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Resistance to Imatinib in Patients with Chronic Myelogenous Leukemia and the Splice Variant BCR-ABL1^{35INS}

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Abstract

Purpose—In patients with chronic myelogenous leukemia (CML), point mutations in the BCR-ABL1 kinase domain are the most common cause of treatment failure with a tyrosine kinase inhibitor (TKI). It is not clear whether the splice variant BCR-ABL1^{35INS} is also associated with treatment failure.

Patients and Methods—We reviewed all CML patients who had BCR-ABL1 kinase mutation analysis performed between August 1, 2007, and January 15, 2014. Patients who had BCR-ABL1^{35INS} detected had their medical records reviewed to determine response to TKI therapy.

Results—Two hundred and eighty four patients had kinase mutation testing performed; of these, 64 patients (23%) had BCR-ABL1^{35INS} detected. Forty-five patients were in chronic phase (70%), 10 were in accelerated phase (16%), 6 were in blastic phase (9%), and 3 were in other settings (5%). Of the 34 chronic phase patients who began therapy with imatinib, 23 patients (68%) failed therapy: 8 patients (24%) had primary refractory disease, 11 patients (32%) progressed, and 4

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patients (12%) had disease progression after dose interruption. In contrast to the patients with disease progression or lack of response, none of 23 patients who were responding to imatinib had BCR-ABL1^{35INS} detected. DNA sequencing of commonly mutated spliceosomal genes SF3B1, U2AF1, SRSF2, ZRSR2, SFA31, PRPF408, U2A565, and SF1 did not reveal mutations in seven BCR-ABL1^{35INS} -positive patients tested.

Conclusions—The splice variant BCR-ABL1^{35INS} is frequently found in patients who are resistant to imatinib. Mutations in the commonly mutated spliceosomal proteins do not contribute to this association.

Keywords

Resistance to imatinib; splice variant; CML

INTRODUCTION

Chronic myelogenous leukemia (CML) results from the balanced translocation of the *ABL1* gene on chromosome 9 to the *BCR* region on chromosome 22, leading to the formation of the fusion protein BCR-ABL1.[1,2] BCR-ABL1 contains a constitutively activated tyrosine kinase domain that is responsible for the malignant transformation in this disease.[3–5] Imatinib, the first generation tyrosine kinase inhibitor (TKI) licensed for treatment of CML, has proven highly effective, as approximately 80% of patients in chronic phase achieve a complete cytogenetic remission within 12 months of therapy.[6] However, approximately 15–20% of patients ultimately develop resistance to imatinib.[6,7] The most common mechanism responsible for imatinib resistance is a point mutation within the ABL1 kinase domain of BCR-ABL1 which either directly interferes with imatinib binding at critical contact points or prevents the BCR-ABL1 molecule from assuming the appropriate conformation that allows imatinib to bind.[8,9]

The BCR-ABL1 splice variant BCR-ABL1^{35INS} was first described in an imatinib-resistant patient with chronic phase CML in 2006.[10] Sequencing of BCR-ABL1^{35INS} revealed that a 35 nucleotide base pair portion of intron 8 is inserted between exons 8 and 9 leading to a frameshift with the insertion of 10 amino acids after amino acid 474, followed by a stop codon. This results in a truncated protein that lacks 653 C-terminal amino acids. Subsequently, a small number of clinical studies reported the relationship between the presence of this splice variant and clinical resistance to imatinib, but results have been inconsistent.[11–13]

In order to clarify the role of BCR-ABL1^{35INS} in patients with CML, we reviewed clinical outcomes of all patients from Memorial Sloan Kettering Cancer Center (MSKCC) who had BCR-ABL1 kinase domain mutation testing performed between August 1, 2007 and January 15, 2014. In addition, we studied 23 sequential patients who were taking imatinib and were in either a partial or major molecular remission on imatinib to determine whether BCR-ABL1^{35INS} could be detected. Lastly, we looked for mutations in genes that encode members of the spliceosome in a subset of patients with BCR-ABL1^{35INS}. All 23 patients who had samples studied while responding to imatinib signed informed consent. The Institutional Review Board at MSKCC approved this study.

