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LETTER TO THE EDITOR

New mutation L324M in the *ABL1* kinase domain: does it confer high resistance to second-generation inhibitors?

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of pluripotent hematopoietic stem cells characterized by the presence of the Philadelphia chromosome (Ph). This chromosome marker results from a reciprocal translocation t(9;22)(q34;q11.2), allowing fusion of the *BCR* at 22q11.2 with *ABL1* at 9q34, resulting in production of a BCR–ABL1 fusion protein with constitutive up-regulated tyrosine kinase (TK) activity [1]. CML is normally a triphasic disease. It starts with a relatively indolent chronic phase (CP) that can progress to an accelerated phase (AP) characterized by the appearance of 10–20% blasts in the blood and bone marrow, and subsequent evolution to blast crisis (BC) [2]. Discovery of the fusion gene *BCR–ABL1*, its TK activity and demonstration that it is the pathogenic event of CML led to the development of specific TK inhibitors (TKIs). The initial efficacy of these inhibitors has been overshadowed mainly due to the appearance of mutations in the TK domain in the *ABL1* gene. Imatinib mesylate (IM) is a selective *BCR–ABL1* kinase inhibitor highly effective in the treatment of CML, particularly in CP. It acts by binding to the receptor of adenosine triphosphate (ATP) in *BCR–ABL1*, competitively inhibiting the phosphorylation of tyrosine residues of the substrate. It has become the first-line agent for the treatment of newly diagnosed patients with CML [3]. Second-generation TKIs, which have increased potency relative to IM and activity against many *BCR–ABL1* kinase domain mutations, have been developed as alternative therapeutic agents [4]. To date, dasatinib, nilotinib and bosutinib have been approved for the treatment of CML in adults with secondary resistance (mainly by acquisition of mutations in the TK domain) [5] or intolerance to previous IM therapy. Recent studies have demonstrated the efficacy of these drugs in the treatment of patients with newly diagnosed CML [6,7]. We report a case of a patient who was diagnosed with CML 16 years ago, who acquired the L324M mutation during dasatinib treatment, with a good response to nilotinib.

Chromosomal analysis was performed in unstimulated cultures of bone marrow (BM). Chromosomal aberrations were identified by G banding and reported according to the International System for Cytogenetic Nomenclature (ISCN).

Total RNA was isolated by conventional extraction technique with TRIzol–chloroform. Quantification of transcript levels of *BCR–ABL1* and *ABL1* (reference gene) was performed by quantitative real-time polymerase chain reaction (QRT-PCR) method using a Rotor-Gene Q cycler (Qiagen) and a One-Step quantitative RT-PCR commercial kit (Molecular MD). The obtained results were expressed as % *BCR–ABL1*/*ABL1* on the International Scale (the conversion factor was obtained from the Institute of Medical and Veterinary Science, Adelaide, South Australia [8]).

High resolution melting (HRM) analysis was performed for mutation screening in the *BCR–ABL1* kinase domain. For this analysis, we used primers previously described by Polàkovà *et al.* [9]. The HRM was analyzed using Rotor-Gene software.

For direct sequencing analysis, *BCR–ABL1* chimeric transcripts were amplified by nested PCR using primers described by Gorre *et al.* [10]. The amplified products were separated through agarose gel, purified and bidirectionally sequenced. Results were analyzed using the Mutation Surveyor® program quantification tool and the ChromasLite® program.

A 44-year-old man was diagnosed with CML in CP (CML-CP) in February 1996. Conventional karyotype and G banding analysis in BM showed the classical t(9;22)(q34;q11.2) in 100% of cells. The BM biopsy showed $<5\%$ blasts. Hematological parameters were: white blood cells: 58×10^9/L (neutrophils: 38, promyelocytes: 6, myelocytes: 34, neutrophil bands: 13), 3 cm splenomegaly, 4.5 cm hepatomegaly. He was treated with 500 mg/day of hydroxyurea $+5$ mIU/day interferon α for 5 days/week, achieving complete hematologic remission as the best response.

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In January 2001 the patient started with 400 mg/day IM, increasing the dose up to 600 mg/day without achieving a complete cytogenetic response (CCyR). Due to the lack of CCyR, in February 2006 the treatment was switched to dasatinib 70 mg/day up to 140 mg/day, and CCyR was achieved. Due to side effects such as pleural effusion, dasatinib was reduced to 100 mg/day, 80 mg/day and 50 mg/day, successively. In December 2010 a cytogenetics study by G banding showed 2/30 (6%) BM cells with t(9;22)(q34;q22.2). In May 2011 a mutational analysis in the *ABL1* domain was determined to be L324M mutation, and the cytogenetics study showed a karyotype with double Ph (46,XY, t(9;22)(q34;q11.2)[25]/47,XY, t(9;22)(q34;q11.2),+ der(22)t(9;22)(q34;q11.2)[14]/46,XY [9]). Since then he has received 600 mg/day of nilotinib. In August 2011 no mutation was observed, and he achieved a CCyR. In May 2012 the molecular study showed a major molecular response (MMR) without mutations (Table I).

