INTRODUCTION

In over 95% of cases, the first step in the pathogenesis of chronic myeloid leukemia (CML) is the formation of the Philadelphia (Ph) chromosome. This is due to reciprocal translocation between chromosomes 9 and 22, designated t(9;22)(q34;q11), which is associated with de novo formation of the BCR-ABL fusion oncogene and oncoprotein with increased tyrosine kinase activity. The 5' region of BCR-ABL oncogene consists of exons derived from the BCR gene, and the 3' part contains sequences of the ABL gene. The breakpoint in the ABL gene is relatively constant and occurs in exon 2 (a2); however, one known exception to the ABL gene breakpoint location is exon 3 (a3) (ref.7). In most CML patients, the breakpoint on the BCR gene occurs in the major breakpoint cluster region (M-BCR), leading to b2a2 (e13a2) or b3a2 (e14a2) fusion transcripts that are translated into the p210 kDa Bcr-Abl protein. Breakpoints outside M-BCR are extremely rare in CML. In contrast, the m-BCR (minor) breakpoint, producing the e1a2 fusion transcript and a p190 kDa fusion protein, is reported in patients with Ph-positive acute lymphoid leukemia. Other atypical BCR-ABL transcripts with fusions, such as e8a2 or e6a2, are very rare and have only
been occasionally described. The \textit{BCR-ABL} transcript e6a2 has been reported in CML cases with aggressive clinical courses and in acute basophilic leukemia.

\section*{MATERIAL AND METHODS, RESULTS

\subsection*{Patient's characteristics}

We describe a 51-year-old male patient previously in good health who presented in January 2010 with marked splenomegaly (12 cm under costal margin), a history of abdominal discomfort lasting 3 weeks, weight loss and night sweats. His peripheral blood showed a hemoglobin concentration of 73 g/l and a platelet count of 36x10^9/l. His white blood cell (WBC) count was 150x10^9/l and included 2% lymphocytes, 4% metamyelocytes, 5% myelocytes, 6% promyelocytes and 2% myeloblasts. The patient’s severe anemia and thrombocytopenia did not cause any clinical complications. The morphology of his bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1). Standard cytogenetics which examined the bone marrow aspirate corresponded to the findings in the peripheral blood (Fig. 1).

\subsection*{Molecular analysis}

The initial \textit{BCR-ABL} analysis was carried out on peripheral blood leukocytes as previously described. Nested reverse transcriptase-polymerase chain reaction (RT-PCR) for e1a2 (minor \textit{BCR-ABL}) and e13a2 or e14a2 (major \textit{BCR-ABL}) fusion transcripts yielded negative results. Because iFISH for \textit{BCR-ABL} (ref.13) was positive, another RT-PCR for a rare e6a2 \textit{BCR-ABL} fusion transcript was performed (forward primer: 5'-GACCTACCTGAGCCACCTGGA G-3', reverse primer: 5'-GTTTGGGCTTACGACCCTCCC-3'). The amplified cDNA product was sequenced with both the forward and reverse primers used for RT-PCR. Sequencing analysis confirmed a rare e6a2 \textit{BCR-ABL} fusion transcript (Fig. 2).

Western blot analysis showed high phosphorylation (activation) of the Crkl and the Src family of kinases (P-SFK). An \textit{in vitro} test of the patients’ leukemic cell sensitivity to TKI, after incubating with or without 10 μM imatinib, demonstrated sensitivity of Bcr-Ab1 tyrosine kinase to imatinib. This was assessed by a decrease in phosphorylated Crkl and the disappearance of P-SFK, suggesting that the P-Src reflects only the Bcr-Abl-dependent Src activity (Fig. 3).

