Clinical outcome of 27 imatinib mesylate-resistant chronic myelogenous leukemia patients harboring a T315I BCR-ABL mutation

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ABSTRACT

We analyzed 27 CML patients treated with imatinib (IM) who developed a BCR-ABL^{T315I} mutation. These patients had poor prognostic features: High or intermediate Sokal index (82%), and lack of CCyR under IM (59%). At T315I discovery, patients were in advanced phase (59%), with clonal evolution (84%). Median time since diagnosis was 39 months, and progression occurred 13 months after IM initiation, regardless of disease phase. Overall survival since IM initiation was 42.5 months for chronic, and 17.5 months for advanced phases, and all patients progressed. This mutation seems related to or (partially?) responsible for progression and poor survival.

Key words: CML, imatinib mesylate, BCR-ABL mutation, T315I

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Imatinib mesylate revolutionized the management of chronic myelogenous leukemia and represents the new standard of care for this disease.[1] Most patients retain a residual disease and increasing numbers of resistant patients accumulate. The onset of ABL point mutations is the most frequent identified mechanism responsible for resistance.[2] Up to 30 different mutations have been described, mostly in advanced phase,[3] and confer different levels of clinical resistance. In this setting, trials with second generation tyrosine kinase inhibitors (TKIs)[4,5] provide encouraging results, however neither *in vitro* nor clinical activity was demonstrated when a T315I was identified.[6,7] In this multicentric retrospective study, we analysed 27 BCR-ABL^{T315I} mutated CML patients exhibiting either clinical, cytological, cytogenetic resistance or molecular progression.

Design and Methods

BCR-ABL^{T315I} mutations have been identified in the 5 different laboratories and clinical data were retrospectively collected according to each centre ethical guidelines. Resistance was defined according to the ELN guidelines[3] and if any hematological, cytogenetic or molecular progression (*i.e.* increasing BCR-ABL/ABL ratio ≥2 logs) of CML in a previously IM-responsive patient was observed.

The mutation was detected on blood samples analyzed according to the methods described by Branford *et al.*[8] by direct sequencing (on both strands) encompassing the entire ABL kinase and ATP-loop domains [residues 242-487].

Survival and time-to-progression curves were established from IM initiation to death or progression, according to Kaplan-Meier method. Progression was defined as any loss of previous response, and transition to a more advanced disease phase.

Calculations were performed using S-plus program (Insightful, Seattle, WA, USA).

Results and Discussion

Twenty-seven CML patients [17 males, 10 females, median age at diagnosis 52 (25-70) months] harboring a BCR-ABL^{T315I} mutation

Table 1. Main characteristics of the 27 CML patients harboring a BCR-ABL^{T315I} mutation. F stands for female, M for male. CP stands for chronic phase, Acc P for accelerated phase, MBC for myeloid blast crisis, LBC for lymphoid blast crisis. H, I, and L stand for high, intermediate and low Sokal scores respectively. CHR stands for complete hematologic remission, PCyR for partial cytogenetic remission, CCyR for complete cytogenetic remission. NA: data not available. This table has been generated by Dr F-E. Nicolini from the raw database.

