

Figure 2. Comparison of the sequence of Hb Lepore-Hollandia derived by cloning and gap-PCR with that of the δ - and β -globin genes. The cross-over took place within a 15 bp window between IVS1-nt 42 – IVS1-nt 56 (boxed region). The intron sequence is given in lower case.

with different haplotype backgrounds suggested independent homologous unequal cross-over events,¹⁰ the present finding of two Lepore-Hollandia mutations resulting from two different cross-over sites in IVS-1 is definitive proof of these being two independent events.

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Chronic Myeloid Leukemia

e6a2 BCR-ABL transcript in chronic myeloid leukemia: is it associated with aggressive disease?

We describe the fourth case of e6a2 BCR-ABL transcript in a patient with chronic myeloid leukemia (CML), using reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing analysis. The clinical and hematologic features and the aggressive course of disease in our patient and in the others reported in literature lead us to hypothesize that this atypical rearrangement may be associated with a worse prognosis.

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Chronic myeloid leukemia (CML) is characterized in 95% of patients by the expression of a large chimeric BCR-ABL fusion transcript arising from the Philadelphia (Ph) translocation t(9;22)(q34;q11). Three breakpoint cluster regions in the BCR gene have been described to date: major (M-bcr),

minor (m-bcr) and micro (μ -bcr). These breakpoints result in BCR-ABL proteins that differ in size and transforming potential.

More than 90% of Ph-positive CML patients have breakpoints in the M-bcr, which typically result in b2a2 (e13a2) and/or b3a2 (e14a2) fusion mRNAs, both of which are translated into p210 BCR-ABL protein. Rarely, CML patients with the p190 BCR-ABL (m-bcr) or p230 BCR-ABL (μ -bcr) fusion genes (e1a2 and e19a2 transcripts, respectively) have been reported. Atypical BCR breakpoints outside these cluster regions have also been described. They involve splicing between whole exons, insertion of small sequences or genomic breakpoints within exons.¹⁻⁸ Despite lacking a variable number of amino acids coded for by the missing exons, BCR-ABL proteins translated from unusual transcripts are still oncogenic and can produce chronic-phase CML.

In this report we describe the fourth case of a CML patient whose chromosome 22 breakpoint was located in bcr intron 6, resulting in an unusual BCR-ABL transcript with an e6a2 junction.

A 76-years old man presented in September 2002 with massive hepatosplenomegaly and a white blood count

Table 1. Clinical and hematologic features of e6a2 CML patients.

	<i>Colla et al.</i> (this report)	<i>Hochhaus et al.</i> 1996	<i>Dupont et al.</i> 2000	<i>Schultheis et al.</i> 2003
Sex/Age	M/76	M/41	M/50	M/65
Presentation	Chronic phase	Chronic phase	Chronic phase	Blastic crisis
Karyotype	46XY,t(9;22)	46XY	46XY,t(9;22)	46XY,t(9;22)
Platelets ($\times 10^9/L$)	43	216	227	88
Hemoglobin (g/dL)	15	15	12.4	6.8
WBC ($\times 10^9/L$)	105	15.6	63.6	157
Basophils (%)	4.9	13	NR	11
Spleen	+++	0	NR	0
Follow-up	Treatment with HU and α IFN; relapse and death from cerebral ictus	AP after 19 months; treatment with HU; allogeneic BM transplantation and death from sepsis	Treatment with autologous PB stem cell transplantation and α IFN plus cytarabine	Treatment with HU and imatinib; death from pneumonia
Survival	64 days	33 months	NR	42 days

NR: not reported; HU: hydroxyurea; α IFN: α interferon; AP: accelerated phase; BM: bone marrow; PB: peripheral blood.

(WBC) of $105 \times 10^9/L$ with 69.9% polymorphonuclear cells, 1.5% lymphocytes, 0.9% monocytes, 8.8% eosinophils, 4.9% basophils, 9% myelocytes and 5% metamyelocytes. His peripheral blood showed a hemoglobin concentration of 15 g/dL and a platelet count of $43 \times 10^9/L$. The diagnosis of chronic-phase CML was confirmed by a bone marrow aspirate. Standard cytogenetic analysis showed a 46,XY, t(9;22) (q34;q11)[20] karyotype.