Point mutations in the *ABL1* kinase domain have been described as the main mechanism of reactivation of *BCR–ABL1* causing a resistant leukemic clone [11,12]. There are currently more than 100 mutations described, some of which seem to occur more frequently in a particular phase of disease. For example, mutations M244, L248, F317, H396 and S417 occur most frequently in the chronic phase, while Q252, Y253, E255, T315, E459 and F486 have been observed mainly in advanced stages of CML. The functional significance of these findings is not yet well defined [13]. Since mutations in the kinase domain show resistance to treatment, different strategies have been designed to overcome the lack of response. Second-generation TKIs such as dasatinib bind to the *ABL1* kinase domain differently from IM, and thereby retain activity against nearly all IM-resistant mutations. The most frequent non-hematologic adverse effect of this inhibitor is pleural effusion. On the other hand, nilotinib therapy achieves and maintains major and complete molecular responses, and has less intolerance and fewer toxic effects than imatinib [14].

Both dasatinib and nilotinib are treatments of choice for Ph(+) CML in chronic and accelerated phase with resistance or intolerance to a prior line of therapy.

We evaluated serial samples of the patient by QRT-PCR, HRM and direct sequencing to quantify *BCR–ABL1* transcripts, perform mutation screening and characterize the mutation, respectively. When the patient's dose of dasatinib was reduced to 50 mg/day (for recurrent pleural effusion), treatment failed. Loss of cytogenetic response was observed, and the karyotype showed the Ph chromosome in 52% (25/48) and double Ph in 29% (14/48) of cells with a null molecular response (*BCR–ABL1*/*ABL1*: 21%), which indicates relapse of the disease. The HRM study presented a profile consistent with the presence of a mutation in the IM binding domain. Due to this finding, we conducted a mutation detection study by direct sequencing, which revealed the presence of a nucleotide change C>A at position 324, resulting in substitution of the amino acid leucine by methionine, mutation L324M [Figures 1(A) and 1(B)]. Taking into account these data and considering the possibility of a secondary resistance to dasatinib, it was decided to change the treatment to 600 mg/day nilotinib (Table I). After 3 months of follow-up, the patient achieved a CCyR and the HRM profile showed a normal sequence compatible with disappearance of the mutation L324M, suggesting that this is sensitive to nilotinib. After 12 months a MMR was reached, indicating an excellent response to this TKI.

Relapse of the disease is often due to the emergence of clones expressing mutant forms of *BCR–ABL1*, which escape inhibition through the exchange of amino acid residues in the *ABL1* kinase domain, producing a reduced binding affinity to the inhibitors.

Crystal structure analysis has revealed the molecular interaction of TKIs with the kinase domain of wild-type and mutant *ABL1*, which is mainly through hydrogen bond sites [15]. Nilotinib binds to the inactive conformation of the *ABL1* kinase domain by undergoing four hydrogen bond

Table I. Monitoring of patient throughout treatment.

		Response to treatment			
				Molecular†	
Date	Treatment	Hematologic	Cytogenetic	QRT-PCR	Mutation
February 1996	500 mg/day hydroxyurea + 5 mIU/day interferon α for 5 days/week				
January 2001	IM 400 mg/day increasing up to 600 mg/day		No CyR		
February 2006	Dasatinib 70 mg/day up to 140 mg/day	CHR	No CyR		
May–June 2007	Dasatinib 140 mg/day	CHR	CCyR		No
July 2008	Dasatinib 100 mg/day	CHR	CCyR		
July 2009	Dasatinib 80 mg/day	CHR	CCyR	0.27%	
November 2009	Dasatinib 100 mg/day	CHR	CCyR		
April 2010	Suspension of dasatinib for pleural effusion				
May 2010	Dasatinib 50 mg/day	CHR	CCyR		
December 2010	Dasatinib 50 mg/day	CHR	Loss CCyR (6% Ph+ cells)		
January 2011	Dasatinib 50 mg/day	CHR		2.43%	
May 2011	Suspension of dasatinib, start nilotinib 600 mg/day	CHR	Loss CyR*	21.00%	L324M
August 2011	Nilotinib 600 mg/day	CHR	CCyR	1.37%	No
May 2012	Nilotinib 600 mg/day	CHR	CCyR	0.035%	No

QRT-PCR, quantitative real-time polymerase chain reaction; CHR, complete hematological response; CyR, cytogenetic response; CCyR, complete cytogenetic response.

*46,XY ,t(9;22)(q34;q11)[25]/47,XY ,t(9;22)(q34;q11)+ der(22)t(9;22)(q34;q11)[14]/46,XY[9].

†Molecular response (MR): complete MR ≤ 0.01%, major MR 0.01–0.1%, minor MR 0.1–1%, minimal MR 1–10%, null MR > 10%.

