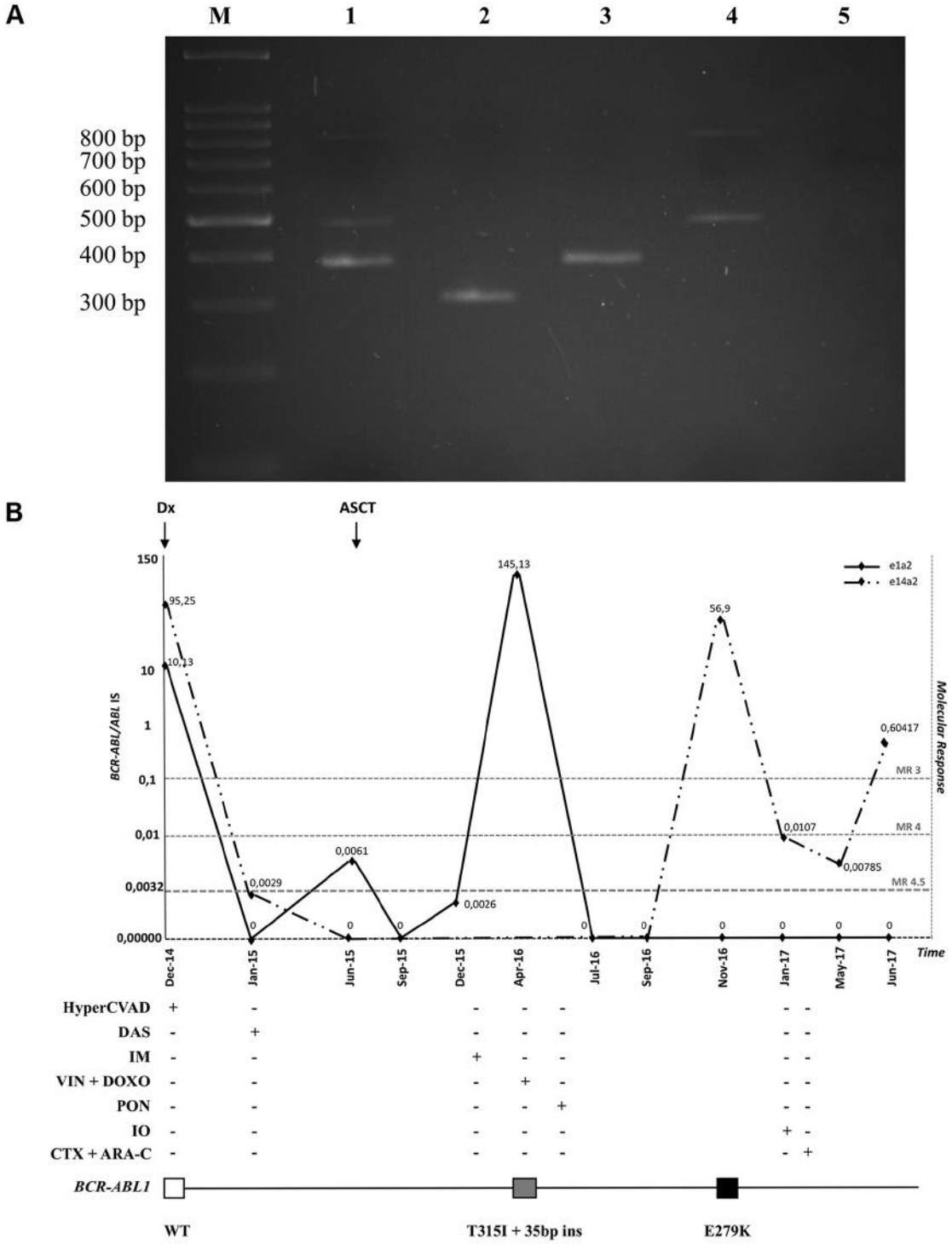

Figure 1. *Clinical evolution of the patient. A. Multiplex RT-PCR analysis of different BCR-ABL fusion transcripts. Lane M=Molecular size marker (100-bp ladder); lane 1=e1a2 (481 bp) and e14a2 (385 bp) from the patient; lane 2=e13a2 (310 pb) positive control; lane 3=e14a2 positive control; lane 4=e1a2 positive control; lane 5=negative control. B. Molecular response to different TKIs in combination with chemotherapy. Monitoring of the patient's disease evolution indicating variations in the e1a2 and e14a2 transcripts (top panel), drug treatments (middle panel) or BCR-ABL mutant clones (bottom panel). Dotted lines represent achievement of a major (MR3) or a deep (MR4; MR4.5) molecular response. A white square indicates wild-type BCR-ABL, a gray square denotes the T315I mutation and the 35-bp insertion and a black square indicates detection of the E279K substitution. ASCT: Autologous stem cell transplant; DAS: dasatinib; IM: imatinib; VIN: vincristine; DOXO: doxorubicin; PON: ponatinib; IO: inotuzumab ozogamicin; CTX: cyclophosphamide; ARA-C: cytarabine.*

Informed consent was received from the patient for the publication of the report. The patient gave written consent to participate in the study as specified in the Declaration of Helsinki.

