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Case Report

Clonal Expansion of Co-Existing Ph-Negative Unrelated Cells in Ph-Positive CML during Imatinib Mesylate Therapy

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Abstract

Chronic myelogenous leukemia (CML) is characterized by the translocation, t (9;22) (q34;q11.2) which gives rise to the *BCR-ABL1* fusion gene. Imatinib mesylate (IM), a tyrosine kinase inhibitor against BCR-ABL1 tyrosine kinase is standard therapy for CML in chronic phase. In the present study, a CML case in chronic phase is reported with evolving Ph-negative clones co-existing with Ph-positive cells during IM treatment. Identification of cytogenetic polyclonality has important implications for monitoring patient underlying tyrosine kinase therapy (TKI). The data revealed importance of periodic cytogenetic studies as part of routine patient management for CML patients, in addition to molecular monitoring.

ABBREVIATIONS

CML: Chronic Myelogeneous Leukemia; ET: Essential Thrombocytopenia; IM: Imatinib Mesylate; MDS: Myelodysplasia; TKI: Tyrosine Kinase.

INTRODUCTION

Chronic myelogenous leukemia (CML) is characterized by t (9;22)(q34;q11.2) translocation resulting in *BCR-ABL1* fusion gene. Imatinib mesylate (IM) is a potent tyrosine kinase inhibitor (TKI) against BCR-ABL1 tyrosine kinase and has become frontline therapy for patients with CML in chronic phase. It showed remarkable efficacy and high rates of cytogenetic response in the treatment of chronic phase CML [1]. However, in spite of being highly effective, IM failure is observed in a substantial proportion of CML patient. Co-existence of Philadelphia-negative and Philadelphia-positive metaphases has been reported in myeloproliferative neoplasms (MPN) [1]. Philadelphia-negative clones have been observed in CML following successful treatment or partial cytogenetic response with IM therapy, or can be associated with myelodysplasia [2-3]. The most common chromosomal aberrations observed in Ph-negative clones are trisomy 8 (34%) and monosomy 7 (20%), [4] while other chromosomal changes were seen at lower frequency. The clinical significance of these abnormal clones is not entirely certain but reports have been published of patients carrying these abnormalities developing myelodysplasia (MDS) [5] or acute leukemia [6].

CASE PRESENTATION

We report a 63-year old male, initially diagnosed with essential thrombocytopenia (ET) in 2006 with multiple medical problems. Previous cytogenetic and molecular results were not available. He was referred to our department to rule out acute leukemia in August 2009. This diagnosis was later revised, to chronic myeloid leukemia (CML) in chronic phase where karyotyping revealed t (9;22)(q34;q11.2) as the sole abnormality. The *e13a2 BCR-ABL1* fusion transcript was detected by real-time PCR (RT-PCR). *JAK2*V617F was not detected. He was given IM, at 200mg/day in September 2009, later increased to 300mg/day in October 2009. A minimal cytogenetic response (83.4%, 26/31) was observed in the marrow at six month, in March 2010, two Ph-negative, unrelated cells were observed: monosomy 7 (4/31 metaphases) and trisomy 8 (1/31 metaphases). The patient developed cytopenias thought to be due to IM. Bone marrow examination revealed hypocellularity, with no evidence of MDS, CML in acceleration or myelofibrosis. Nevertheless, IM dosage was increased to 400mg/day in May 2010. The patient continued to experience cytopenias. He had co-existing diabetes mellitus, coronary artery disease, previous cerebral infarction and chronic kindney diseases (CKD), with a creatinine clearance of 21 mL/minutes, and was given subcutaneous erythropoietin

4000 units twice weekly for anemia. In May 2011, his marrow was still hypocellular, with no dysplasia. Peripheral blood (PB) karyotyping showed 5.9% Ph-positive metaphase (1/17 cells) and Ph-negative clones (3/17 cells) with persistence of trisomy 8. Peripheral blood at this time point showed *BCR-ABL1/ABL1* ratio (0.4752% (IS)).

In May 2012, his marrow remained hypocellular with no apparent MDS, but his karyotype showed expansion of Ph-negative, with both monosomy 7 and loss of Y chromosome (20/49 metaphases), as well as persistence of the Ph-negative clone with trisomy 8 (7/49 metaphases). Along with Ph-negative clones the number of Ph-positive clones also increased (19/49 metaphases). The *BCR-ABL1/ABL1* ratio increased (4.0660% (IS)) (Table 1). After 33 months of therapy, he was diagnosed with IM failure. He had never attained a hematological response. In June 2012, IM was discontinued, leading to partial recovery of his pancytopenia a month later. Mutation analysis did not demonstrate a tyrosine kinase inhibitor (TKI) domain mutation. Second generation of TKI therapy was considered not feasible with the evolution of abnormal cytogenetics in the Ph-negative cells. Allogeneic stem cell transplantation was not feasible in view of multiple co-morbidities. He was treated with Thalidomide 100mg/day and hydroxyurea 500mg/day to control cell counts. Supportive care management was planned as he was not considered able chemotherapy. In April 2013, patient remained in CML in chronic phase. Complete blood count demonstrated hemoglobin 2.93g/dL, platelet count 369 x10^3 /µL, total white blood count (WBC) 5.95 x 10^3/µL. There was no hepatosplenomegaly. Clinically he remained well during follow-up for 27 months of treatment until 5th August 2015; circulating blasts (24%) were detected in blood consistent with transformation to acute leukemia. His WBC was 9.55 x 10^3/µL and rose to 36.50 x 10^3/µL in two weeks. Palliative care was offered.