UPN#	*Age (y)*	*Sex*	*Phase at diagnosis*	*Sokal index*	*Karyotype at diagnosis with IFN**	*Treatment IM-Prog**	*Interval response to IM*	*Best at T315I*	*Phase Mutation identified*	*BCR-ABL after T315 identification**	*Survival at latest follow-up*	*Outcome since IM initiation**	*Follow-up*
1	52	F	CP	NA	NA	49	29	CHR	CP	T315I	7.5	Deceased	29
2	53	M	CP	I	NA	NA	39	CCyR	CP	T315I	27.5	Alive	44.5
3	52	F	CP	L	46,XX,t(9;22)[20]	112	3	CHR	LBC	T315I	9	Deceased	112
4	61	M	CP	H	46,XY,t(9;22;12)(q34;q11;q11)/ 48,idem,+8,+der(22)	6	32	CHR	CP	T315I /Y253H	41	Alive	48
5	37	M	CP	L	46,XY,t(9;22)(q34;q11)[20]	10	12.5	PCyR	LBC	T315I	11	Deceased	24
6	54	M	CP	NA	46,XX, t(9;22)(q34;q11)[22]	131	10	CHR	CP	T315I	18	Deceased	48
7	55	M	CP	I	NA	6	12	CHR	CP	T315I /M351T	7	Deceased	12.5
8	56	M	CP	NA	NA	9	18	CHR	CP	T315I /M351T	1	Deceased	30
9	53	F	CP	NA	46,XY,t(3;7;12),t(9;22) (q34;q11)[20]	20	3	CP	MBC	T315I	7	Deceased	12.5
10	52	M	CP	H	45,X,-Y,t(9;22)(q34;q11)[20]	4	5	CHR	AccP	T315I	13	Deceased	18
11	58	F	CP	H	46,XX, t(9;22)(q34;q11) [20]	8	11	CHR	AccP	T315I	28	Deceased	43
12	52	M	MBC	NA	NA	0	NA	NA	MBC	T315I	3	Deceased	7
13	50	M	CP	I	NA	NA	0	CHR	MBC	T315I	5	Deceased	5
14	58	M	MBC	NA	NA	0	2	CHR	MBC	T315I/E255K/E255V	0.5	Deceased	2
15	48	M	CP	NA	46,XY,t(9;22),t(13;14)[20]	64	20	CCyR	LBC	T315I	4.5	Deceased	8.5
16	25	F	CP	H	ND	0	14	CHR	LBC	T315I	0.5	Deceased	23.5
17	56	F	CP	NA	46,XX,t(9;22)(q34;q11)[20]	84	25	CCyR	AccP	T315I	7	Alive	65
18	28	M	CP	L	NA	NA	50	CHR	Acc P	T315I	29	Deceased	61
19	34	M	CP	H	NA	0	20	CCyR	CP	T315I	7	Alive	28
20	43	M	CP	H	46,XY,t(9;22)(q34;q11)[20]	9	12	CHR	CP	T315I	0.5	Alive	28
21	60	F	CP	NA	NA	NA	5	CHR	MBC	T315I	0.5	Deceased	10
22	53	M	CP	I	46,XY,t(9;22),(q34;q11)[20]	NA	22	PCyR	MBC	T315I /L324Q	1	Alive	36
23	35	F	CP	I	46,XX,t(9;22),(q34;q11)[30]	35	10	None	CP	T315I	1	Alive	37
24	57	F	CP	I	46,XX,t(9;22),(q34;q11)[20]	35	18	CCyR	LBC	T315I /F311L	15	Deceased	57.5
25	38	F	CP	NA	NA	3	36	CHR	CP	T315I	1.5	Alive	47
26	70	M	CP	H	45,XY,-7,t(9;22)(q34;q11) [20]/46,XY[3]	0	0.5	CCyR	MBC	T315I	6	Deceased	9
27	48	M	CP	H	46,XY,t(9;22)(q34;q11)[9]/47, XY,idem,+der(22)t(9;22)[11]	0	15,2	CCyR	CP	T315I	1	Alive	24

** Months*

were recorded. The patients' characteristics are summarized in Table 1. All patients were in chronic phase (CP) at diagnosis with a majority of unfavorable Sokal scores [high and intermediate for 8 and 6 patients respectively and low for 3 patients, among 17 evaluable (82%)]. Despite the limitations of such a retrospective analysis on a small series of patients, this repartition remains curious and possibly related to this population of patients harboring a BCR-ABL^{T315I} mutation, because in France scores are high for 21%, intermediate for 39% and low for 40% of patients (FE. Nicolini, French CML registry, personal communication 2005). All patients had *M*-BCR transcripts except 2 harboring *m*-BCR transcripts, and 3 unknown. Strikingly, at CML diagnosis, 6/15 evaluable patients had additional chromosomal abnormalities such as a variant Ph1+trisomy 8+Ph1 duplication, a chromosome 7 deletion, a chromosome Y deletion, a Ph1 duplication alone, and 2 patients had additional translocations: t(3;7;12), t(13;14) (UPN#4, 9, 26, 10, 27, 9 and 15, respectively). The median time between diagnosis and IM initiation was 20 (0-145.2) months with a majority of patients treated with interferon prior to IM (17/25 evaluable patients, 68%) for a median time of 9 (0-112) months. The initial median dose of IM was 464 mg/day and all but 3 [1 AP, 2 blast crisis (BC)] were in CP at IM initiation. Most patients were poor responders to IM,[3] with, no responses in 2 (7.5%), 14 (52%) CHR, 2 (7.5%) PCyR, 9 (33%) CCyR, 2 of whom 2 were in major molecular response. With a median follow-up of 21 months. Since IM initiation, and worryingly, all patients progressed after a median of 13 (0-49.6) months for all phases (Figure 1A), with 11 (41%) patients in CP and 16 (59%) in advanced phase [4 accelerated, 12 (7 myeloid + 5 lymphoid) BC] at progression. Time-to-progression

