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A case of acute myeloid leukemia with e6a2 BCR-ABL fusion transcript acquired after progressing from chronic myelomonocytic leukemia

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ABSTRACT

Philadelphia (Ph) chromosome is a cytogenetic hallmark of chronic myeloid leukemia (CML). Most patients with CML harbor either the e13a2 or e14a2 BCR-ABL fusion product, while a small subset of the cases expresses e1a2 or e19a2 transcripts. We report a patient with chronic myelomonocytic leukemia (CMML), initially Ph chromosome negative at presentation, with rapid disease progression to acute myeloid leukemia (AML) and appearance of Ph chromosome and BCR-ABL e6a2, a very uncommon fusion transcript. The AML was refractory to treatment with subsequent emergence and dominance of a Ph negative leukemic clone. The patient expired shortly after disease progression.

Philadelphia (Ph) chromosome results from the reciprocal translocation t(9;22)(q34.1;q11.2), and is a diagnostic feature for chronic myeloid leukemia (CML). In most cases, the breakpoint in *BCR* occurs in M-bcr region, leading to production of e13a2 and/or e14a2 fusion transcripts. The breakpoints infrequently occur in either m-bcr or μ-bcr, producing e1a2 or long e19a2 fusion transcripts. Several other variant transcripts, such as e8a2, e13a3, e14a3, and e6a2 have also been identified and account for < 1% of CML cases. Late appearing Ph chromosome may be acquired over the course of myelodysplastic/myeloproliferative neoplasm (MDS/MPN) or MDS, representing disease progression and often signifying poor prognosis. In this report we describe a patient with chronic myelomonocytic leukemia (CMML), a subtype of MDS/MPN, which progressed to AML with gain of the rare BCR-ABL1 fusion transcript e6a2 and died shortly of tumor lysis syndrome.

The patient is a 58 year old man who presented on 9/2012 at an outside institution with WBC 24.3 K/ul, Hgb 7.6 g/dl, and Platelets 56.0 K/ul; differential: neutrophils 53%, lymphocytes 10%, monocytes 35%, eosinophils 2%, and blasts 0%. The aspirate smear was hemodilute and inadequate for differential, but showed dysgranulopoiesis. The bone marrow (BM) biopsy was markedly hypercellular (approximately 100%) with hyperplastic granulopoiesis and dysplastic megakaryocytes (monolobation, widely separated nuclei). Immunohistochemistry revealed 5-10% blasts expressing CD34 and CD117, which by flow

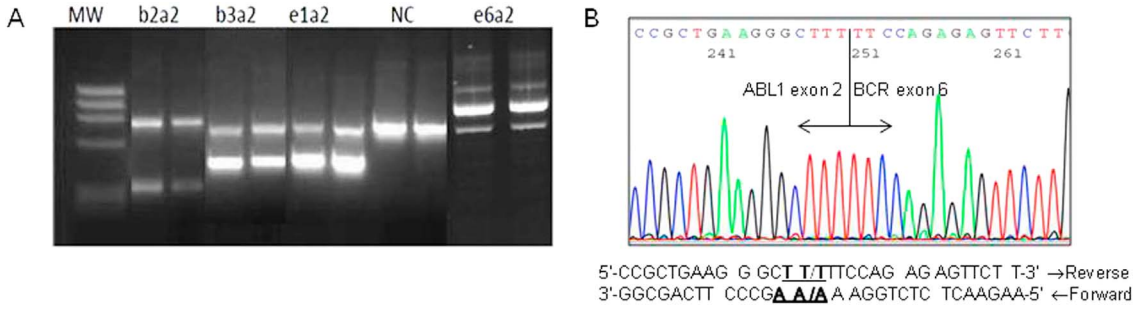

Fig. 1. A; larger BCR-ABL1 fusion product is amplified by multiplex PCR, which is confirmed to be e6a2 by Sanger sequencing. B; Sanger sequencing (reverse read) showed the fusion is between BCR exon 6 and ABL1 exon 2 (corresponding forward read is illustrated under the sequence graph).

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Table 1
Clinicopathologic features of reported chronic myeloid neoplasm cases harboring e6a2 fusion transcript.