PATIENTS AND METHODS

Identification of Patients with BCR-ABL1^{35INS}

All bone marrow or blood test results from patients with CML who had BCR-ABL1 mutation testing performed between August 1, 2007, and January 15, 2014, were reviewed to determine whether BCR-ABL1^{35INS} was present. Patient charts were then reviewed to assess response to TKI therapy which was classified according to standard criteria.[14]

BCR-ABL1 kinase mutation analysis by direct sequencing

All samples were analyzed at Quest Diagnostics- Nichols Institute (San Juan Capistrano, CA); samples were not accompanied by any clinical history. RNA was extracted using MagNA Pure instrument (Roche, Indianapolis, IN). BCR-ABL1 fusion transcripts were amplified in the first round of RT-PCR using a reverse primer annealed at the BCR-ABL1 exon 9/exon 10 junction and two forward primers annealed at BCR exon b2 and e1, respectively. The multiplexed RT-PCR was designed to ensure that b2a2/b3a2 and e1a2, the most frequent BCR-ABL1 transcripts, were all amplified. Amplifying the fusion transcript also assured that the fusion transcript and not the native ABL1 transcript was sequenced. A semi-nested PCR was followed to amplify BCR-ABL1 kinase domain (exon4-exon9). The nested PCR products were then purified and sequenced in both forward and reverse directions. Four sequencing primers were used to ensure that the entire BCR-ABL1 kinase domain sequence had 2X coverage.

DNA Sequencing analysis to determine presence of spliceosome mutation

DNA was isolated from peripheral blood granulocytes from seven patients on this study with BCR-ABL1^{35INS}. DNA re-sequencing of coding exons of known mutations in *SF3B1, U2AF1, SRSF2, ZRSR2, SF3A1, PRPF408, U2AF65*, and *SF1* was performed on unamplified genomic DNA.

RESULTS

Patient characteristics—During the time period of this study, 545 patients with CML were seen at MSKCC. Of these, 284 patients (52%) were screened for a BCR-ABL1 kinase domain mutation. Mutation testing was done according to standard indications: no response, slow response, or loss of response[15]; a few patients had testing in the setting of imatinib toxicity prior to changing TKI therapy. Sixty-four five of the 284 patients (23%) were found to have BCR-ABL1^{35INS}; 43 samples (67%) were from peripheral blood and 21 samples (33%) were from bone marrow. Forty of the 64 patients (63%) had the diagnosis of CML made prior to their initial visit at MSKCC. The median time from start of imatinib therapy to detection of BCR-ABL1^{35INS} was 23 months (range 0–233). The median age of all patients with BCR-ABL1^{35INS} was 51years (range 13–76*)* and 36 patients were male (56%) (Table 1).

BCR-ABL1^{35INS} was the first mutation detected in 48 of the 64 patients (75%). Thirteen of the 16 remaining patients had had one prior mutation test that was negative (this includes 3

patients whose first test was a technical failure); 2 patients had had 2 prior mutation tests, and 1 patients had had 3.

At the time of BCR-ABL1^{35INS} detection, 45 patients (70%) were in chronic phase, 10 patients (15%) were in accelerated phase, and 6 patients (9%) were in blast crisis. Two additional patients were in a molecular relapse post stem cell transplant, and one patient had a preceding Philadelphia chromosome negative myeloproliferative disorder. Of the 45 patients in chronic phase, 34 (76%) had begun therapy with imatinib, 6 patients (13%) had begun dasatinib, and 5 patients (11%) had begun nilotinib as first line treatment.

Chromosomal progression at the time of BCR-ABL1^{35INS} detection—A total of 44 patients, or 69% of the 64 patients with the splice variant, had either a recurrent Ph chromosome by karyotype (n=7), FISH (n=26), had an increase in PCR (n=2), or had additional chromosomal abnormalities (n=9) at the time BCR-ABL1^{35INS} was detected (Table 2). Nineteen patients had no change in karyotype, FISH, or PCR, and one patient had no concomitant testing done.

