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Hematological Malignancies

A Retrospective Analysis of *BCR-ABL1* Kinase Domain Mutations in the Frontline Drug Intolerant or Resistant Chronic Myeloid Leukemia Patients: An Indian Experience from a High-End Referral Laboratory

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Abstract


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Objective This article identifies and evaluates the frequency of mutations in the BCR-ABL1 kinase domain (KD) of chronic myeloid leukemia (CML) patients who showed suboptimal response to their current tyrosine kinase inhibitor (TKI) regime and assesses their clinical value in further treatment decisions.

Materials and Methods Peripheral and/or bone marrow were collected from 791 CML patients. Ribonucleic acid was extracted, reverse transcribed, and Sanger sequencing method was utilized to detect single-nucleotide variants (SNVs) in BCR-ABL1 KD.

Results Thirty-eight different SNVs were identified in 29.8% ($n = 236/791$) patients. T315I, E255K, and M244V were among the most frequent mutations detected. In addition, one patient harbored a novel L298P mutation. A subset of patients from the abovementioned harbored compound mutations (13.3%, $n = 33/236$). Follow-up data was available in 28 patients that demonstrated the efficacy of TKIs in correlation with mutation analysis and *BCR-ABL1* quantitation. Molecular response was attained in 50% patients following an appropriate TKI shift. A dismal survival rate of 40% was observed in T315I-harboring patients on follow-up.

Conclusion This study shows the incidence and pattern of mutations in one of the largest sets of Indian CML patients. In addition, our findings strengthen the prognostic value of KD mutation analysis among strategies to overcome TKI resistance.

Keywords

- chronic myeloid leukemia
- kinase domain mutations
- BCR-ABL1
- tyrosine kinase inhibitors
- Sanger sequencing
- therapy resistance

* *These authors have contributed equally and share the first authorship.*

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Introduction

Chronic myeloid leukemia (CML) accounts for 15 to 20% of all leukemia in adults and is characterized by the Philadelphia chromosome (Ph), formed by the translocation of genetic material between the long arms of chromosomes 9 and 22 [t(9; 22)(q34;q11)].[1] At a molecular level this leads to the fusion of two genes, breakpoint cluster region (BCR) and Abelson murine leukemia gene (ABL), a proto-oncogene that encodes a tyrosine kinase involved in cell division, differentiation, apoptosis, and deoxyribonucleic acid (DNA) damage repair. The BCR-ABL1 gene fusion leads to a constitutively active ABL1 kinase that impairs terminal differentiation in the myeloid series cells leading to accumulation of cells of granulocytic lineage at variable stages of maturation ranging from blasts to mature neutrophils depending on the clinical stage of CML.[2]

CML is considered as a "poster child" for precision medicine since 2001 when the first tyrosine kinase inhibitor (TKI) imatinib was introduced as the frontline therapy to target Ph+ leukemic cells with minimal damage to the normal human cells.[3] Its mechanism of action is through a competitive binding at adenosine triphosphate-binding sites of the tyrosine kinase,[4] and was the first Food and Drug Administration-approved drug that has emerged as a therapeutic "gold standard." Further, second-generation TKIs (2GTKIs) such as nilotinib, dasatinib, and bosutinib were developed to alleviate imatinib resistant or intolerant CML and were eventually approved for first-line therapy. Given the relative predictability of CML management with TKIs, a precise treatment guideline rendered by the National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet (ELN) for the CML patients has been widely accepted.[5]

Despite the promising efficacy of imatinib, 33 to 40% of the patients acquire resistance or intolerance to the drug and are unable to reach the desired milestones.[6,7] Most importantly, up to 50% patients even acquire resistance to the second or third line drug. Based on the criteria established by the NCCN guidelines and ELN, resistance is defined as: less than a complete hematologic response (CHR) and/or no cytogenetic response (CyR: Ph+ bone marrow metaphases > 95%) at 3 months, BCR-ABL1 transcript levels > 10%, and/or less than a partial CyR (≤ 35% Ph+ metaphases) at 6 months, BCR-ABL1 transcript levels above 1% and/or less than a complete CyR (CCyR; no Ph+ metaphases) at 12 months, and BCR-ABL1 transcript levels > 0.1%, or loss of a CHR or CCyR or confirmed loss of major molecular response (MMR; *BCR-ABL1* transcript levels < 0.1%), mutations, or clonal chromosome abnormalities in Ph-cells at any subsequent time during therapy.[5,8]