Case No.	Reference (year)	Age/Sex	Diagnosis	BCR/ABL Transcript(s)	Treatment[a]	Response to TKI	Clinical course/outcome	Survival
1	Hochhaus [3]	41/M	CML-CP	e6a2	HU, allo-BMT	NA	AP after 19 months Death from sepsis 16 days after BMT	33 mo.
2	Dupont [4]	50/M	CML-CP	e6a2	ASCT, αIFN+ara-C	NA	NR	NR
3	Schultheis [5]	65/M	CML-BP	e6a2	HU, Imatinib	Reduction of WBC after 30 days	Death from pneumonia	42 d
4	Colla [2]	76/M	CML-CP	e6a2	HU, αIFN	NA	Relapsed and death from cerebral ictus	64 d
5	Popovici [6]	67/M	CML-CP	e6a2	Imatinib	Complete hematologic and cytogenetic response	Hematologic remission	Alive at 18 mo.
6	Roti [10]	37/M	CML-CP	e6a2, e1a2	Imatinib	Partial molecular response	Disease stabilized on imatinib	Alive at 21 mo.
7	Schnittger [1]	48/M	CML-CP	e6a2	Imatinib, HU, dasatinib	Imatinib: disease progression with clonal evolution and resistence mutations Dasatinib: Initial hematologic and cytogenetic remission followed by blast crisis and new resistance mutation	Death from blast crisis	10 mo.
8	Vefring [7]	42/M	CML-AP	e6a2	Imatinib, ASCT, dasatinib	Imatinib: persistent disease Dasatinib: effective on subsequent myeloid sarcoma	Developed myeloid sarcoma (CML-BP) after ASCT Death from hematemesis	45 mo.
9	Vefring [7]	48/M	CML-CP	e6a2	Imatinib, CDA, αIFN, ASCT	Disease progression	Hematologic remission after ASCT	Alive at 62 mo.
10	Langabeer [8]	36/M	CML-CP	e6a2, e1a2	Imatinib, nilotinib, ASCT	Imatinib: progression to AP with clonal evolution Nilotinib: complete cytogenetic remission	Complete molecular remission	Alive at 28 mo.
11	Hayette [9]	64/F	CMML	e6a2 (acquired)	Imatinib	Reduction of BCR/ABL transcript after 3 months	NR	Alive at 3 mo.
12	Present case	58/M	CMML	e6a2 (acquired)	Induction chemotherapy, dasatinib, nilotinib	Dasatinib: received only 3 doses due to pneumonitis Nilotinib: disease progression despite drastic reduction of Ph+ clone	Disease progression to AML after one month Death due to rapid disease progression and tumor lysis syndrome	15 mo.

[a] Listed temporally. M, male; F, female; CML, chronic myelogenous leukemia; CP, chronic phase; AP, accelerated phase; BP, blast phase; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; HU, hydroxyurea; allo, allogenic; BMT, bone marrow transplant; ASCT, allogenic stem cell transplant; SCT, stem cell transplant; αIFN, alpha interferon; ara-C, cytarabine; CDA, chlorodeoxyadenosine; TKI, tyrosine kinase inhibitor; mo., months; d, days; NR, not.

