Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain

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ABSTRACT

The emergence of resistance to the Bcr-Abl inhibitor imatinib mesylate in patients with Philadelphia chromosome-positive (Ph^+) leukemia has prompted the development of second-generation compounds active against several imatinib-insensitive mutant forms of Bcr-Abl, including dasatinib (BMS-354825; Bristol-Myers Squibb). In order to assess which pre-existent or emerging kinase domain mutations are associated with decreased clinical efficacy of desatinib, we analyzed BCR-ABL kinase sequences before and during treatment in 21 Ph^+ patients who failed to respond to or relapsed during dasatinib therapy. In all patients but one, resistance to dasatinib was invariably found to be associated with mutations at residue 315 and/or at residue 317.

Key words: chronic myeloid leukemia, acute lymphoblastic leukemia, imatinib, dasatinib, resistance, Bcr-Abl, mutations

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The striking efficacy of imatinib mesylate in chronic myeloid leukemia (CML)[1,2] has established this therapy as the new standard of care for the disease.[3] However, resistance is an emerging problem which has prompted the design of several second-generation Bcr-Abl inhibitors. One of these, dasatinib (BMS-354825; Bristol-Myers Squibb), is now in advanced clinical development.[4] *In vitro* assays[5,6] and crystallographic studies[7] have suggested that the less stringent conformational requirements for Bcr-Abl binding are likely to render dasatinib active against many of the kinase domain mutants responsible for imatinib resistance. One remarkable exception appears to be the T315I, which has been shown to disrupt a hydrogen bond critical for dasatinib binding and to create steric hindrance which interferes with the entrance of the inhibitor into the ATP-binding site. In order to assess which pre-existent or emerging mutations are associated with decreased clinical efficacy of dasatinib, we analyzed BCR-ABL kinase domain sequences before and during treatment in Philadelphia chromosome-positive (Ph^+) leukemia patients who failed to respond to or relapsed during dasatinib therapy.

Design and Methods

In a phase II program (sponsored by Bristol-Myers Squibb) we treated with dasatinib 70 mg twice daily a total of 45 patients with either CML (n=35) or Ph^+ acute lymphoblastic leukemia (ALL) (n=10) who were resistant to or intolerant of imatinib. Their median age was 50 years (range, 18-74), the median duration since the diagnosis of CML was 32 months (range, 4-158); and the median duration of imatinib treatment was 17 months (2-57). At the time of writing, with a median follow-up of 12 months (range, 1-19), 21 patients have shown evidence of either primary or acquired resistance, defined as a failure to achieve a hematologic response or loss of hematologic response during treatment, respectively. All the patients provided written informed consent to their participation in this study. Hematologic response was assessed according to the criteria already described for the phase I study.[4] Cytogenetic

Table 1. Summary of patients' characteristics and response to dasatinib according to mutation status.

Pt no.	*Sex*	*Age*	*Disease phase or type*	*Reason for imatinib discontinuation*	*Mutation status before starting dasatinib*	*Best HR on dasatinib*	*Best CgR on dasatinib*	*Months on dasatinib*	*Mutation status at progression*
					Primary resistance				
1	M	48	CML/AP	resistance	WT	NR	NR	4	T315I
2	F	61	CML/AP	resistance	T315I	NR	N.E.	1	T315I
3	F	33	CML/myBC	resistance	T315I	NR	N.E.	1	T315I
4	M	62	CML/myBC	resistance	F317L	NR	NR	3	F317L
5	F	38	CML/lyBC	resistance	T315I	NR	N.E.	1	T315I
6	F	21	Ph$^+$ ALL	resistance	T315I, M351T, L387M	NR	N.E.	2	T315I, M351T, L387M
7	M	40	Ph$^+$ ALL	resistance	T315I	NR	N.E.	1	T315I
8	M	37	Ph$^+$ ALL	resistance	F359V	NR	N.E.	2	F359V, T315I
					Acquired resistance				
9	M	35	CP	intolerance	WT	CHR	mCgR	15	T315I
10	F	60	CML/myBC	intolerance	G250E	NEL	NR	8	F317L
11	M	25	CML/lyBC	resistance	Y253H	CHR	CCgR	9	T315I
12	M	27	CML/lyBC	resistance	WT	CHR	CCgR	4	T315I
13	F	26	CML/lyBC	resistance	E255K	CHR	NR	3	E255K, T315I
14	F	60	CML/lyBC	intolerance	D276G	CHR	CCgR	7	T315I
15	M	37	CML/lyBC	resistance	WT	RTC	PCgR	9	F317L
16	M	41	Ph$^+$ ALL	resistance	E255K	CHR	N.E.	4	T315I
17	M	18	Ph$^+$ ALL	resistance	Y253H	CHR	CCgR	13	T315A, F317L
18	M	64	Ph$^+$ ALL	resistance	M351T	CHR	CCgR	13	M351T, F317L
19	M	73	Ph$^+$ ALL	intolerance	WT	CHR	CCgR	6	F317I
20	F	55	Ph$^+$ ALL	intolerance	WT	CHR	CCgR	4	K356R
21	F	63	Ph$^+$ ALL	resistance	L387M	CHR	N.E.	5	F317L, L387M

