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CASE REPORT

Coexistence of inversion 16 in chronic myeloid leukaemia in blast crisis

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Abstract Chronic myeloid leukaemia (CML) is consistently associated with the *BCR-ABL*1 fusion gene located on the derivative chromosome 22 (Philadelphia chromosome, Ph + ve) as a result of a t(9;22)(q34;q11.2) translocation. We describe a 36-year-old male in blast phase CML presenting with acute myelomonocytic leukaemia and eosinophilia. Five months after initial disease diagnosis and despite being treated with standard imatinib therapy (400 mg daily) conventional cytogenetic analysis (CCA) demonstrated the evolution of a chromosome 16 pericentric inversion (inv(16)(p13q22)) within the already Ph + ve cells. Bone marrow aspiration and biopsy revealed 60% blasts, positive for CD34, HLADR, CD33 and CD13. Fluorescence in situ hybridization (FISH) and reverse transcription–polymerase chain reaction (RT-

PCR) confirmed presence of a *CBFβ-MHY*11 rearrangement whilst next generation sequencing (NGS) detected an E255K point mutation within the *BCR-ABL*1 tyrosine kinase domain. Neither the *CBFβ-MHY*11 rearrangement nor the E225K point mutation were present at diagnosis. The patient received a combination of daunorubicin (60 mg/m^2) and cytarabine (100 mg/m^2) and had his tyrosine kinase inhibitor (TKI) changed to nilotinib before undergoing a male unrelated donor (MUD) stem cell transplant (SCT). The t(9;22) and inv(16) rearrangements persisted at day 100 post MUD SCT and his TKI was further switched to dasatinib. The patient presented with pleural effusions and had subsequent CNS relapse and passed away from relapsed disease and infective complications, 19-month post MUD allograft.

Keywords Chronic myeloid leukaemia · Blast phase · Inv(16)(p13q22)/CBFβ-MHY11 · Next generation sequencing · Conventional cytogenetic analysis · Imatinib · Nilotinib · Dasatinib

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Introduction

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative neoplasm characterized by the presence of the t(9;22)(q34;q11.2) also known as the Philadelphia (Ph) chromosome which results in the formation of the *BCR-ABL1* fusion gene. *BCR-ABL*1 protein has constitutive tyrosine kinase activity and plays a pivotal role in the pathogenesis of CML [1]. At diagnosis, 90–95% of cases of CML have the typical t(9;22) rearrangement while the remaining cases either have variant translocations involving a third or fourth chromosome (3- or 4-way reciprocal translocation variants), or have a cryptic translocation of 9q34 (ABL) and 22q11.2 (BCR) that can only be detected by fluorescence in situ

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hybridization (FISH), and reverse transcription–polymerase chain reaction (RT-PCR) [2].

Tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL1 oncoprotein are the standard therapy for patients with CML. However, it has been estimated that 20–40% of patients receiving first-line imatinib therapy will eventually require an alternative treatment because of imatinib intolerance or resistance [3]. The best characterized mechanism of resistance is point mutations within the *BCR-ABL1* kinase domain that impair or prevent TKI binding [4].

Depending on the clinical setting (primary or secondary resistance and chronic or advanced phase), kinase domain mutations have been detected in 30–90% of patients with imatinib resistance and in 20–80% of patients who failed to respond to nilotinib or dasatinib therapy [4].