Discussion and Conclusion

Adults diagnosed with Ph-positive ALL display poor overall survival, as expression of the *BCR-ABL* oncoprotein confers an inferior prognosis compared to other ALL subtypes. In this disease, the e1a2 or the e13/e14a2 *BCR-ABL* transcripts are commonly detected in the leukemic clones although less frequent breakpoints on both the *BCR* and *ABL* genes may also occur generating additional atypical transcripts (23, 24). Moreover, different investigators have previously described the coexistence of e1a2 and e13/e14a2 BCR-ABL transcripts in the same patients (25, 26).

In this report, we describe the case of a patient with Ph-positive ALL that, at diagnosis, co-expressed both the e1a2 and e14a2 *BCR-ABL* variants. Although the patient underwent ASCT, his disease relapsed twice after this procedure. Interestingly, the first relapse was attributable to a leukemic clone only expressing the e1a2 *BCR-ABL* transcript while the second relapse was due to an e14a2-positive clone in the absence of the previous shorter *BCR-ABL* mRNA. While we did not formally establish if the two different *BCR-ABL* transcripts were expressed by the same clone (*via* alternative splicing mechanisms) or represented two distinct cellular types each characterized by a specific *BCR-ABL* rearrangement (e1a2 or e14a2), the occurrence of two different relapses always involving the emergence of a different single *BCR-ABL* variant strongly supports the second hypothesis. It remains to be seen if co-expression of multiple *BCR-ABL* transcripts in the same or in different leukemic clones modifies the prognosis of Ph-positive ALL patients.

As identification of the T315I mutation in Ph-positive ALL or CML is associated with an inferior prognosis, each time the patient lost a previously acquired response he was subjected to a timely molecular analysis in order to take the appropriate therapeutic decisions (27, 28).

As the patient received dasatinib, imatinib and ponatinib during the course of his disease, the clones causing the two ALL relapses displayed mutations conferring either insensitivity (T315I) or limited sensitivity (E279K) to the different TKIs prescribed. Hence, at least initially, even when acquiring TKI resistance the disease remained dependent on BCR-ABL kinase activity.

Unfortunately, CNS relapse is still extremely difficult to treat and is often associated with a dismal outcome as in the case of this patient that had previously benefited from multiple chemotherapeutic regimens associated with different TKIs.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

References

1 Fujimaki K, Hattori Y and Nakajima H: 10-year complete remission in a philadelphia chromosome-positive acute lymphoblastic leukemia patient using imatinib without high-intensity chemotherapy or allogeneic stem cell transplantation. Int J Hematol *107(6)*: 709-711, 2018.

2 Stagno F, Vigneri P, Del Fabro V, Stella S, Restuccia N, Giallongo C, Massimino M, Berretta S, Pennisi MS, Tibullo D, Tirro E, Buscarino C, Messina A and Di Raimondo F: Concomitant and feasible treatment with dasatinib and the anti-egfr antibody cetuximab plus radiotherapy in a cml patient with multiple squamous neoplasias. Acta Oncol *49(1)*: 109-110, 2010.

3 Massimino M, Consoli ML, Mesuraca M, Stagno F, Tirro E, Stella S, Pennisi MS, Romano C, Buffa P, Bond HM, Morrone G, Sciacca L, Di Raimondo F, Manzella L and Vigneri P: Irf5 is a target of bcr-abl kinase activity and reduces cml cell proliferation. Carcinogenesis *35(5)*: 1132-1143, 2014.

4 Castagnetti F, Gugliotta G, Baccarani M, Breccia M, Specchia G, Levato L, Abruzzese E, Rossi G, Iurlo A, Martino B, Pregno P, Stagno F, Cuneo A, Bonifacio M, Gobbi M, Russo D, Gozzini A, Tiribelli M, de Vivo A, Alimena G, Cavo M, Martinelli G, Pane F, Saglio G, Rosti G and Party GCW: Differences among young adults, adults and elderly chronic myeloid leukemia patients. Ann Oncol *26(1)*: 185-192, 2015.

5 Massimino M, Stella S, Tirro E, Romano C, Pennisi MS, Puma A, Manzella L, Zanghi A, Stagno F, Di Raimondo F and Vigneri P: Non abl-directed inhibitors as alternative treatment strategies for chronic myeloid leukemia. Mol Cancer *17(1)*: 56, 2018.