Acquisition of mutations in the tyrosine kinase domain (TKD) is the best characterized among the BCR-ABL1-dependent mechanism.[9,10] Mutations in the TKD interfere with the binding of TKI molecule to their targeted site in the BCR-ABL1 protein.[5,11] Detection of mutations in BCR-ABL1 gene can predict the outcome and risk of relapse of a CML patient. The presence of a TKD mutation or compound mutations can aid in prognostication and appropriate TKI selection. More than 100 mutations have been identified in the kinase domain (KD) region and the current 2GTKIs and third-generation (3G)-TKIs have shown variable sensitivity toward specific mutation profiles.[5,12] T315I, the "gatekeeper mutation" is one of the most frequently detected single-nucleotide variants that evades all TKIs except the 3GTKI, ponatinib.[13] The spectrum of KD mutations is expanding, that makes the assessment of resistant CML critical and warrants tailored treatment for individual patients keeping in mind the goal of therapy, that is, overall survival or treatment-free remission.

Although there have been a few studies describing the BCR-ABL1 KD mutation patterns from India; however, these studies are restricted to specific geographic locations.[7,14–19] Herein, we sought to identify and characterize the patterns of KD mutation in one of the largest cohorts of Indian CML patients across the country, especially those who presented with resistance or intolerance to frontline therapy.

Materials and Methods

Patient Characteristics and Data Collection

This retrospective study included a total of 791 patients of CML referred to our laboratory during the period of August 2018 to August 2019 for *BCR-ABL1* KD mutation test upon approval from the institutional review board. The diagnoses of CML were based on clinical, hematologic, and cytogenetic profile of the patient. All patients included in the cohort met the two following criteria: (1) confirmed CML diagnosis undergoing TKI therapy, (2) BCR-ABL1 mutation analysis performed in our laboratory during the fixed time period of the data analysis, that is, August 2018 to August 2019. Data collection for each patient included age, gender, contact information, geographical location, clinical history, ongoing therapy, BCR-ABL1 quantitative test results, if any, and BCR-ABL1 KD mutation test results. The clinical, treatment-related, and follow-up parameters wherever available were charted (►Table 2).

BCR-ABL1 KD Mutational Analysis

The tests were prescribed under the suspicion of resistance to ongoing therapy. Indications of mutation analysis were based on the clinical practice guidelines.[1,8,20] Loss of hematologic response (HR), CyR, or molecular response or delay in attaining defined landmarks was indications for recommending TKD mutation analysis. Poor response was defined as not achieving HR, CyR, and MMR at the defined time points by ELN.[21]

Sample Procurement

For each patient, 3 to 5 mL blood or bone marrow sample was collected in an ethylenediaminetetraacetic acid vial. Each sample was checked for cell count using an automated cell counter (Sysmex, USA). Blood/bone marrow sample acceptance criteria includes observed stability of specimen, ≥ 3 mL of sample in the vial, and total leucocyte count ≥ 1,000/µL.[22] Samples not meeting the criteria were duly rejected.

Ribonucleic acid Extraction and Complementary DNA Preparation

Total ribonucleic acid (RNA) was extracted using QiaAmp RNA blood mini kit (Qiagen, United States) as per the manufacturer's protocol from the 3 to 5 mL whole blood or bone marrow. The purified total RNA was evaluated for concentration (OD260) and purity (OD260/280) by spectrophotometric method (Nanodrop, Thermoscientific, United States). Since optimal assay sensitivity is dependent on the RNA input, it was ensured to use an RNA isolation method yielding greater than 200 ng/µL and to target greater than 1,000 ng total RNA input per test. Purified total RNA was analyzed immediately after extraction or stored below –20°C until ready for testing.