Patients were treated with dasatinib 70 mg twice daily. Definitions of hematologic and cytogenetic response as reported in[4] Pt, patient; M, male; F, female; AP: accelerated phase; myBC, myeloid blast crisis; lyBC, lymphoid blast crisis; WT, wild-type; NR, no response; N.E., not evaluated; CHR, complete hematologic response; NEL, no evidence of leukemia; RTC, return to chronic phase; CCgR, complete cytogenetic response; PCgR, partial cytogenetic response; mCgR: minor cytogenetic response. Patients no. 4, 10, 15, 18 and 21 were the subject of a previous report.[11]

response was assessed on 30 bone marrow metaphases using standard banding techniques.[8] Mutation analysis of the BCR-ABL kinase domain was performed as previously reported, with minor modifications.[9,10] Briefly, after total RNA extraction from mononuclear cells and reverse transcription, three overlapping fragments covering the entire kinase domain (amino acids 206 through 524) were generated by nested polymerase chain reaction and screened for the presence of sequence variations by denaturing-high performance liquid chromatography (D-HPLC)(WAVE 3500-HT; Transgenomic, Cramlington, UK). In D-HPLC-positive cases, subsequent sequencing was performed on an ABI PRISM 3730 (Applied Biosystems, Foster City, CA, USA) to characterize the precise nucleotide substitution(s). Five of these patients (#4, 10, 15, 18 and 21 in Table 1) have been previously described.[11]

Results and Discussion

Eight patients had primary resistance to dasatinib (patients #1-8 in Table 1 and Figure 1). In all these patients, a T315I or a F317L mutation was already detectable before the onset of treatment or became detectable after 1 month. The mutations persisted up to the time of disease progression (Figure 1), which occurred at a median of 1.5 months (range, 1-4) after starting dasatinib despite the fact that in five out of eight cases (patients #1, 3, 4, 6 and 8; Figure 1), a dose increase to 90 or 100 mg twice a day was attempted.

Thirteen patients had acquired resistance to dasatinib (patients #9-21 in Table 1 and Figure 1). Relapse occurred a median of 7 months (range, 3-15) after starting dasatinib. Mutation analysis performed before the onset of treatment showed that five of these patients had no evidence of nucleotide changes, while the remaining eight patients harbored various imatinib-resistant kinase domain mutations (G250E, Y253H, E255K, D276G, M351T, L387M; Table 1 and Figure 1). At the time of relapse, however, most of the original mutant clones had disappeared whereas mutations either at codon 315 or at codon 317 had invariably emerged in 20/21 patients (Table 1 and Figure 1). Interestingly, in two of these cases relapse was associated with the selection of novel amino acid substitutions – a threonine to alanine change at codon 315 (T315A) and a phenylalanine to isoleucine change at codon 317 (F317I). In the remaining patient (#20 in Table 1 and Figure 1) a previously unreported K356R mutation was detected.