\subsection*{Treatment}

The initial cytoreduction was performed with a daily dosage of 6 g of hydroxyurea that was administered with standard therapy to prevent the tumour lysis syndrome. After 7 days of cytoreduction, imatinib therapy was initiated at a reduced daily dosage of 400 mg due to the patient’s severe thrombocytopenia. The imatinib-induced neutropenia that occurred 2 weeks after the initiation of treatment with imatinib led to the introduction of therapy with G-CSF and anti-infective prophylaxis. One transfusion unit of platelets was administered because of cutaneous petechiae. The imatinib therapy was limited to 5 days per week. While drug therapy was ongoing, a search for an unrelated hematopoietic stem cell donor was initiated, as no suitable donor was identified in the patient’s family. After 3 months of treatment with imatinib, the patient did not achieve any hematologic response, as there was a persistence of basophilia in the peripheral blood and bone marrow. Nor did the patient achieve a cytogenetic response, as the Ph chromosome was found in all 12 metaphases analyzed. After the identification of a suitable donor, the patient was allografted with peripheral stem cells from an HLA-identical male donor after a conditioning regimen of Flu+Bu12+ATG (fludarabine, busulphan 12mg/ kg, antithymocytic globuline) in June 2010. Graft-versus-host disease (GVHD) prevention was achieved with the cyclosporine A therapy. The posttransplantation course was complicated by grade 1 mucositis, grade 1 renal insufficiency and a fever of unknown origin. The patient showed 90% donor chimerism in the peripheral blood on day +15 and was engrafted again on day +18 after transplantation. Further chimerism analysis showed 96% donor hematopoiesis in the marrow on day +28. A check of the bone marrow on day +50 showed hypocellularity with a decreased basophil count of 1% and regeneration changes. Standard cytogenetic analysis was not available.\section*{Fig. 1. Hypercellular bone marrow aspirate with predominance of granulopoiesis at the time of diagnosis. The number of myeloblasts was not elevated. There was an increased population of basophils, including their immature forms. The number of eosinophils was increased as well.
Identification of E6A2 BCR-ABL fusion in a philadelphia-positive CML with marked Basophilia: implications for treatment strategy.

Fig. 2. Sequencing analysis confirmed a rare e6a2 BCR-ABL fusion transcript.

Fig. 3. High phosphorylation of SFK and Crkl as detected by Western analysis in the patient’s leukemic cells at the time of diagnosis. Without the addition of imatinib (0) both phosphorylated proteins (P-SFK and P-Crkl) were detected. Incubation with imatinib (IM; 10 μM) in vitro inhibited the phosphorylation of Crkl and SFK. β-actin was used as a gel loading control.

due to cultivation failure. However, the e6a2 BCR-ABL fusion gene was present in the second round of nested RT-PCR. Clinically, there was no evidence of GVHD, and the dose of cyclosporine A was reduced. However, on day +70, the patient experienced pancytopenia. The bone marrow examination showed only 47% donor chimera, suggesting that a graft failure had occurred. Despite the withdrawal of cyclosporine A and growth factors, an attempted rescue of the graft failed, possibly due to Epstein-Barr virus (EBV) reactivation and gradually increasing number of EBV DNA copies in plasma. Anti-CD20 monoclonal antibody (rituximab) was administered to prevent the development of EBV-associated lymphoproliferative disease in a severely immunocompromised host. Only one administration of rituximab led to a complete resolution of EBV DNA in the recipient plasma. Nevertheless, the patient required a second transplant from another donor. After identification of a suitable alternative donor, the patient was allografted with peripheral stem cells from HLA-identical female donor after a conditioning regimen of Flu+CTX+LD-TBI (fludarabine, cyclophosphamide, low dose total body irradiation) in September 2010. Prevention of GVHD was achieved again with cyclosporine A. The patient was engrafted again on day +15 and showed 100% donor chimerism. There was no evidence of the presence of the e6a2 BCR-ABL fusion transcript when testing the bone marrow using nested RT-PCR on day +30 after the second transplant.

DISCUSSION AND CONCLUSION

The presence of atypical, shorter BCR-ABL transcripts in CML is associated with an aggressive clinical course5-8,16,17. It has been suggested that the poor clinical outcome associated with the e6a2 Bcr-Abl protein could be the result of increased kinase activity due to the partial loss of the guanine exchange factor/dbl-like domain6. This domain, also completely absent in the p190 Bcr-Abl protein, mediates the interaction with several Ras-like G-proteins involved in cell proliferation, signal transduction, and cytoskeletal organization18,19. The patient described here adds to our knowledge of e6a2-associated CML. He presented with marked splenomegaly, basophilia, severe thrombocytopenia, anemia and a high phosphorylation of SFK downstream of Bcr-Abl. In agreement with other authors, we believe that patients with the rare e6a2 BCR-ABL transcript variant encoding Bcr-Abl kinase, possibly with enhanced oncogenic potential, cannot be treated with standard imatinib therapy. Instead, they should receive a stem cell transplant right away after a short course of treatment with imatinib or with a dual Src/Abl kinase inhibitor or, they should be registered in clinical trials with experimental agents.
ACKNOWLEDGEMENTS

This research was supported by the Ministry of Health of the Czech Republic (grant NS 9949-3), by student project LF_2011_006 of Palacky University, by Bristol-Myers Squibb Research Grant (to V.D.) and by the grant MSM 6198959205 of the Ministry of Education, Youth and Sports of the Czech Republic. We would like to thank the personnel at the Laboratory of Cytogenetics and at the Laboratory of Molecular Genetics at the Department of Hemato-Oncology for their expert technical assistance.

REFERENCES