Polymerase Chain Reaction and Sanger Sequencing

As the first step toward polymerase chain reaction (PCR) analysis, complementary DNA was prepared from a minimum of 100 ng quality-checked RNA using reverse transcriptase (3B Black Biotech, India) according to the manufacturer's protocol. A two-step PCR assay is conducted for our in-house routine KD mutation analysis. The first step included the amplification of the BCR-ABL fusion transcript followed by a second PCR reaction which amplified 579 base pair of the ABL1 KD region for any mutations present. Finally, the amplicon was subjected to Sanger sequencing using the SeqStudio genetic analyzer (Applied Biosystems, United States) to identify point mutations. The generated sequencing data was analyzed using SeqStudio analysis software (Applied Biosystems).

Statistical Analysis

All statistical analysis and calculations were done using GraphPad prism8 software and Microsoft Excel. Mutation rates were calculated and frequency patterns were compared in each mutation group. Mann–Whitney test and two-tailed Student's *t*-test were used to assess association of variables wherever applicable. A p-value of < 0.05 was considered to indicate statistically significant difference.

Ethical Approval

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013.

Results

A total of 791 CML patient samples reported in this study were screened for *BCR-ABL1* KD mutations following TKI resistance of which 236 (29.8%) presented with single or multiple mutations in the KD. In the overall cohort, the male: female ratio was 1.8:1 and median age was 42 years. A majority ($n = 106$, 45%) of the patients presenting with resistance was between 30 and 50 years. In mutation positive patients ($n = 236$), the male:female ratio was 2.4:1 and median age was 41 years (data not shown). Mutation positive cases were subjected to further analysis (►Fig. 1).

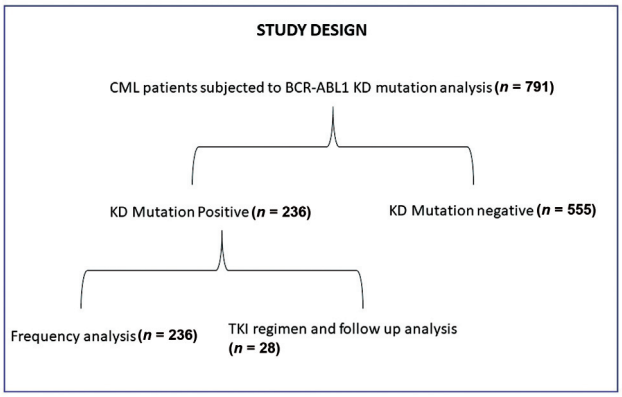

Fig. 1 Flowchart describing study design: Distribution and segregation of patients according to kinase domain (KD) mutation status and number of patients selected for each analysis step.

Distribution of Kinase Domain Mutation

In this cohort, 38 types of mutations were detected (►Table 1). Among the most prevalent mutations, single-occurring T315I was the most frequent as observed in 15.67% ($n = 37/236$) cases followed by E255K (10.16%, $n = 24/236$) and M244V (9.32%, $n = 22/236$) (►Fig 2). Each specific mutation, categorized based on their functional position in the KD, were identified and listed along with the number of cases that harbored them (►Table 1).

A subset of cases (13.3%, $n = 33/236$) harbored compound mutations, that is, two or more mutations (►Supplementary Table S1, available online only). A novel mutation L298P was detected in one case with T3151 as comutant. Three cases harbored triple mutations, E255K/T315I/F317L, E279-K/G250E/M244V, and G250E/L248V/H396R. In patients with complex mutations, T3151 was the most frequent mutation partner observed ($n = 11/33$) followed by M244V ($n = 8/33$) and G250 ($n = 6/33$) (►Supplementary Table S1).

Treatment Follow-Up

Therapy timeline and molecular response data since diagnosis was available for 28 patients with detected mutations detailed in ►Table 2. The average time taken to assess for a TKD mutation was 3 years after start of first-line TKI. Imatinib 400 mg was the choice of first-line therapy for most of these cases (89.2%). Postdetection of resistant mutation, patients with T315I mutations were administered the 3G ponatinib 45 mg ($n = 4$). All other TKD mutation carrier patients were administered a 2G TKI dasatinib with dosage range of 50 to 100 mg once daily ($n = 10$) or nilotinib 400 mg twice daily ($n = 9$) and 300 mg twice daily ($n = 1$). A few patients continued on imatinib at the same or higher dose of 600 to 800 mg ($n = 4$). A third TKI was required by eight patients after their previous two TKIs produced suboptimal response. One patient (E255K) chose to discontinue TKI therapy and has not been included in ►Table 2. There was no significant association of mutation with age or gender. Among patients with TKD mutation, 50% ($n = 12/24$) patients reached MMR after a TKI