These data complement *in vitro* observations[2,3] as well as structural studies,[4] confirming that the T315I mutation is highly resistant to dasatinib. In imatinib-resistant/intolerant CML or Ph$^+$ ALL patients, the T315I accounted for dasatinib treatment failure in 13 of 21 cases. All the five

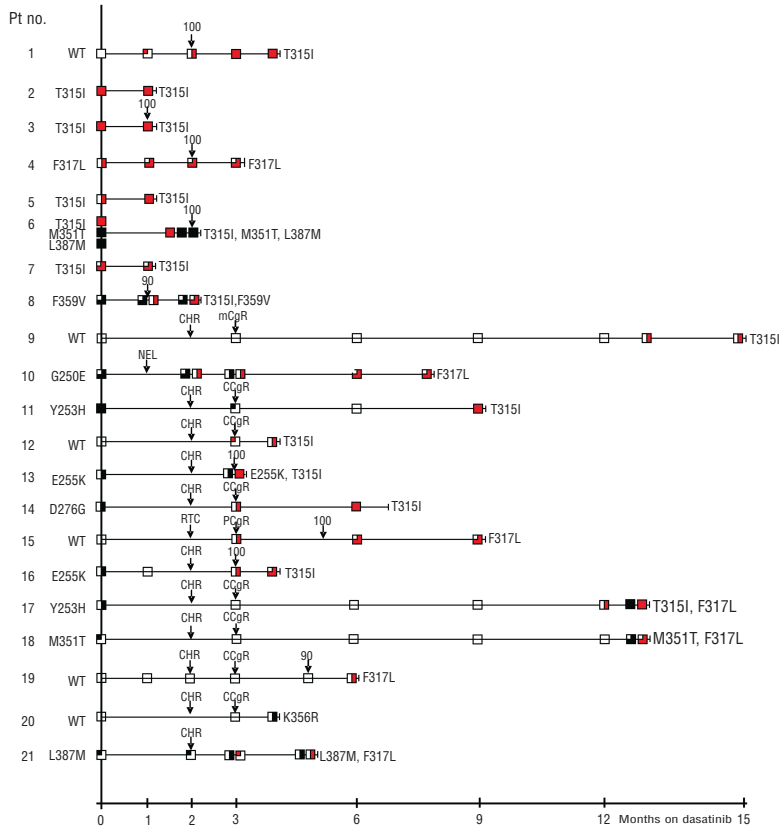

Figure 1. Mutation monitoring and follow-up of patients. Open squares represent wild-type ABL; full squares represent mutated ABL, as detected by direct sequencing. Mutations at codons 315 and 317 are highlighted in red. The degree of shading indicates the relative proportion of mutant with respect to wild-type, as estimated by relative peak heights in sequence chromatograms. Dasatinib was started at 70 mg twice daily; dose increases to 90 or 100 mg twice daily were attempted in nine cases, as indicated. The times the best hematologic and cytogenetic responses were achieved are indicated by an arrow. Abbreviations as in Table 1.

patients (#2, 3, 5, 6 and 7 in Table 1 and Figure 1) who already had evidence of a T315I before the onset of dasatinib – at least by direct sequencing – failed to achieve any response. In the remaining eight patients, the T315I outgrew during treatment. In patients #1 and 8 it became detectable after only 1 month of therapy and no hematologic response could be observed. In patients #9, 11, 12, 13, 14 and 16, the T315I emerged after the patient had achieved a hematologic and in most cases even a cytogenetic response, and invariably preceded or accompanied relapse. Interestingly, an additional patient (#17) was found to harbor a variant amino acid substitution at codon 315 (T315A) at the time of relapse. The T315A has never been reported in patients, but has recently been identified in an *in vitro* saturation mutagenesis screening for Bcr-Abl mutants conferring resistance to dasatinib.[12] Ba/F3 cells expressing the T315A-Bcr-Abl were shown to have a 90-fold higher IC_{50} with respect to those expressing unmutated Bcr-Abl when incubated with dasatinib.[12] Of note, the increase in imatinib IC_{50} for this mutant was only 2.4-fold, suggesting that in such a case resuming imatinib alone or in combination might prove effective. In our patient, rechallenge with imatinib at the dose of 800 mg/die was actually attempted after dasatinib discontinuation and resulted in a reduction of leukocytosis and a decrease of the proportion of T315A- and F317L-positive cells, but at the same time promoted the rapid selection of an additional G250E mutation. For this reason, and because of disease persistence, imatinib was withdrawn after 7 weeks. The patient is now being treated with nilotinib which produced normalization of the blood cell count, but the T315A and the G250E were still detectable after 2 months of treatment.