Molecular analysis by RT-PCR (according to the BIOMED-1 Concerted Action protocol)⁹ showed no amplification product with primers for the detection of the b2a2 (e13a2) and b3a2 (e14a2) transcripts (corresponding to p210 protein), whereas with primers e1C (bcr exon e1) and a2B (abl exon a2), used to detect the e1a2 (p190) transcript, an atypical band larger than e1a2 but smaller than e13a2 and e14a2, was detected (Figure 1). Direct sequencing of the RT-PCR product, using the ABI/Prism 310 Sequencer (PE Applied Biosystems, Warrington, United Kingdom), revealed a corresponding fusion between exon e6 of bcr gene and exon a2 of the abl gene, resulting in an e6a2 BCR-ABL transcript, 642 bp larger than expected e1a2, but 786 bp and 881bp smaller than e13a2 and e14a2, respectively.

The patient was treated with hydroxyurea and subsequently α -interferon (IFN) with an initial hematologic response. The dose of IFN administered was 9 mIU daily; during this continued treatment no significant side effects were observed. However after only 2 months, the patient relapsed and presented with a WBC of $110 \times 10^9/L$, a hemoglobin concentration of 6.5 g/dL and a platelet count of $16 \times 10^9/L$. Although the patient was treated with oncocarbide and blood product support, he died of a cerebral ictus 64 days after the initial diagnosis.

Analyzing the clinical and hematologic features of e6a2 CML patients reported so far in literature (Table 1), despite the small number of cases described, it can be hypothesized that this type of CML may represent a different biological entity associated with a worse prognosis. In fact 2 out of the 3 patients studied had an aggressive type of disease: one presented in blast crisis and the second had rapidly accelerating disease with extramedullary manifestations.^{6,8} The third patient was initially treated with unpurged autologous peripheral blood stem cell transplantation followed by α interferon plus cytarabine: the authors did not report his clinical outcome.⁷

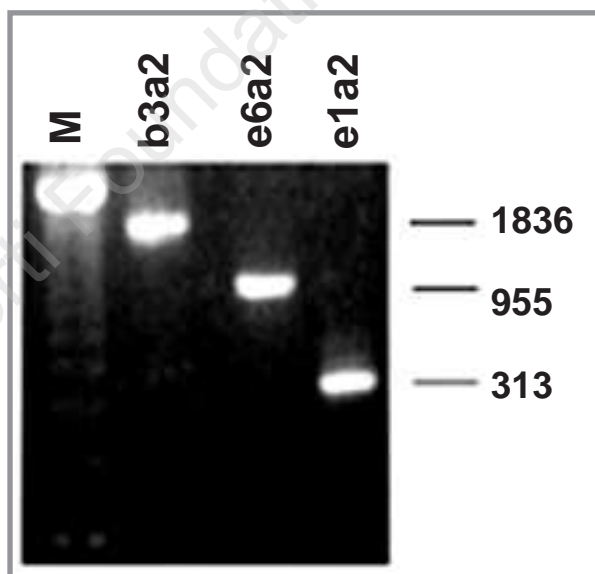


Figure 1. Single-step RT-PCR for BCR-ABL transcripts using primers e1C (bcr exon 1) and a2B (abl exon 2). Lane 1, b3a2 BCR-ABL CML patient; lane 2, e6a2 BCR-ABL patient described in this report; lane 3, e1a2 BCR-ABL CML patient; lane M, molecular marker.