Table 1 Distribution and frequency of mutations based on their structural positions in the kinase domain with most frequent (in bold) and novel mutation (*in bold italics*)

Structure	Hotspot mutation	No. of cases[a] (*N* = 236)	Frequency
	M244V	**30**	**12.7118644**
P-loop	L248V	5	2.11864407
	G250E	19	8.05084746
	Q252H	6	2.54237288
	Y253F	1	0.42372881
	Y253H	15	6.3559322
	E255K	**32**	**13.559322**
	E255V	6	2.54237288
C-helix	E275K	2	0.84745763
	D276G	4	1.69491525
	E279K	6	2.54237288
	I293V	1	0.42372881
	L298P	***1***	***0.42372881***
	V299L	2	0.84745763
ATP-binding site	F311L	3	1.27118644
	F311I	11	4.66101695
	T315I	**48**	**20.3389831**
	F317L	17	7.20338983
SH2	M351T	10	4.23728814
	E355G	4	1.69491525
Substrate binding site	**F359V**	**22**	**9.3220339**
	F359I	3	1.27118644
A-loop	E373K	1	0.42372881
	L387M	3	1.27118644
	M388L	2	0.84745763
	H396P	3	1.27118644
	H396R	9	3.81355932
	H396Y	1	0.42372881
	A397P	1	0.42372881

[a]Total number of cases (with frequency) in which mutation was detected either single or with a comutation.

shift within the median time of 7 months and median time of 40 months (~3.5 years) since diagnosis. Death occurred in 60% ($n = 3/5$) T315I carriers. Patients with F317L, F359V showed 100% survival and E255K/V showed 66% survival post-TKI shift. The survival was not statistically significant between mutant groups F359V/F317L versus T315I (p-value = 0.07) or E255K versus T315I (p-value =0.3) (► **Table 3**).

Discussion

CML treatment has undergone a sea change since the 20th century, beginning with radiotherapy, busulfan, and hydroxycarbamide, followed by interferon-alpha. During the 1980s, allogeneic stem cell transplant became the treatment of choice for eligible patients but not without risk of mortality.[23] In 1998, introduction of the first TKI, imatinib, revolutionized treatment for newly diagnosed CML by bringing up the survival of treated CML patients comparable to a healthy individual.[24] This TKI has shown promising results and still remains a first-line therapy for CML,[5] as in our patient group where we observe 90% patients being treated with imatinib as first-line TKI. Despite the achievements, there has been a major hurdle in the complete success of imatinib, in the form of acquired resistance and intolerance to the drug.

Over the past two decades, studies have already identified the mechanisms of drug resistance, that include drug metabolism, bioavailability, leukemic cell evolution, and the most frequently responsible KD point mutations or

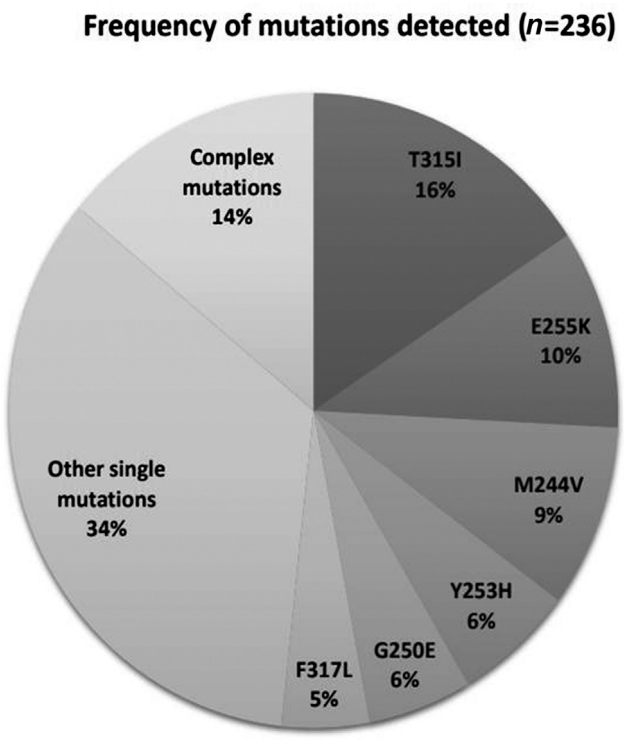

Fig. 2 Mutations detected in BCR-ABL1 kinase domain (KD). Pie chart representation of KD mutations detected across the cohort in order of their frequency and type.