Additional kinase domain mutations—Twelve of the 64 patients (19%) with BCR-ABL1^{35INS} had point mutations in addition to BCR-ABL1^{35INS}; 7 patients were in chronic phase, one was in accelerated phase, 2 had Ph+ ALL, and 2 were in blastic phase (Table 3). Five of the 12 mutations (42%) were T315I and in 3 instances, BCR-ABL1^{35INS} appeared at least 3 months before T315I was noted (UPIN 057, 046, and 026).

Response to imatinib given as first line therapy in chronic phase patients with BCR-ABL1^{35INS}—Of the 34 patients who began imatinib in chronic phase, 23 patients (68%) were considered treatment failures for the following reasons: 8 patients (24%) had primary refractory disease, 11 patients (32%) progressed while on imatinib, and 4 patients (12%) had disease progression after dose interruption. Among the 34 imatinib-treated patients with the splice variant were 7 (21%) who developed CTCAE grade 3–4 toxicity (mostly hematologic)[16] that necessitated a change in therapy. Four slow-responding patients (12%) remain on imatinib, now in a major molecular response (one patient died from lung cancer while on imatinib).

Response to dasatinib or nilotinib given as first line therapy in chronic phase patients with BCR-ABL1^{35INS}—Six patients began dasatinib as first line therapy at the time of BCR-ABL1^{35INS} detection: 3 remain on therapy but only one has a major molecular response. Two patients had a complete molecular response but then progressed and were lost to follow up; another patient had no response and was switched to another TKI. Five patients began nilotinib as first line therapy: 2 patients had no response and were changed to another TKI, one patient progressed, one patient achieved a complete molecular response but was lost to follow up, and one patient remains on therapy with a major molecular response.

Response in patients who relapsed following stem cell transplant with BCR-ABL1^{35INS}—Two patients had BCR-ABL1^{35INS} detected at the time of molecular relapse following stem cell transplant after long disease-free intervals (96 and 165 months). Both are now in a complete molecular remission on nilotinib.

Response to Change in TKI Therapy—Table 4 shows the clinical response in chronic phase patients with BCR-ABL1^{35INS} who had received imatinib as first line therapy and were changed to either dasatinib or nilotinib because of either lack of response, disease progression, or toxicity. Seven patients were changed to dasatinib as second line therapy: 4 of these responded and are maintained on dasatinib (1with a complete molecular remission, 2 with major molecular remissions, and one with a partial molecular remission) and 3 patients stopped dasatinib because of toxicity. Sixteen patients were changed from imatinib to nilotinib as second line therapy: 3 patients responded and maintained their response (one complete molecular response, one major molecular response, and one partial molecular response). Seven patients stopped nilotinib because of toxicity, 2 responded to nilotinib but then progressed, 2 were non-compliant, and 2 were lost to follow up.

Frequency of BCR-ABL1^{35INS} in patients responding to imatinib—In order to determine whether BCR-ABL1^{35INS} was found in patients who were responding to imatinib, 23 sequential samples were obtained from patients who had achieved a complete (n=15) or major molecular response (n=7) on imatinib as first line therapy had peripheral blood testing for mutation analysis; one additional patient had a 2-log reduction after 6 months of imatinib. The median age of this group was 61 years (range 37–82), and the median time from diagnosis to BCR-ABL1^{35INS} testing was 87 months (range 6–151). None of the 23 patients had BCR-ABL1^{35INS} detected.

Levels of BCR-ABL1 ^{53IN5} transcript—RNA levels were estimated from sequencing traces from 30 chronic phase patients who either progressed on or never responded to imatinib and had sequencing tracings available for review. The quantity of BCR-ABL1^{35INS} detected from these patients was variable and ranged from 10% to 100%, average 34% (data not shown).

Results of Spliceosome Mutational Analysis

Sequencing Analysis of Genes Encoding Spliceosomal Proteins in Patients with BCR-ABL1^{35INS}—We searched for mutations in commonly mutated genes encoding spliceosomal proteins; this included mutational analysis for *SF3B1, U2AF1, SRSF2, ZRSR2, SF3A1, PRPF408, U2AF65, and SF1* in seven patients with chronic phase CML and BCR-ABL1^{35INS} who had bone marrow samples available for study. No mutations in any of these genes were found in DNA extracted from granulocytes in any of these patients.

