Complete molecular response of e6a2 BCR-ABL positive acute myeloid leukemia to Imatinib then Dasatinib.


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Abstract:

De novo presentation of acute myeloid leukaemia (AML) expressing the Philadelphia (Ph) chromosomal abnormality is rare and is associated with a dismal prognosis. To date reported cases of Ph+ AML have expressed either the e13a2 or e14a2 BCR-ABL fusion transcripts. We report a unique case of de novo AML expressing the e6a2 fusion transcript and describe disease sensitivity to both Imatinib prior to allogeneic stem cell transplant (SCT) and Dasatinib for AML relapse after allogeneic SCT. Furthermore we report that sustained molecular remission has been achieved despite withdrawal of tyrosine kinase inhibitor therapy.
Introduction:
More than 90% of patients with chronic myeloid leukemia (CML) have b2a2 (e13a2) and/or b3a2 (e14a2) BCR-ABL fusion transcripts, which are translated into the p210 bcr-abl protein. CML with p190 BCR-ABL (breakpoints in mbcr) or p230 BCR-ABL (breakpoints in µbcr) fusion genes (e1a2 and e19a2 transcripts, respectively) are less frequent. Further variant BCR-ABL rearrangements affecting other regions of the BCR gene have also been described.¹⁻¹²

A very rare fusion transcript joining the first 6 exons of BCR to exon 2 of ABL (e6a2) has been reported in four cases of CML with an aggressive clinical course.²⁻⁵ More recently, this translocation has been reported in single cases of chronic myelomonocytic leukemia,¹³ T-ALL¹⁴ and acute basophilic leukemia.¹⁵ We describe a unique case in which the e6a2 was found in a patient with de novo Ph⁺ acute myeloid leukemia (AML). Despite the previously described poor prognosis of this rare group of patients, the current patient achieved sustained disease control with imatinib, reduced intensity allogeneic stem cell transplantation and subsequently dasatinib.

Materials and Methods:
Reverse transcriptase polymerase chain reaction (RT-PCR)
RNA was prepared from peripheral blood or marrow. After red cell lysis, pelleted white cells were resuspended in Trizol (Invitrogen, Carlsbad, CA) and cDNA prepared by reverse transcription (RT) with Superscript II (Invitrogen). 2 µl of cDNA was used in a single round of PCR amplification using AmpliTaq Gold (Applied Biosystems, Foster City, CA) with 200 nM of each gene specific primer pair with appropriate RT negative and positive controls were used. Primers for the CML p210 b2a2 (e13a2) and b3a2 (e14a2) transcripts were used in addition to primers e1C (BCR exon e1) and a2B (ABL exon a2) for detection the e1a2 (p190) transcript.¹⁶ Amplification products were visualised by ethidium bromide staining of agarose gels.

PCR products were column purified using the PCR-M kit (Viogene, Taipei, Taiwan) and were eluted in a 30µl volume, 6µl was treated with ExoSapIT (GE Healthcare, Buckinghamshire, England). The PCR product was then used as template in cycle sequencing with the Big Dye Terminator v3.1 kit (Applied Biosystems). The reactions were run on a GeneAmp 9700 thermocycler (Applied Biosystems). The sequencing reactions were ethanol precipitated and run on a 3100 Genetic Analyser (Applied
Biosystems,) and analysed using Sequencher™ 4.6 (Gene Codes Corporation, Ann Arbor, MI).

Case Report
Computed tomography and isotope bone scanning of the painful right hip in a 53 year-old woman demonstrated a solitary destructive lesion of the femoral head, a core biopsy of which showed myeloblastic chloroma. At presentation, there was a normocytic anaemia (Hb=90 g/l) with anisopoikilocytosis, mild thrombocytopenia (133 x 10^6/l) and a white blood cell (WBC) count of 4.3 x 10^9/l with normal differential and morphology. Splenomegaly was absent.

Cytogenetic assessment by FISH of the core biopsy revealed t(9:22) with a deletion of the derivative chromosome 9. Bone marrow aspiration was a dry tap. The trephine biopsy demonstrated acute panmyelosis with myelofibrosis along with abnormal localization of immature precursors (ALIP) with an absence of typical CML megakaryocyte morphology (figure 1).

RT-PCR on peripheral blood was negative for the p210 e13a2 (e14a2) transcripts. Primers to detect the e1a2 (p190) transcript, revealed an atypical band, 642 bp larger than the e1a2 PCR product (Figure 2). Direct sequencing of this product, revealed an e6a2 BCR- ABL transcript.

Two weeks after total hip joint replacement and commencement of imatinib mesylate 400mg/d, induction chemotherapy with cytarabine (3g/m^2 bd days 1, 3, 5, 7) and idarubicin (12mg/m^2 d1-3) was administered. Imatinib mesylate continued until the development of neutropenic enterocolitis on d22. A bone marrow biopsy on d37 demonstrated no blasts, residual fibrosis and absence of the e6a2 BCR-ABL transcript by RT-PCR. Imatinib was recommenced on d45.