6 Preyer M, Vigneri P and Wang JY: Interplay between kinase domain autophosphorylation and f-actin binding domain in regulating imatinib sensitivity and nuclear import of bcr-abl. PLoS One *6(2)*: e17020, 2011.

7 Giallongo C, Tibullo D, La Cava P, Branca A, Parrinello N, Spina P, Stagno F, Conticello C, Chiarenza A, Vigneri P, Palumbo GA and Di Raimondo F: Brit1/mcph1 expression in chronic myeloid leukemia and its regulation of the g2/m checkpoint. Acta Haematol *126(4)*: 205-210, 2011.

8 Stagno F, Stella S, Spitaleri A, Pennisi MS, Di Raimondo F and Vigneri P: Imatinib mesylate in chronic myeloid leukemia: Frontline treatment and long-term outcomes. Expert Rev Anticancer Ther *16(3)*: 273-278, 2016.

9 Li Y, Zou D, Zhao Y, Mi Y, Wang J and Qiu L: Clinical characteristics and outcomes of adults with philadelphia chromosome positive and/or bcr-abl positive acute lymphoblastic leukemia: A single center study from china. Leuk Lymphoma *51(3)*: 488-496, 2010.

10 Cimino G, Pane F, Elia L, Finolezzi E, Fazi P, Annino L, Meloni G, Mancini M, Tedeschi A, Di Raimondo F, Specchia G, Fioritoni G, Leoni P, Cuneo A, Mecucci C, Saglio G, Mandelli F, Foa R and Party GLW: The role of bcr/abl isoforms in the presentation and outcome of patients with philadelphia-positive acute lymphoblastic leukemia: A seven-year update of the gimema 0496 trial. Haematologica *91(3)*: 377-380, 2006.