The natural history of untreated CML is usually bi- or triphasic: an initial indolent chronic phase (CML-CP) characterised by a high white blood cell (WBC) count and a hypercellular bone marrow composed of maturing granulocytes is followed by an ill-defined accelerated phase (CML-AP), a terminal blast phase (CML-BC) or both. Both CML-AP and CML-BC are associated with disease progression, resistance to therapy and poor clinical outcome [5]. Transition to CML-BC can be accompanied by cytogenetic clonal evolution of additional acquired chromosome abnormalities. The most commonly observed acquired abnormalities include an extra copy of the Philadelphia chromosome, trisomy 8, isochromosome17q, or trisomy 19 [6]. However, in rare cases, patients have chromosomal translocations characteristic of certain types
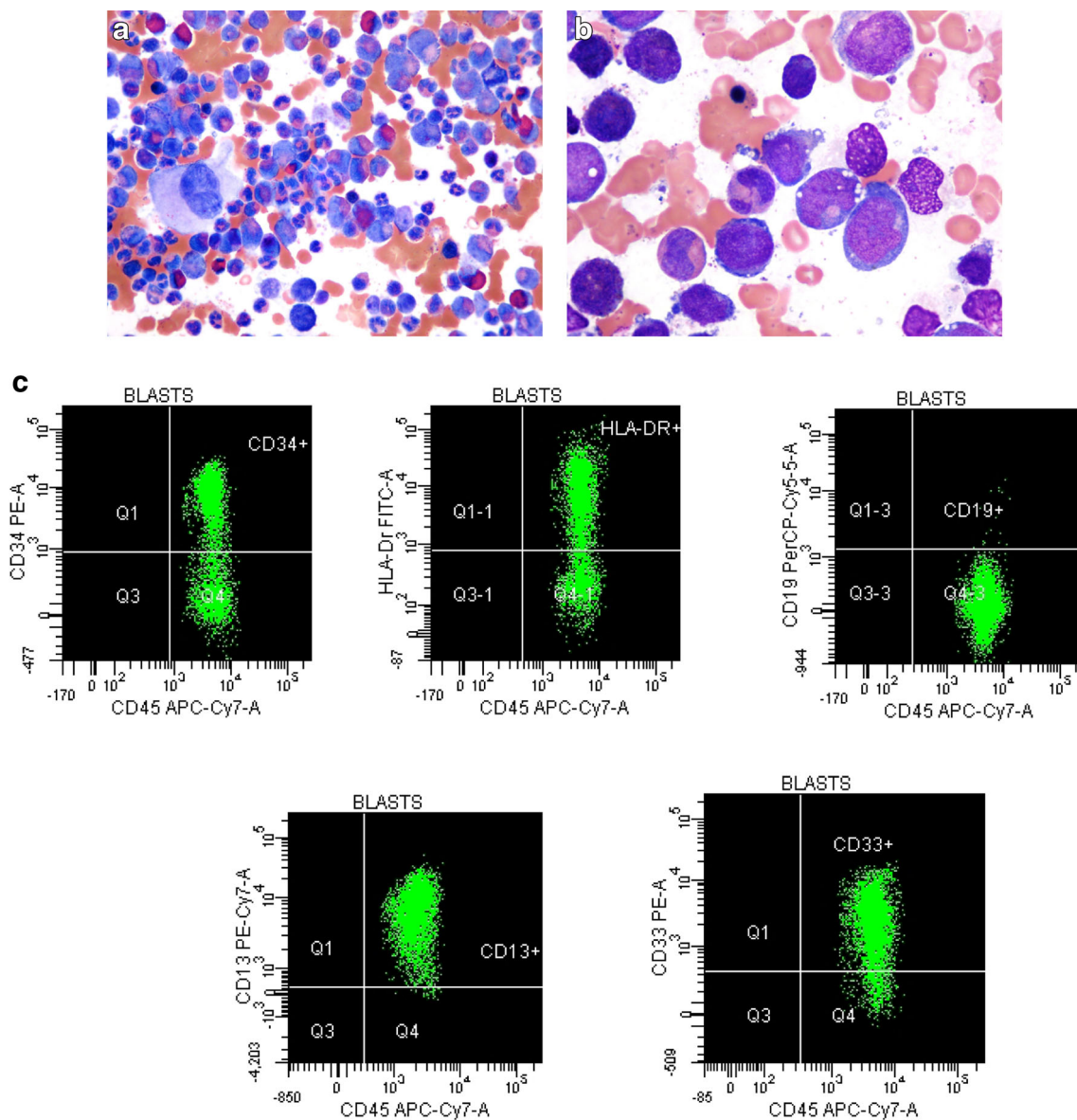
Fig. 1 **a** BM at diagnosis. **b** BM illustrating CML in blast crisis showing hypercellular bone marrow with 60% myeloid blasts and 5% eosinophils. **c** PB flow cytometry showing immunophenotyping data