cytometry were also positive for CD13, CD33, and MPO; but negative for B/T-lymphoid markers. The karyotype was normal. FISH study was negative for t(9;22) and chromosome 5, 7, 8, and 20 aberrations. Molecular studies were positive for RUNX1 and TP53 mutations, but negative for *BCR-ABL1* fusion transcripts and FLT3, NPM1, ASXL1, EZH2, and ETV6 mutations. Based on these findings and a history of persistent monocytosis, a diagnosis of CMML-1 was rendered. He was refractory to 4 cycles of azacytidine and vorinostat and rapidly progressed to AML 8 months later in 5/2013 with 20% marrow blasts, which by flow cytometry were positive for CD34 (small subset), CD13, and CD33; while negative for CD117 and B/T-lymphoid markers including CD19 and cCD3, thereby excluding mixed phenotype acute leukemia. Cytogenetics study again showed a normal karyotype: 46,XY[20]. No molecular testing was performed. Despite an AML induction with cytarabine and idarubicin (standard "7+3" regimen) on 5/16/2013, his disease further progressed with 41% marrow blasts, which responded with re-induction with high-dose cytarabine on 5/30/2013 with reduction of blasts to 5%. Although he never achieved complete remission, his blast count remained stable and he did not receive further treatment or had interval biopsies until 9/2013, when his disease progressed again with 83% marrow blasts and 63% circulating blasts, and received fludarabine, cytarabine, idarubicin, and G-CSF (FLAG-IDA salvage regimen) and hydroxyurea. The patient was transferred to our institution on 9/30/2013 where a bone marrow biopsy showed 34% blasts. Karyotype analysis detected Ph chromosome: 46,XY,t(9;22)(q34.1;q11.2)[11]/46,XY[9]; and FISH showed BCR/ABL1 fusion signal in 6% cells: nuc ish(ABL1x3,BCRx3)(ABL1 con BCRx1)[30/500]. RT-PCR was negative for p210 and p190 *BCR-ABL1* fusion transcripts, but revealed a much larger product (~800bp) suggesting a variant fusion with estimated molecular weight of 191 kDa (Fig. 1A). Sanger sequencing confirmed a fusion of BCR exon e6 and ABL exon a2, generating e6a2 fusion transcript (Fig. 1B). Molecular/NGS studies were positive for DNMT3A, RUNX1, and SUZ12 mutations; and negative for FLT3, NPM1, CEPBA, NRAS, KRAS, TET2, ASXL1, IDH1/2, and JAK2 mutations. Dasatinib (140 mg/day) was initiated on 10/28/2013. However, FISH study from a 11/5/2013 blood sample showed BCR/ABL1 in 475/500 (95%) of the cells, indicating expansion of the Ph+ clone. Due to dasatinib-induced pneumonitis dasatinib (3 doses received) was discontinued on 11/17/2013 and switched to nilotinib (800 mg/day) on 11/19/2013. His WBC was 17.6 K/uL in pre-dasatinib and 4.6 K/uL pre-nilotinib (but post- dasatinib) periods, during both of which ABL kinase mutation by Sanger sequencing of ABL exon 4–9 were negative. His WBC further increased to 91.3 K/uL with 80% circulating blasts on 11/24/2013. However, FISH revealed *BCR-ABL1* fusion in only 17/508 (3.4%) of peripheral blood cells. The patient was given palliative care and expired on 12/04/13 from tumor lysis syndrome.

This report illustrates an unusual case of CMML which transformed to AML rapidly with emergence of the rare *BCR-ABL1* e6a2 fusion transcript. The possibility CML in blasts crisis was excluded as the CMML biopsy showed dysgranulopoiesis and lacked Ph chromosome and BCR-ABL1 fusion transcript.

We further reviewed the previous reports of *BCR-ABL1* e6a2 fusion transcript, the vast majority of which were CML with male predominance (Table 1) [1–10]. Two of these cases also co-expressed e1a2 transcript, likely representing low level alternative exon splicing [8,10]. The other reported CMML case harboring *BCR-ABL1* e6a2 fusion transcript, similar to our case, acquired the Ph chromosome later in the disease course [9].

CML with *BCR-ABL1* e6a2 fusion transcript appears to be associated with more aggressive clinical features, including accelerated/blastic phase on presentation [5,7], rapid disease progression [1,3,8], and imatinib treatment failure [1,7,8]. Their response to TKI treatment is variable (Table 1). Briefly, 3 of 7 patients who received a TKI (#5, #6, #10) responded to imatinib, nilotinib, or dasatinib; with disease stabilization or hematologic/molecular remission. Three patients (#7–9) showed persistent/progressive disease, while one died early from infection (#3). The other reported CMML case (#11) showed molecular response to imatinib after 3 months, although long term followup is not available. Our patient is unique since although his Ph+ clone drastically diminished in response to nilotinib (95% to 3.4%), his blast burden remained high (80%) with dominance of the Ph negative clone. Although it is tempting to speculate that the Ph negative clone may have been selected from suppression of the Ph+ clone by nilotinib, the patient's rapidly progressive disease may be due to an inherently aggressive biological behavior of his antecedent CMML and unrelated to TKI therapy, a notion that appears to be supported by the presence of RUNX1 and TP53 mutations in the CMML specimen.

It was hypothesized that shorter BCR-ABL transcripts are associated with a more aggressive clinical course due to lack of important regulatory bcr sequences within the fusion proteins [2]. Specifically in e6a2 transcript, the breakpoint in bcr intron 6 may result in partial loss of GEF/dbl-like domain that mediates interaction with several Ras-like G proteins involved in cell proliferation and signal transduction, which leads to enhanced tyrosine kinase activity of the BCR-ABL1 fusion protein.

In summary, our case illustrates the importance of combining conventional karyotype/FISH and appropriate molecular studies to detect rare BCR-ABL fusion transcripts such as e6a2. As a late appearing secondary Ph chromosome is usually considered a poor prognostic factor in hematopoietic malignancies, individualized therapeutic strategies such as newer TKIs and allogeneic stem cell transplantation may be warranted.

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