DISCUSSION

Our case illustrates a CML patient with evolving Ph-negative clones co-existing with Ph-positive cells during IM treatment. Cases of Ph-negative clones have been reported in the literature with an incidence of 2-17% in patients on IM [6]. The detection of Ph-negative clones, simultaneously growing with Ph-positive cells in our case, may support the hypothesis of two-step pathogenesis of CML in some patients, where Ph-negative clones may represent an existing pre-leukemic stage [7]. These may be the first step in leukemogenesis, providing fertile ground for the subsequent development of the Ph-translocation. The initial loss or failure of detection of these pre-existing Ph-negative clones may be the result from the growth advantage of Ph-positive cells [7]. The selective suppression of Ph-positive cells by TKI therapy may then confer growth advantage to these pre-existing clones. Alternatively, this may be the result of a growth advantage of cytogenetically abnormal polyclonal hematopoiesis as a result of IM therapy [8].

Our patient acquired chromosomal abnormalities in Ph-negative typical of treatment-related MDS, including monosomy 7, trisomy 8 and loss of Y, often associated with an unfavorable prognosis [2,4,8]. The chromosomal abnormalities in Ph-negative cells may be signatures of genetic instability caused by TKI therapy [5] and may therefore demonstrate poor clinical prognosis. Alternatively acquisition of Ph-negative cells could be secondary TKI therapy-induced suppression of Ph-positive cells [7]. It is less likely that patient has developed *de novo* leukemic or myelodysplastic disease, but cannot be excluded. Although some patients with Ph-negative clonal evolution after TKI therapy have been reported as exhibiting dysplastic features, most of them showed clonal evolution to clinical MDS after years of therapy.

In a case reported by Chee *et al*., [9] one patient developed MDS associated with Ph-negative clonal evolution after during maintenance IM monotherapy following allogeneic transplantation, and progressed rapidly to a fatal acute myeloid leukemia. The question arises whether the emergence of Ph-negative clones might predict the development of a new pathogenic complication. Conversely clonal evolution may represent the unmasking of an underlying de novo MDS, or treatment-related MDS; or alternatively, the clonal evolution may simply be a transient phenomenon that has not been elucidated. Cytogenetic polyclonality in CML treated with IM has importance implications for monitoring of TKI treatment. This observation suggests benefit in regular monitoring by conventional cytogenetic, at diagnosis and during the course of the disease, even if the patient achieves complete cytogenetic response. This monitoring would aim to accumulate information to determine the frequency of abnormal Ph-negative clones, and to correlate these cytogenetic findings with morphologic features, prognosis, and treatment choices, during the long-term follow-up in patients with CML. This would be informative for clinical management, particularly with regard to decisions on allogeneic hematologic stem cell transplantation. This case was also misdiagnosed at initial presentation as ET due to lack of cytogenetic and molecular

Table 1: Diagnostic results and cytogenetic initiation during follow-up.

Time	IM, mg/daily	WCC x10^9/L	Hb, g/L	Platelet, x10^9/L	Karyotyping	Ph +ve clone	Ph -ve clone	*BCR-ABL1/ ABL1*, % (IS)
11-Aug-2009	200	14.80	9.6	581	46,XY,t(9;22)(q34;q11.2)[27]	100%	0%	54.3686
15-Mar-2010	400	2.11	8.9	88	46,XY, t(9;22)(q34;q11.2)[26] / 45, XY, -7 [4] / 47,XY,+8 [1]	83.9%	16.1%	10.1116
11-May-2011	400	4.06	8.2	263	46, XY, t(9;22)(q34;q11.2) [1] / 47,XY,+8 [3]/ 46, XY [13]	5.9%	94.1%	0.4752
15-May-2012	400	1.70	8.7	23	44, X, -Y, -7 [20] / 46,XY, t(9;22)(q34;q11.2) [19] / 47,XY,+8 [7] / 46, XY [3]	38.8%	61.2%	4.0660

Abbreviations: WCC: White Cell Count; Hb: Hemoglobin; Ph+ve: Philadephia Positive; Ph-ve: Philadephia Negative; IS=International Standardization

results. The WHO 2008 Classification requires four criteria to be met in the diagnosis of essential thrombocythaemia which includes the exclusion of Ph-positive chromosome or *BCR-ABL1*. We recommended for all cases suspected of MPN, the presence of *BCR-ABL1* rearrangements shall be determined.

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