DISCUSSION

Approximately 40–60% of human genes undergo alternative splicing, a process that generates multiple mRNA isoforms from the same gene; however, most isoforms are degraded by the non-sense mediated mRNA decay pathway before being translated into functional proteins.[17–19] Given the recent discovery of mutations in genes which normally encode members of the spliceosome in patients with both myeloid and lymphoid malignancies.[20–23], we wanted to clarify whether the presence of the splice variant BCR-ABL1^{35INS} was a common occurrence in patients who had BCR-ABL1 mutation testing

performed, whether BCR-ABL1^{35INS} was associated with resistance to TKI therapy, and whether a mutation in the spliceosomes was responsible for this variant.

Mutation screening was performed in 284 of the 545 patients with CML (52%) seen at this center during the time period of this study, which while high, is in accordance with the nature of our referral population. The group of patients with the splice variant had a median age, 51 years, which is lower than the median age of patients with CML in the general population, which is 66 years[24]; this may be a function of the relatively small number of patients with *BCR-ABL1^{35INS}* in this study. We found that 64 patients, or 23% of the 284 patients screened for a BCR-ABL1 mutation had BCR-ABL1^{35INS} present which is within the reported mutation rate of other studies in chronic phase imatinib-treated patients.[25] However, in the only other large screening study by Laudadio et al[12] that looked just for BCR-ABL1^{35INS}, the incidence of detection was much lower, only 1.7% of 174 patients tested.[12] This discrepancy may reflect the difficulty in quantifying BCR-ABL1^{35INS} mRNA. Early termination of translation in eukaryotes has been shown to lead to RNA instability[26, 27] and it is possible that BCR-ABL1^{35INS} mRNA is unstable, leading to the low levels detected in our patient samples. Instability of this mRNA may also explain why this splice variant is not reported by many laboratories that test for BCR-ABL1 mutations. Unlike point mutations, where the abnormally spliced BCR-ABL1 is in the genome and all expressed protein is mutated, patients with the abnormally spliced BCR-ABL1^{35INS} mRNA express a mixture of truncated and full length protein at different levels. This may explain the variation in phenotype and the inconsistency in the literature concerning the clinical relevance of this mutation. In our study, 14 of 29 patients (48%) had levels < 20% BCR-ABL1^{35INS} while in the O'Hare study that reported clinical outcomes of 20 patients with BCR-ABL1^{35INS}, 18 of these patients (90%) had < 20% of BCR-ABL1^{35INS} transcripts.[13]

Our results also suggest that the presence of BCR-ABL1^{35INS} is associated with imatinib resistance; of the 34 patients with BCR-ABL1^{35INS} who had begun imatinib as first line therapy, 23 patients (68%) had either had no response, had a response but then progressed while on imatinib, or interrupted their treatment (Table 1). It is important to note that note that compared to imatinib-treated patients who progressed/did not respond to imatinib, none of the 23 sequentially tested patients who were responding to imatinib had BCR-ABL1^{35INS} detected ($p<0.001$ by Fisher's exact test). Too few patients with BCR-ABL1^{35INS} had begun therapy with either dasatinib (n=6) or nilotinib (n=5) to be able to state whether these second generation TKIs can overcome this resistance.

Adding support to this conclusion is the finding that chromosomal progression was detected in 44 of the 64 patients (69%) simultaneously with BCR-ABL^{35INS}. As shown in Table 2, seven patients had recurrence of the Ph chromosome by karyotype, 26 patients by FISH, and 2 by PCR. Nine patients had chromosomal abnormalities in addition to the Ph chromosome including a double Ph, + 8, + 17, among others.

The presence of an additional point mutation that could also explain resistance was found in 12 of the 64 patients (19%) with BCR-ABL1^{35INS}, although 5 of the 12 patients were in either accelerated or blastic phase, or had Ph+ acute lymphoblastic leukemia, settings where additional mutations are frequently seen (Table 3). Five of these 12 patients had the T315I

mutation; interestingly, 2 of these patients had BCR-ABL1^{35INS} detected before the T315I mutation as found.