of de novo acute myeloid leukaemia (AML) such as the t(8;21)(q21;q21), t(15;17)(q24;q21) and the inv(16)(p13q22)/t(16;16)(p13;q22) have been described in CML-BC [7–9].

AML with inv(16) has been defined as a distinctive morphological subtype by French-American-British classification and is designated M4Eo [10]. The World Health Organization classification has since included in AML with recurrent cytogenetic abnormalities AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11* in which there is characteristic abnormal eosinophil morphology [11]. The core-binding factor β (*CBF*β) gene at

16q22 and the myosin heavy chain gene (*MYH11*) at 16p13 are both disrupted and recombined to create a novel fusion gene, *CBF*β-*MYH11* [12]. This fusion gene can be detected by conventional cytogenetics, FISH and RT-PCR. Unlike de novo AML, inv(16)(p13q22) in CML-BC is not associated with a favourable prognosis [13].

We describe a CML patient in BC at which time the blasts had morphologic features of myelomonocytic leukaemia with excess eosinophils. Conventional cytogenetics showed inv(16)(p13q22) and FISH and RT-PCR proved the presence of *CBF*β-*MHY*11 which was absent at diagnosis.

Fig. 2 Deep sequencing of the *BCR-ABL*1 kinase domain—screenshots showing a portion of the global alignment of sequence reads to the reference *ABL1* sequence obtained with AVA software (Roche Applied Science) around codons 253–255. *Upper panel*: sample at diagnosis, no evidence of nucleotide substitutions were detected (sequencing depth, 3432 reads as shown on the right *Y* axis). *Lower panel*: relapse sample showing an E255K (88.32%; *green bar*) and a Y253F (3.28%; *red bar*) kinase domain mutation

Case report

A 36-year-old male patient presented with a 3-month history of weight loss, drenching night sweats and left upper quadrant pain. On examination he was noted to have splenomegaly with haemoglobin 77 g/L; white cell count (WCC) 366 × 10⁹/L and a platelet count of 988 × 10⁹/L.

Bone marrow (BM) biopsy revealed a hypercellular marrow with myeloid hyperplasia, increased megakaryocytes and suppression of erythropoiesis but no excess blasts (Fig. 1). RT-PCR on PB was positive for the p210 $BCR\text{-}ABL$1 transcript. BM chromosome analysis revealed a male 46,XYt(9;22)(q34;q11) karyotype in all ten metaphases examined. FISH analysis demonstrated a typical $BCR\text{-}ABL$1 rearrangement in 99.2% of the 250 BM nuclei examined.

A diagnosis of CML in chronic phase was established and he was treated with standard dose imatinib (400 mg daily) with normalization of counts. Five months later, his WCC was 115 × 10⁹/L. His blood film revealed leucoerythroblastic changes with a large number of circulating blasts. Upon admission to hospital, he deteriorated quite rapidly, developed pneumonia, type I respiratory failure and sustained a splenic infarct. He was admitted to intensive care for stabilisation and underwent embolisation of a splenic artery pseudoaneurysm.

BM biopsy and aspirate confirmed myeloid blast crisis with a hypercellular aspirate containing approximately 60% blasts and 5% eosinophils (Fig. 2). Flow cytometry detected a population of myeloblasts (60%), positive for CD34, HLADR, CD33 and CD13. BM chromosome analysis showed a male karyotype with the t(9;22) Philadelphia

rearrangement previously detected at diagnosis and a pericentric inversion of chromosome 16 (p13p22) (inv(16)) in all ten metaphases examined. FISH/RT-PCR analysis confirmed a CBFβ-MHY11 rearrangement. Next generation sequencing (NGS) [14] detected an E255K point mutation within the $BCR\text{-}ABL$1 tyrosine kinase domain. Neither the CBFβ-MHY11 rearrangement nor the E225K point mutation were present at disease diagnosis.