overexpression of the BCR-ABL1 gene.[9] In one of the earliest comprehensive analyses, 144 Australian patients were evaluated for BCR-ABL1 kinase mutations and a highly significant number of patients (89%) with an underlying mutation developed resistance to imatinib therapy.[25] Ever since, multiple studies have proven the point that close molecular monitoring and prompt mutation analysis is critical for disease management and long-term remission.

There are limited studies available on Indian patients[15,17,19,26]; with the most recent next-generation sequencing (NGS)-based study by Chandrasekhar et al that identifies TKD mutations in 50% ($n = 21/41$) patients, with 13 patients harboring 6 novel missense mutations,[2] a relatively high incidence possibly owing to deep sequencing methodology (►**Table 4**). In this report, we present data of one of the largest subsets of CML patients ($n = 791$), who were assessed after failing to achieve response milestones post-first-line TKI and observed the presence of TKD mutations in 29.8% patients.

This is in corroboration with previous Indian patient reports by Chaitanya et al[16] and Rajappa et al[15] describing TKD mutations in 33 and 29% CML patients, respectively. Overall, the incidence of TKD mutation has been reported in 30 to 50% imatinib-intolerant patients worldwide.[27–30] Gender distribution of the present cohort is comparable to Chandrasekhar et al,[2] Tripathi et al,[7] and Chaitanya et al[16] but is significantly different from Patkar et al[19] and Rajappa et al.[15] We also observe a slightly higher median age in comparison to other studies (►**Tables 2** and **4**). In addition, treatment resistant cases that harbored a mutation ($n = 236$) in the *BCR-ABL1* KD show that the female gender has a markedly lower presentation of resistant mutations suggesting alternative mechanisms that may possibly be contributing to their suboptimal response to therapy.[9,31]

In the spectrum of mutations found, we found one novel missense mutation L298P whose clinical significance warrants further investigation. It was observed with T315I as a comutant which may possibly add to the complexity while deciding the therapy options. In consistency with worldwide data,[32] the "gatekeeper mutation" T315I in the substrate binding site occurs most frequently in our patients (20%) as it is considered the primary mechanism of the BCR-ABL1 positive cells to escape TKI pressure with enhanced leukemogenesis.[33] T315I+ cells renders all TKIs ineffective, except ponatinib due to a different binding mechanism of the drug to the tyrosine kinase protein.[34] The T315I evidently confers poor prognosis in CML patients as seen in our patients with majority (60%) of deaths as compared to other mutations. However, this observation is based on a small subset of patients ($n = 5$) and warrants a clinical follow-up of a bigger cohort. It is important to mention that one of the factors behind this dismal outcome was the socioeconomic status of the patients who could not afford or procure ponatinib, a situation that needs to be addressed for CML management in India.

Apart from imatinib, resistant mutations have been observed postadministration of 2G TKIs as well. The F317L, V299L, and T315A mutations are reported to impart a high degree of resistance to dasatinib.[5] In our patients with F317L on follow-up, all three patients with the mutation (►**Table 2**) showed a significant response with nilotinib, with one of them unresponsive to dasatinib before shifting to nilotinib, while one completely failing on continued imatinib leading to death. We observed no incidence of T315A mutation in this cohort. V299L was detected in two patients and this mutation rarely emerges after imatinib therapy and is reported to emerge post-dasatinib as second-line therapy. We lost these two patients to follow-up and could not assess their clinical course. The more frequent mutations within the P-loop (Y253H/F, E255V/K) and F359 mutations were associated with a high degree of resistance to nicotinic in this study. The three patients who harbored F359V mutation (►**Table 2**) showed a trend of shifting to dasatinib to reach CyR or MMR. E255K/V was the second most frequent mutation (16%; $n = 38$) observed in our patient group, similar to incidence reported in previous patient studies (8–17%) and is associated with poor prognosis.[13,35] We observe a variable trend in TKI shift in the six patients with E255K/V mutations with varying response to both dasatinib and nilotinib, and one continuing on a higher imatinib dose without 2GTKI shift. One patient with Y253H who is currently on dasatinib as second-line therapy after 30 months of resistance to imatinib is stable and awaiting BCR-ABL1 transcript levels. However, a larger patient set would be needed to assess the statistical significance of a specific mutation and their TKI course.