Consolidation chemotherapy (cytarabine 100mg/m^2 continuous intravenous infusion daily for 5d and idarubicin 9mg/m^2 for 2d) began on d56. Imatinib was withheld from the onset of neutropenia until completion of an autologous stem cell collection yielding 5 x10^6 CD34^+ cells/kg. The stem cell product was e6a2 negative.

A second identical consolidation chemotherapy cycle commenced on d106. Marrow examination following recovery from the second consolidation demonstrated ongoing remission with reduced fibrosis.
Given the poor prognosis of Ph+ AML, a matched sibling (sister) allogeneic SCT was undertaken in first remission with 3.6 x 10^6/kg CD34+ peripheral blood cells following reduced intensity conditioning with Fludarabine (25mg/m² d -6 to -2) and Cyclophosphamide (1g/m² d -2 and -1). Rapid engraftment ensued and peripheral blood and bone marrow chimerism analysis confirmed 100% donor hematopoiesis at d28. In the absence of acute graft-versus-host disease (GVHD) cyclosporin was tapered from d100 and fully withdrawn by 5 months post-transplant. Imatinib 400mg/d maintenance, started at 1 month post-transplant, was poorly tolerated due to peripheral edema, neutropenia and disturbed liver function tests and was discontinued at 4 months post-transplant.

At 6 months post-transplant recurrent neutropenia and thrombocytopenia occurred. Marrow examination revealed relapsed AML identical to diagnosis and reappearance of the e6a2 BCR-ABL transcript. Chimerism analysis on peripheral blood CD3+ lymphocytes was 100% donor.

Donor ill health made donor lymphocyte infusion impossible. Dasatinib 70mg BD was commenced on the START-B study17. Improvement of peripheral blood parameters, complete suppression of the e6a2 BCR-ABL transcript, a return to morphological marrow remission and 100% donor myeloid (CD15) and lymphoid (CD3) chimerism were achieved after 1 month of therapy. Subsequent intolerance to Dasatinib including transfusion dependence, edema, anorexia and diarrhoea necessitated dose reduction and then cessation after 6 months therapy.

Despite remaining off all tyrosine kinase inhibitor (TKI) therapy for a total of 18 months, the patient remains in complete molecular remission, with normal peripheral blood counts, 100% donor chimerism and full resolution of myelofibrosis on bone marrow examination. There has been no GVHD.

**Discussion**

The presence of shorter BCR-ABL transcripts in CML have been associated with aggressive clinical phenotype and early transformation, perhaps due to the lack of important regulatory bcr sequences within the fusion proteins8,5. The observed poor clinical outcome associated with e6a2 BCR-ABL protein is likely to be due to increased kinase activity due to the partial loss of the guanine exchange factor (GEF)/dbl-like domain, which is also completely absent in p190 BCR-ABL protein, and which mediates the interaction with several Ras-like G proteins involved in cell
proliferation, signal transduction and cytoskeletal organization

Our case is unusual, as the presence of the e6a2 transcript and the development of AML occurred without evidence of preceding CML. The absence of prior clinical or laboratory features of CML, such as pre-existing leukocytosis or basophilia, along with absence of splenomegaly at presentation are all supportive of a diagnosis of Ph+ AML\textsuperscript{18}. Furthermore, the achievement of early complete hematological response without residual features of CML also support this diagnosis.

We have demonstrated the sensitivity of this disease to two separate TKI in the clinical course of our patient. Critically, despite the withdrawal of TKI therapy due to poor tolerance post-SCT, there has been no recurrence of the e6a2 clone possibly due to a sustained allogeneic graft-versus-leukemia effect even in the absence of GVHD. Alternatively, the brief exposure to Dasatinib may have lead to disease eradication. This however is less likely in light of the demonstrated moderate rate of hematological remissions induced by Dasatinib in myeloid blast crisis of CML and the lack of reported complete molecular responses in this setting\textsuperscript{17}.

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**Authorship:**

Contribution; DSR, JFS, AD drafted the paper. MM, DAW, SK, AD performed the assays and reported results. All authors approved the final version.

Conflict-of-interest; The authors declare no competing financial interests.

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Figure 1. 100x magnification of a section of bone marrow trephine taken at diagnosis, demonstrating hyperplasia, areas of marked panmyelosis and fibrosis and clusters of blasts.
Figure 2: Detection of the e6a2 transcript:
M is a 100bp ladder marker. A no RNA negative control (nr) and a no template (nt) negative control are shown. Amplification of the usual e1a2 transcript (PCR product of 389bp) from the ALL-1 cell line, and absence of product from the HL-60 cell line are shown in lanes 7 and 8 respectively. The abnormal 1033 bp band seen at presentation in blood and bone marrow is demonstrated in lanes 1 and 2.
Lanes 3 reveals the absence of abnormal band from the remission bone marrow following initial therapy. A positive control sample is shown in lane 4.
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