Standard AML induction chemotherapy DA3 +10 (daunorubicin 60 mg/m² and cytarabine 100 mg/m²) was started and his TKI therapy was switched to nilotinib (400 mg bd daily). He was also tissue-typed and referred for a matched-unrelated bone marrow transplant as he had no sibling donors.

He recovered his blood counts suggesting transient remission however, for 7 weeks post-DA3 +10 induction chemotherapy; he relapsed again with blast crisis. He received salvage FLAG chemotherapy (fludarabine, cytarabine and idarubicin). A post FLAG chemotherapy BM biopsy revealed morphological remission chromosome analysis still showed persistence of the t(9;22) and inv(16) rearrangements 46,XY,t(9;22)(q34;q11),inv(16)(p13q22) with ten cells having both t(9;22) and inv(16) rearrangements, therefore he was not in remission.

Pre-SCT cytogenetic analysis done immediately on recovery of counts from induction showed the diagnostic t(9;22) rearrangement in 83.3% (25 cells) of the 30 metaphases examined. Three (12%) of these Ph + ve cells also showed the inv(16) rearrangement 46,XY,t(9;22)(q34;q11, 22]/46idem,inv(16)(p13q22, 3]/46,XY{5]. The marrow was relatively cellular with 2% blasts, 9% myelocytes,

Table 1 Summary of reported cases of inv(16) in CML in blast crisis

Reference	Age/gender	Time to BC	Treatment	Survival
Wu et al. 2006 [7]	33, M	NA	Allo-BMT	Died 2 failed allo-BMT
Wu et al. 2006 [7]	41, M	3	Allo-BMT	Alive 4 years post allo-BMT
Wu et al. 2006 [7]	62, F	4	Imatinib	Died 24 months
Merzianu et al. 2005 [8]	43, F	11	Imatinib	Died 3 months
Merzianu et al. 2005 [8]	61, F	0	Hydroxyurea	Died 7 months
Merzianu et al. 2005 [8]	47, M	8	Interferon	Died 1 months
Merzianu et al. 2005 [8]	36, F	7	Hydroxyurea	Died 1 months
Patel et al. 2006 [13]	64, M	6	Allo-SCT	Died 16 months
Patel et al. 2006 [13]	56, M	0	Hydroxyurea	Died 3 months
Tsuboi et al. 2002 [15]	44, M	84	Hydroxyurea	Died 3 months
Myint et al. 1997 [16]	29, M	3	Hydroxyurea	Died 0 months
Evers et al. 1992 [17]	39, M	3	Hydroxyurea	Died 10 months
Asou et al. 1992 [18]	51, M	1	Hydroxyurea	Died 3 months
Enright et al. 1992 [19]	78, M	4	Hydroxyurea	Died 3 months
Heim et al. 1992 [20]	21, M	2	Busulfan	Alive 48 months
Secker-Walker et al. [21]	25, M	0	Allo-BMT	Died 15 months
Ninomiya et al. 2011 [22]	63, F	0	Imatinib	Died 7 months

M male, F female, BC blast crisis, NA not available, BMT bone marrow transplantation, $Allo\text{-}SCT$ allogeneic stem cell transplantation

13% promyelocytes but no eosinophils or basophils and consistent with chronic phase CML. He proceeded directly to a myeloablative MUD SCT, with cyclophosphamide and total body irradiation (TBI) conditioning. He engrafted by day 21. Post-transplant he suffered from severe mucositis. He also required foscarnet for cytomegalovirus (CMV) reactivation. He developed Grade I cutaneous graft versus host disease. The treatment plan was to restart his nilotinib therapy post SCT but this was delayed due to deranged liver function tests (LFT).