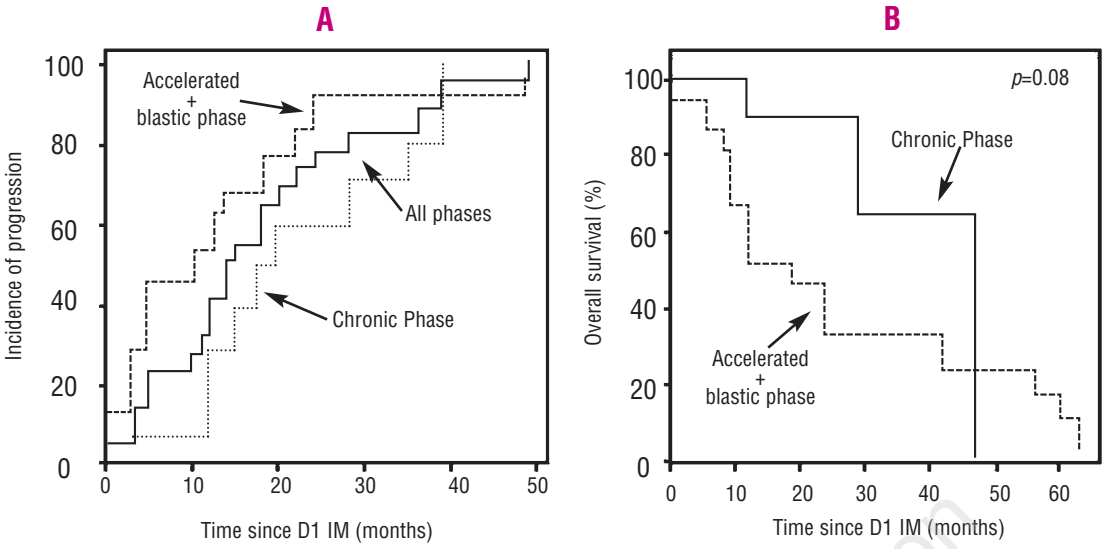

Figure 1. (A) Time to progression of CML since IM initiation, in patients harboring a BCR-ABLT315I mutation. Plain line represents all phases of CML, dotted line chronic phase only and dashed line accelerated + blastic phases at onset of T315I mutation. (B) Kaplan-Meier overall survival since initiation of IM in patients harboring a BCR-ABL^{T315I} mutation, broken down by disease phase at onset of T315I mutation. These 2 figures have been generated through a S-plus program by Dr. *Q-H. Lê* and reformatted to Powerpoint by *F-E. Nicolini*.

was significantly longer for patients in CP (18 months) versus advanced phases (12 months, p=0.04). Twelve/15 evaluable patients (80%) presented a clonal evolution at T315I identification (4 of which were already present at diagnosis) with no significant impact on survival (p=0.22). Likewise, the T315I mutation was discovered with additional mutations in 6 patients (1 Y253H, 2 M351T, 1 E255K and E255V, 1 L324Q, and 1 F311L), but these had no impact on overall survival (p= 0.91).

In this retrospective analysis, fourteen/18 (78%) evaluable patients were found challenged with higher doses of IM (600-800 mg/day), with one return to CP and one transient CCyR after IM combined with chemotherapy, and 12 failures. Six patients (1 CP, 5 BC) were treated with dasatinib, and no difference in survival was seen between dasatinib-treated and non-treated patients (p=0.15). None of the patients received nilotinib. Additionally, 3 patients underwent allogeneic stem cell transplantation with 2 remaining alive at 1 and 14 months follow-up. Finally, at latest follow-up, overall survival since IM initiation (Figure 1B), however longer for CP (42.5 Mo.) was not statistically different than that for AP+BC (17.5 Mo., p=0.08) patients.