11 Verma D, Kantarjian HM, Jones D, Luthra R, Borthakur G, Verstovsek S, Rios MB and Cortes J: Chronic myeloid leukemia (cml) with p190 bcr-abl: Analysis of characteristics, outcomes, and prognostic significance. Blood *114(11)*: 2232-2235, 2009.
12 Brissot E, Labopin M, Beckers MM, Socie G, Rambaldi A, Volin L, Finke J, Lenhoff S, Kroger N, Ossenkoppele GJ, Craddock CF, Yakoub-Agha I, Gurman G, Russell NH, Aljurf M, Potter MN, Nagler A, Ottmann O, Cornelissen JJ, Esteve J and Mohty M: Tyrosine kinase inhibitors improve long-term outcome of allogeneic hematopoietic stem cell transplantation for adult patients with philadelphia chromosome positive acute lymphoblastic leukemia. Haematologica *100(3)*: 392-399, 2015.
13 Giebel S, Labopin M, Gorin NC, Caillot D, Leguay T, Schaap N, Michallet M, Dombret H and Mohty M: Improving results of autologous stem cell transplantation for philadelphia-positive acute lymphoblastic leukaemia in the era of tyrosine kinase inhibitors: A report from the acute leukaemia working party of the european group for blood and marrow transplantation. Eur J Cancer *50(2)*: 411-417, 2014.
14 Vigneri P, Stagno F, Stella S, Cupri A, Forte S, Massimino M, Antolino A, Siragusa S, Mannina D, Impera SS, Musolino C, Malato A, Mineo G, Tomaselli C, Murgano P, Musso M, Morabito F, Molica S, Martino B, Manzella L, Muller MC, Hochhaus A and Raimondo FD: High bcr-abl/gus(is) levels at diagnosis of chronic phase cml are associated with unfavorable responses to standard-dose imatinib. Clin Cancer Res *23(23)*: 7189-7198, 2017.
15 Thomas DA, O'Brien S, Cortes J, Giles FJ, Faderl S, Verstovsek S, Ferrajoli A, Koller C, Beran M, Pierce S, Ha CS, Cabanillas F, Keating MJ and Kantarjian H: Outcome with the hyper-cvad regimens in lymphoblastic lymphoma. Blood *104(6)*: 1624-1630, 2004.
16 Redaelli S, Mologni L, Rostagno R, Piazza R, Magistroni V, Ceccon M, Viltadi M, Flynn D and Gambacorti-Passerini C: Three novel patient-derived bcr/abl mutants show different sensitivity to second and third generation tyrosine kinase inhibitors. Am J Hematol *87(11)*: E125-128, 2012.
17 Patel AB, O'Hare T and Deininger MW: Mechanisms of resistance to abl kinase inhibition in chronic myeloid leukemia and the development of next generation abl kinase inhibitors. Hematol Oncol Clin North Am *31(4)*: 589-612, 2017.
18 Pirosa MC, Leotta S, Cupri A, Stella S, Martino EA, Scalise L, Sapienza G, Calafiore V, Mauro E, Spadaro A, Vigneri P, Di Raimondo F and Milone G: Long-term molecular remission achieved by antibody anti-cd22 and ponatinib in a patient affected by ph'+ acute lymphoblastic leukemia relapsed after second allogeneic hematopoietic stem cell transplantation: A case report. Chemotherapy *63(4)*: 220-224, 2018.
19 Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, Baccarani M, Cortes J, Cross NC, Druker BJ, Gabert J, Grimwade D, Hehlmann R, Kamel-Reid S, Lipton JH, Longtine J, Martinelli G, Saglio G, Soverini S, Stock W and Goldman JM: Monitoring cml patients responding to treatment with tyrosine kinase inhibitors: Review and recommendations for harmonizing current methodology for detecting bcr-abl transcripts and kinase domain mutations and for expressing results. Blood *108(1)*: 28-37, 2006.
20 Stagno F, Vigneri P, Del Fabro V, Stella S, Cupri A, Massimino M, Consoli C, Tambe L, Consoli ML, Antolino A and Di Raimondo F: Influence of complex variant chromosomal translocations in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. Acta Oncol *49(4)*: 506-508, 2010.
21 Stagno F, Vigneri P, Del Fabro V, Stella S, Massimino M, Berretta S, Cupri A, Consoli C, Messina L, Tirro E, Messina A and Di Raimondo F: Successful nilotinib therapy in an imatinib-resistant chronic myeloid leukemia patient displaying an intron-derived insertion/truncation mutation in the bcr-abl kinase domain. Leuk Res *33(9)*: e157-158, 2009.
22 Soverini S, De Benedittis C, Polakova KM, Linhartova J, Castagnetti F, Gugliotta G, Papayannidis C, Mancini M, Klamova H, Salvucci M, Crugnola M, Iurlo A, Albano F, Russo D, Rosti G, Cavo M, Baccarani M and Martinelli G: Next-generation sequencing for sensitive detection of bcr-abl1 mutations relevant to tyrosine kinase inhibitor choice in imatinib-resistant patients. Oncotarget *7(16)*: 21982-21990, 2016.
23 Tan Y, Chen X, Qi X, Li G, Wang J, Bian S, Xu Z and Wang H: Acute lymphoblastic leukemia expressing b3a2 (p210), e1a2 (p190), and variant e1a2 bcr-abl transcripts: A case report and review of the literature. Acta Haematol *128(2)*: 119-123, 2012.
24 Shin SY, Cho JH, Kim HJ, Jang JH, Lee ST and Kim SH: Two cases of acute lymphoblastic leukemia with an e1a3 bcr-abl1 fusion transcript. Ann Lab Med *35(1)*: 159-161, 2015.
25 Agirre X, Roman-Gomez J, Vazquez I, Jimenez-Velasco A, Larrayoz MJ, Lahortiga I, Andreu EJ, Marquez J, Beltran de Heredia JM, Odero MD, Prosper F and Calasanz MJ: Coexistence of different clonal populations harboring the b3a2 (p210) and e1a2 (p190) bcr-abl1 fusion transcripts in chronic myelogenous leukemia resistant to imatinib. Cancer Genet Cytogenet *160(1)*: 22-26, 2005.
26 Arana-Trejo RM, Ruiz Sanchez E, Ignacio-Ibarra G, Baez de la Fuente E, Garces O, Gomez Morales E, Castro Granados M, Ovilla Martinez R, Rubio-Borja ME, Solis Anaya L, Herrera P, Delgado Llamas J and Kofman S: Bcr/abl p210, p190 and p230 fusion genes in 250 mexican patients with chronic myeloid leukaemia (cml). Clin Lab Haematol *24(3)*: 145-150, 2002.
27 Watanabe K, Minami Y, Ozawa Y, Miyamura K and Naoe T: T315i mutation in ph-positive acute lymphoblastic leukemia is associated with a highly aggressive disease phenotype: Three case reports. Anticancer Res *32(5)*: 1779-1783, 2012.
28 Liu YC, Hsiao HH, Chang CS, Liu TC, Yang WC, Hsu JF, Huang CT, Cho SF, Wu CH, Tsai YF and Lin SF: Outcome of allotransplants in patients with chronic-phase chronic myeloid leukemia following imatinib failure: Prognosis revisited. Anticancer Res *33(10)*: 4663-4667, 2013.

Received November 9, 2018
Revised November 18, 2018
Accepted November 19, 2018