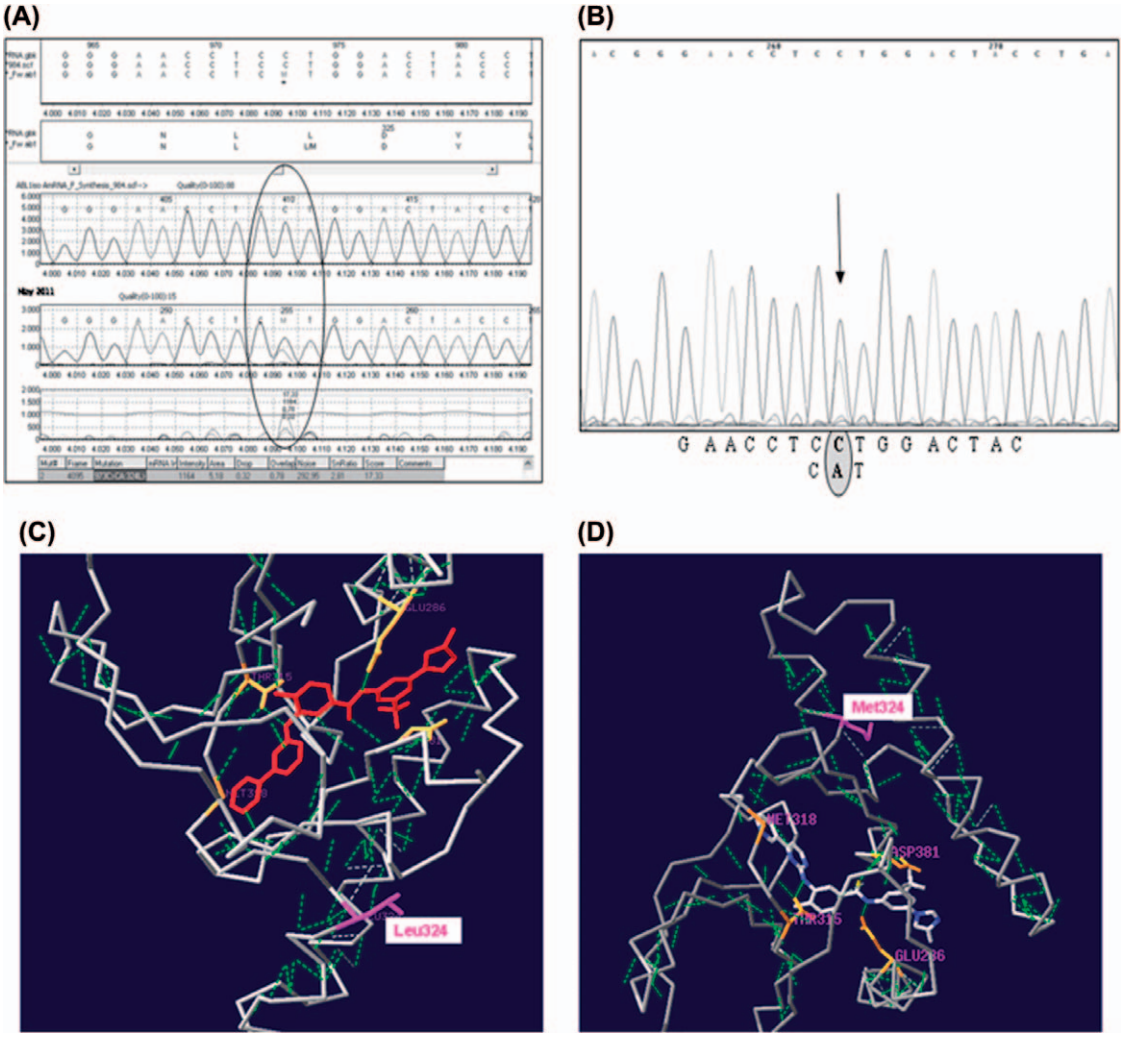

Figure 1. Point mutation in the *ABL1* kinase domain. (A) Electropherogram showing mutation L324M. It was generated by the nucleotide change C>A (CCT>CAT) at position 324 (NM_005157.4) giving rise to amino acid substitution of leucine by methionine. Percentage of mutated allele was estimated at 35.47% using software Mutation Surveyor® quantification tool (SoftGenetics). (B) Sequence diagram of same sample analyzed by ChromasLite® (Technelysium Pty Ltd) program. (C) Model of nilotinib binding to *ABL1* kinase domain using program Swiss PdbViewer 4.1.0. Left: *ABL1* wild type (L324), right: *ABL1* L324M mutated.

interactions involving the pyridyl-N and the backbone NH of Met-318, the anilino-NH and the side chain OH of Thr-315, the amido-NH and side chain carboxylate of Glu-286, and the amido carbonyl with the backbone NH of the Asp-381. The modeling program Swiss-PdbViewer 4.1.0 allowed analysis of the substitution of leucine by methionine at position 324 of the *ABL1* kinase. This nucleotide change does not modify the interaction with nilotinib [Figure 1(C)], allowing the drug to continue exerting its inhibitory action.

Too low doses of dasatinib due to drug intolerance could be the cause of treatment failure, while the good tolerance to nilotinib probably allowed action on the mutated clone with high effectiveness.

In conclusion, to our knowledge this is the first report of the L324M mutation, which was sensitive to nilotinib 600 mg/day. Our case achieved CCyR, MMR and elimination of the mutated clone after 1 year's treatment. More reports on the clinical characteristics of similar cases will be necessary to determine its prognostic significance.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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