The striking relationship described between the type of BCR-ABL fusion gene and the leukemia phenotype has led us to hypothesize that shorter BCR-ABL transcripts give rise to a more aggressive clinical phenotype and early transformation due to the lack of important regulatory bcr sequences within the fusion proteins.¹⁰ Therefore, in the e6a2 BCR-ABL protein, the breakpoint in bcr intron 6 could be responsible for increased kinase activity and greater transforming potential because of the partial loss of the guanine exchange factor (GEF)/*dbl*-like domain, completely absent in p190 BCR-ABL protein. The truncation of this bcr domain, able to mediate the interaction with several Ras-like G proteins involved in cell proliferation, signal transduction and cytoskeletal organization, could increase the oncogenic potential of BCR-ABL.⁸

Nevertheless the molecular and hematologic characterization of more patients with e6a2 transcript will be needed to verify the real correlation of this transcript with more aggressive disease. This knowledge is important in order to improve the choice of treatment and thus the clinical outcome of single patients.

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Chronic Myeloproliferative Disorders

No mutations in the GATA-1 gene detected in patients with acquired essential thrombocythemia

Mutations in the GATA-1 gene have been identified in patients with familial macrothrombocytopenia and Down's syndrome patients with a transient myeloproliferative disorder and/or acute megakaryoblastic leukemia. We screened this gene in 46 patients with essential thrombocythemia and identified only a common single nucleotide polymorphism that is unlikely to be of pathological significance.

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Although GATA-1 was originally described as an erythroid-specific nuclear binding factor, it is now known to play a critical role in megakaryocytic proliferation and differentiation. Over-expression of GATA-1 in a mouse pluripotent myeloid cell line 416B induces megakaryocytic differentiation¹ and megakaryocyte-specific loss of GATA-1 expression in a knock-out mouse leads to thrombocytopenia with hyperproliferation of morphologically retarded megakaryocytes.² Furthermore, two different types of mutation in GATA-1 have recently been shown to have pathologic consequences for the megakaryocytic lineage. Firstly, five different inherited point mutations identified in exon 4, which codes for the N-terminal zinc finger, lead to disrupted binding to either its essential co-factor FOG-1 or DNA and cause macrothrombocytopenia with variable degrees of dyserythropoiesis and anemia in affected individuals.³⁻⁷ Secondly, acquired mutations have been detected in exon 2 in most patients with the transient myeloproliferative disorder (TMD) and/or acute megakaryoblastic leukemia (AMKL) associated with Down's syndrome. The mutations include deletions,

insertions, splice mutations, nonsense and missense mutations, and cause loss of the full length GATA-1 protein but not of a shorter length variant lacking the N-terminal activation domain which can still bind DNA and interact with FOG-1 but has reduced transactivation potential.⁸

The etiology of the sustained thrombocytosis and megakaryocytic hyperproliferation characteristic of the acquired myeloproliferative disorder essential thrombocythemia (ET) is not known. No specific cytogenetic abnormalities or molecular markers have been associated with the disorder and its diagnosis remains one of exclusion. There is, however, evidence that it is biologically heterogeneous, with patients having either polyclonal or clonal myelopoiesis as demonstrated by X-chromosome inactivation patterns (XCIPs) in informative females, and normal or elevated PRV-1 expression.⁹ We would hypothesize that, especially in patients with polyclonal myelopoiesis, mutations in genes implicated in megakaryocytopoiesis which cause subtle differences in function may contribute to increased megakaryocytic production. We therefore looked for mutations in the GATA-1 gene in DNA from neutrophils of 46 female ET patients. The DNA had been previously used to investigate clonality in these patients, whose demographic and clinical details have been previously published.¹⁰

Polymerase chain reaction (PCR) analysis was performed using Optimase Polymerase™ (Transgenomic Limited, Crewe, UK) with 7 different primer sets covering the GATA-1 coding region (exons 2-6), the hematopoietic-specific promoter (IE, exon 1) and a 317 bp cis-acting regulatory element, HS1, which is associated with a hematopoietic-specific DNase I hypersensitive site and is required in full for megakaryocytic- but not erythroid-specific expression¹¹ (Table 1). PCR products were analyzed using denaturing high performance liquid chromatography (DHPLC) on a Transgenomic WAVE®, DNA fragment analysis system at optimal temperatures for each fragment as determined by WAVE-MAKER® software (Table 1). Patterns from patients' sam-