In six of 21 patients, lack of response or relapse were strikingly associated with the presence or the selection of a mutation at codon 317 (F317L in five cases, F317I in one case). Co-crystal studies have demonstrated that the aromatic ring in the side chain of phenylalanine 317 directly interacts with the pyrimidine and thiazole rings of dasatinib.[7] Accordingly, several amino acid substitutions affecting residue 317 were observed in the *in vitro* saturation mutagenesis screening for dasatinib-resistant mutants, including both the imatinib-resistant F317L and presently unreported variants such as F317V, F317I and F317S.[12] The F317L has been shown to induce a 9 to 13.5-fold increase of dasatinib IC_{50} in cellular assays.[5,6,12] In two patients, a dose increase from 70 to 90 or 100 mg twice daily was attempted but this did not succeed in eliminating the F317L/I-positive mutant clone.

Finally, in one patient of our series (#20) a K356R mutation became detectable by direct sequencing at the time of relapse. This mutation has never been reported, either *in vitro* or *in vivo*, in association with imatinib or dasatinib resistance. It is unclear whether in this patient the emergence of the K356R was the actual determinant of resistance, or some other mechanism might rather have intervened and ultimately determined the expansion of the Bcr-Abl-positive cells harboring the mutation. In some

cases (patients #10, 11, 14, 16 and 17), pre-existing dasatinib-sensitive mutant clones (i.e., E255K, Y253H, D276G) were selectively eliminated, but soon replaced by a newly-emerged dasatinib-resistant T315I- or F317L-positive clone. In other cases (patients #8, 13, 18 and 21), the T315I- or F317L-positive cells were selected by dasatinib treatment as a subclone of the original, imatinib-resistant mutated clone, since both the original and the newly outgrown mutations were detected on the same allele (*data not shown*). Given that this was invariably observed in advanced CML and Ph+ ALL cases, it can be hypothesized that in this clinical setting the high rate of genomic instability may foster the development over time of multiple mutations within the same or in different Bcr-Abl-positive sub-clones, which will then be selected or de-selected depending on the spectrum of sensitivity and resistance to the inhibitor employed.

At the time of relapse, resistance-associated mutations were clearly detected by direct sequencing since they always accounted for at least 50% of the Bcr-Abl-positive cells (Figure 1). However, the more sensitive D-HPLC analysis[9,10] could in some cases (patients 9, 11, 19, 20 and 21) detect the emergence of the mutations 1 to 3 months earlier than sequencing (*data not shown* in Figure 1), thus proving a particularly valuable tool for monitoring imatinib-resistant patients treated with second-generation inhibitors.

The T315I is usually observed in approximately 15% of Ph+ leukemia patients who are resistant to imatinib,[13-15] but is likely to become the prevalent mutation in those who fail to benefit from second-line treatment with dasatinib or other novel inhibitors for which threonine 315 is a critical binding residue.[16] At present, there is only one compound (MK-0457, also known as VX-680) in clinical development in the field of leukemias that has been documented to be effective in CML and Ph+ ALL patients harboring the T315I mutation.[17] MK-0457 has been shown not to require interaction with threonine 315 for efficient binding and inhibition in recent co-crystal studies.[18] Observation of a larger series of patients is required to assess whether the F317L will turn out to be another problematic mutant in dasatinib-treated patients.

Authors' Contributions

SS, GM, GR: designed the research; SS, SC, AG, GM, SL, NT, II: performed the research; FC, GR, CB, SP, MR, PPP, FP, PG: collected clinical data; SS, SC, AG, DC and GS: analyzed the data; SS, GM, MB: wrote the manuscript; MB gave final approval for submission.

Conflict of Interest

The authors reported no potential conflicts of interest.

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