In the only other report that examined clinical response in patients with BCR-ABL1^{35INS}, only 4 of 20 patients (19 in chronic phase) who began imatinib as first line therapy were felt to have BCR-ABL1^{35INS} as the sole cause of TKI resistance; of these, 3 had already been changed to dasatinib when BCR-ABL1^{35INS} was detected.[13] One possible explanation for the discrepancy between this study and ours is we studied more than three times the numbers of patients with BCR-ABL1^{35INS}.

Our study had several limitations. The first is that because more than 60% of our patients had the diagnosis of CML made and TKI therapy begun at an outside institution, we were unable to determine whether BCR-ABL1^{35INS} was present at diagnosis. However, previous studies have shown that the incidence of kinase domain mutations is low at diagnosis and does not appear to correlate with response to imatinib with the exception of the T315I mutation.[28, 29] It is also not possible to determine whether dasatinib or nilotinib are effective in this particular group of imatinib-resistant patients as only a relatively small number of patients could be assessed. We did not screen patients who were responding to either dasatinib or nilotinib for BCR-ABL1^{35INS}. Our rational was that since the depth of response is deeper on second generation TKIs[30, 31], and because we did not find BCR-ABL1^{35INS} among the 23 patients who were doing well on imatinib, the likelihood of finding this mutation in these two additional populations was extremely low.

Currently, we have little insight into the mechanism(s) of resistance associated with BCR-ABL1^{35INS}. O'Hare et al[13] showed that BCR-ABL1^{35INS} did not confer resistance to imatinib in stably transfected Ba/F3 and K562 cells; however, the possibility remains that the splice variant of BCR-ABL1 may confer TKI resistance in the presence of wild type BCR-ABL1, a hypothesis which has not been directly tested in prior work. Indeed, precedence for splice variants in kinase proteins resulting in increased dimerization and activation of signaling has recently been published. Poulikakos et al[32] showed that p61BRAF^{V600E}, a splice variant that is a truncated version of BRAF^{V600E}, has an increased propensity to form dimers and is associated with resistance to the BRAF inhibitor vemurafenib. Moreover, when p61BRAF^{V600E} is expressed in the presence of full-length BRAF^{V600E}, ERK signaling becomes resistant to pharmacologic BRAF inhibition.

In summary, results from this study show that the presence of the splice variant BCR-ABL1^{35INS} is a common finding in patients who do not respond or lose their response to imatinib, particularly after a period of non-compliance, and should alert the clinician that a change in TKI therapy may be warranted. It is not yet clear whether the use of dasatinib or nilotinib allows for a better therapeutic response. Mutations in the spliceosome do not appear to contribute to its presence, at least in the spliceosomes components studied. We suggest that the association of BCR-ABL1^{35INS} with imatinib resistance as shown here calls for a re-evaluation of the possible mechanism of resistance associated with expression of this splice isoform.

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Highlights

- The splice variant BCR-ABL1^{35INS} is associated with imatinib failure
- 69% of patients with BCR-ABL1^{35INS} had a simultaneous recurrent Ph chromosome
- BCR- ABL1^{35INS} was not seen in patients who were responding to imatinib
- Mutations in spliceosomal proteins do not contribute to this association.

Table 1

Patient Characteristics

Total # pts screened for BCR-ABL1 mutation[1]	**284**
# Pts with BCR-ABL^{135ins}	64 (23%)
Median age (range)	51 (13–76)
Males no. (%)	36 (56%)
CML phase at time of BCR-ABL^{135ins} detection	
Chronic	45 (70%)
Accelerated	10 (15%)
Blastic	6 (9%)
Other[2]	3 (5%)
Time (mos) from diagnosis of CML to detection of BCR-ABL^{135ins}	23 (0–233)
TKI used 1st line in chronic phase	
Imatinib	34 (76%)
No response	8 (22 %)
Response-progressed	11 (33%)
Non compliant-progressed	4 (11%)
Total # patients	23(68%)
Toxicity-changed TKI	7 (19%)
Remain on imatinib[3]	4 (11%)
Dasatinib	6 (13%)
Nilotinib	5 (11%)
Ponatinib	0