On day 100 post SCT he relapsed again, the t(9;22) and inv(16) rearrangements detected at CML-BC were again present. His tyrosine kinase inhibitor was switched from nilotinib to dasatinib (100/150 mg alternate days). He was treated with mitoxantrone to control his white cell count and then received two donor lymphocyte infusions (DLI) which has been successful in achieving a fourth haematological remission. A bone marrow aspirate at this stage revealed a hypocellular aspirate with no excess of blasts. Cytogenetics confirmed a complete remission with normal cytogenetics, karyotype 46,XY.

The patient presented with headaches and MRI and lumbar puncture confirmed CNS relapse of disease. The CNS relapse consisted of 37% blasts and 1% eosinophils. He received intrathecal chemotherapy (cytarabine, hydrocortisone and methotrexate), but required cranial irradiation to eradicate disease. BM and BCR-ABL/ABL testing confirmed ongoing morphological and molecular remission, respectively. Dasatinib TKI was increased to 100 mg/150 mg alternate days. During outpatient follow-up, low molecular evidence (BCR-ABL/ABL 0.08%) of relapse on two consecutive tests were noted. Three months later, the patient, presented as an emergency with shortness of breath, was found to have not only pleural effusions, but haematological evidence of disease relapse in blast crisis with mainly blasts in his blood. He had subsequent CNS relapse, and passed away shortly after this from infective complications (*Staphylococcus aureus* bacteraemia) 19 months post MUD allograft.

Discussion

The cellular mechanism which drives the transition of CML-CP to CML-BC is currently unknown. However, it is well recognized that during this transition common additional abnormalities in CML-BC lead to a gain or loss of genetic material such as extra copies of Ph, trisomy 8, isochromosome17q, or trisomy 19 inferring these additional hits are in part responsible for the transition [6]. The coexistence of inv(16) and t(9;22) is rare with a limited number of reports in the literature over the past 25 years (Table 1). These cases have both been described

in blast crisis of CML and also de novo AML. Most of the de novo forms of AML with the t(9;22) and inv(16) have a favourable prognosis. CML-BC with these abnormalities are typically characterized by an aggressive clinical course with rapid disease progression and resistance to treatment [7, 8, 13]. In common with this case, most have shown a sudden onset with only several months from first presentation to CML-BC (Table 1).

It is well recognized that the presence of *BCR-ABL1* kinase domain point mutations are suggestive of genetic instability and of increased risk of progression and are detectable in up to 50% of patients with treatment failure and progression [4]. In our case, imatinib was only effective for 5 months for the control of chronic phase before transformation. At BC, we detected the presence of *CBFβ-MHY*11 and E255K point mutation within the *BCR-ABL*1 tyrosine kinase domain. To investigate, this further we used NGS (22) comparing diagnostic and BC samples. Of interest, the BC sample consisted of an E255K clone representing 90.5% and a smaller clone consisting of Y253F (3.69%) which would be below the threshold of conventional Sanger sequencing. However, the diagnostic sample did not contain either mutated clone at a sensitivity of 1% over 4000 reads. This sample also did not contain the *CBFβ-MHY11* transcript.

The E255K mutation occurs within the P-loop of the kinase domain and is a common feature in imatinib resistance cases and is well recognized to have increased oncogenic potency despite reduced kinase activity compared with unmutated BCR-ABL.

Our patient survived 23 months from his presentation in BC. This is at the longer end of the survival spectrum in the case series but despite bone marrow transplant, DLIs and TKIs he did not achieve anymore prolonged survival.

In summary, although the presence of inv(16) is a rare occurrence in CML-BC, this is still associated with a poor prognosis even in the TKI era. The vast majority of patients have succumbed to their disease and highlights the need for further treatment options in this group of patients and the value of continued monitoring for disease progression.

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