The onset of BCR-ABL^{T315I} mutations during the treatment of CML with TKIs remains challenging, because this mutation is the most frequently identified in IM–treated patients[6], and none of the TKIs clinically available to date[4,5,6] retain any activity *in vitro*. More over, the continuation of these inhibitors might elicit the outgrowth of T315I clones as shown in the past.[9,10,11] In 5/6 patients receiving dasatinib, the BCR-ABL^{T315I} mutation was assessed (through direct sequencing method) and absent before the introduction of this TKI, but present after a median of 4 months. The PCR-ASO was retrospectively performed in 2 patients (UPN#16 and 17) and demonstrated that the *BCR-ABL^{T315I}* mutation was already present at low levels before dasatinib. Two/6 patients remained alive at latest follow-up, as dasatinib was immediately withdrawn when the T315I was identified. Thus, if a *BCR-ABL^{T315I}* mutation is identified in a CML patient, TKI treatment withdrawal should be the first therapeutic intervention recommended.

Despite low number of patients and the limitations of such an analysis, we were able to show in this study that the onset of *BCR-AB^{LT315I}* mutations occurs in a category of CML patients with poor initial prognostic features (poor Sokal scores, additional chromosomal abnormalities, poor response to IM), mostly in advanced phases. It is unknown whether the presence of a BCR-ABL^{T315I} mutation is actually responsible for progression or if this mutation simply co-migrates with other general factors of progression such as clonal evolution or additional mutations. The first hypothesis is supported by *in vitro* studies[12] which demonstrate that a BCR-ABL mutation provides the mutated clone with a proliferative advantage over BCR-ABLWT cells and favors disease transformation potency. In our study, it is of note that 3/16 evaluable patients (UPN# 11, 17, 19) had neither chromosomal abnormalities other than the Ph1, nor additional mutation at T315I identification. In addition, previous studies[13,14] have failed to detect c-kit and PDGF-R mutations in patients resistant to IM, suggesting that disease progression might be induced solely by BCR-ABL modifications. However, additional genetics abnormalities affecting other not-investigated/unknown BCR-ABL independent mechanisms of IM resistance/progression

cannot be excluded. Furthermore, in addition to the disruption of IM binding, it is debatable if a *BCR-ABL*^{T315I} mutation increases ABL tyrosine kinase activity and enhances progression as suggested by some authors[15] though not by others[12]. The second hypothesis is supported by crystallographic analysis[16] showing that the lack of the critical hydrogen bond that disrupts IM binding during T315I residues exchanges is associated with the modification of multiple other protein-drug interactions that could participate to the deregulation of ABL tyrosine kinase activity. Others have shown[15] that various intracellular proteins, including STAT-5, might be altered in *BCR-ABL*$^{T315I+}$ cells.

In conclusion, despite the obvious limits of such a retrospective study, we show that the *BCR-ABLT*^{315I} mutation occurs probably in CML patients with poor initial prognostic features, and at high risk of disease progression. Subsequently, serial BCR-ABL mutation assessments throughout the course of the disease is recommended for IM-resistant patients for early detection of the BCR-ABL mutations.

Simple TKI withdrawal might-at least transiently-slow down disease progression and allow a sufficient timeframe for the seek of alternative therapeutic options such as the identification of a suitable donor for allogeneic stem cell transplantation, a suitable option to consider[17] or the use of aurora-kinase inhibitors (i. e. MK 0457) that might be active in this setting.[18]

Authors' Contributions

F-EN and SC designed the study, acquired, analyzed and interpreted the data, F-EN and CR-L wrote the paper. SH, MM participated in the acquisition, analysis and interpretation of data. EB, DB, MT, FG, LL, FX-M, FM, CR-L participated in the acquisition of the data. F-EN and Q-HL performed statistical analysis. CP helped with the analysis of the data and proofread the final manuscript version.

Conflict of Interest

The authors reported no potential conflicts of interest.

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