[1] Between 8/1/2007–1/15/2014

[2] Two pts had prior stem cell transplant, one had prior Philadelphia chromosome negative myeloproliferative neoplasm

[3] One additional patient died from lung cancer while on imatinib

Table 2

Chromosomal or Molecular Progression While on Therapy at the Time of BCR-ABL^{35INS} Detection

	Peripheral Blood	Bone Marrow	Total n=64	
Increase in Ph by karyotype only	0	7	7	n=44
Increase in Ph by FISH only	21	5	26	
Increase in PCR only	1	1	2	
No. Pts with additional chromosomal abnormalities[1]	1[2]	8	9	
No change in FISH or PCR	13	6	19	
No additional testing done	1	0	1	

[1] 46,XX, t(9;22)(q34.1;q11.2), t(10;12)(p11.2;q21)[3]; 46,XX, t(10;12)(p11.2;q21)[17]

46,XX, t(9;22)(q34.1;q11.2), der(6)t(3;6) (q21;p23)[19] 46,XX, t(9;22)(q34.1;q11.2)[1]

46,XY, t(9;22)(q34.1;q11.2), t(1;5)(q25;q13)[20]

46,XX, t(9;22)(q34.1;q11.2) [18];47,XX,+8[2]

49,XY,+8,t(9;22)(q34.1;q11.2), +17,der(22),t(9;22)(q34.1;q11.2) [4]/46,XY[16]

46,XX, der(9) t(9;22)(q34.1;q11.2)t(6;18;22)(p21;q11.2;q12)[19]/46,XX[1]

48,XX,t(3;21)(q26;q22), ider(22)(q10) t(9;22)(q34.1;q11.2), +12,+ider(22)t(9;22)(q34.1;q11.2)[20]

[2] Peripheral blood FISH: double Ph chromosome 250/500 cells (50%)

48,XY,+8, ider(22)t(9;22)(q34.1;q11.2) +19[20]

Table 3

Patients on Imatinib with BCR-ABL1^{35INS} and Additional Mutations

UPIN	Date Dx CML	Phase at time of BCR-ABL^{ins35}	Date BCR-ABL^{ins35}	Point Mutation/Date	Setting
008	1/1994	CP	6/2009	E450E; 6/2009	Progression on IM
013	1/2006	CP	11/2009	E255V; 11/2009	Progression on IM
031	1/2002	CP	6/2011	V299L: 6/2011	Progression on IM
051	1/2012	CP	10/2012	K247R; 10/2012	Slow response to IM
053	10/2007	CP	4/2013	Y449H; 4/2013	Slow response to IM
058	3/2013	CP	11/2013	K271R; 11/2013	Transient increase in PCR
057	11/2011	CP	7/2013	T315I; 11/2013	Progression on IM
012	1/1999	AP	9/2009	E355G; 9/2009	No response to IM or DA
046	10/2010	Ph+ ALL	7/2012	V299L 7/2012; T315I; 12/2013	Progression on DA + decadron
058	6/2013	L-BC	10/2013	T315I 10/2013	Dx of L-BC
062	5/2013	Ph+ ALL	12/2013	T315I; 12/2013	Dx of L-BC
037	7/2008 (CP)	M-BC	9/2011	T315I; 1/2012	No response to chemotherapy +DA

IM: imatinib

DA: dasatinib

CP: chronic phase

AP: accelerated phase

L-BC: lymphoid blast crisis

M-BC: myeloid blast crisis

Ph+ ALL: Philadelphia chromosome positive acute lymphoblastic leukemia

PCR: polymerase chain reaction

Table 4

Response to Dasatinib or Nilotinib After Progression or Lack of Response to Imatinib in Patients with BCR-ABL1^{35INS}

	Dasatinib n=7	Nilotinib n=16
Maintained response	4 (1CMR, 2 MMR; 1PMR)	3 (1 CMR, 1MMR; 1 PMR)
Stopped 2^0 toxicity	3	7
Responded, then progressed	0	2
No response	0	0
Lost to follow up	0	2
Non-compliant	0	2

PMR: partial molecular response

MMR: major molecular response

CMR: complete molecular response