Table 2 Details of tyrosine kinase inhibitor shifts and associated response in 28 patients on follow-up

Age	Gender	Disease phase	1st line TKI	Time of first mutation analysis since diagnosis (mo)	Mutation detected	2nd line TKI	Time of follow-up since TKI shift (mo)	BCR-ABL1 transcript level % (other symptom)	TKD analysis repeated (months since diagnosis)	Mutation detected	3rd line TKI	Time of follow-up since TKI shift (mo)	BCR-ABL1 transcript level % (other symptom)	Other	Status (March 2020)
51	M	CML-CP	Imatinib	16	No mutation	Dasatinib	14	0.3 MR	Yes (36)	E255K	Nilotinib	5	0.1 MMR		Alive
59	M	CML-CP	Imatinib	24	E255K	Dasatinib	3	19.1 (*cytopenia*)	Not tested	Not tested	Nilotinib	5	Not tested		Alive
36	M	CML-CP	Imatinib	24	F359V, G250E	Dasatinib	24	33.4	Yes (48)	E255K	Bosutinib	1	No response		Dead
28	M	CML-CP	Imatinib	96	E255K	Imatinib 600 mg	13	Not tested							Alive
80	M	CML-CP	Imatinib	84	E255V	Dasatinib	12	Not tested							Alive
33	M	CML-CP	Imatinib	24	E255V	Nilotinib	2	No response							Dead
16	M	CML-CP	Imatinib	19	E279K	Nilotinib	7	0.1 MR							Alive
46	M	CML-CP	Imatinib	132	E355G	Dasatinib	7	0.003 MR 4.5							Alive
40	M	CML-CP	Nilotinib	56	F311I	Nilotinib resumed	12	0.12 MR							Alive
45	M	CML-CP	Imatinib	204	F311L	Imatinib 800 mg	4	No response							Dead
37	M	CML-CP	Imatinib	36	F317L	Nilotinib	4	0.6 ~CCyR							Alive
48	F	CML-CP	Imatinib	60	F317L	Nilotinib	4	Not tested							Alive
42	M	CML-CP	Imatinib	27	F317L	Dasatinib	7	23.9	No	No	Nilotinib	6	5.21 ~PCyR		Alive
25	F	CML-CP	Imatinib	144	F359V	Nilotinib	48	Not tested (*leukocytosis*)			Dasatinib	12	Not tested		Alive
47	F	CML-CP	Dasatinib	168	F359V	Nilotinib	3	1.96 > PCyR							Alive
39	M	CML-CP	Nilotinib	15	F359V	Dasatinib	3	0 DMR							Alive
29	M	CML-CP	Imatinib	48	G250E	Nilotinib	8	0 DMR							Alive
45	M	CML-CP	Imatinib	Not tested	–	Nilotinib	Not tested	Not tested (*intolerance*)	Yes (9)	G250E	Dasatinib	15	2.9 ~CCyR		Alive
51	M	CML-CP	Imatinib	144	H396P	Dasatinib	12	0.29 ~MR							Alive
39	F	CML-CP	Imatinib	72	H396R	Nilotinib	6	No response							Dead

(*Continued*)

Table 2 (*Continued*)

Age	Gender	Disease phase	1st line TKI	Time of first mutation analysis since diagnosis (mo)	Mutation detected	2nd line TKI	Time of follow-up since TKI shift (mo)	BCR-ABL1 transcript level % (other symptom)	TKD analysis repeated (months since diagnosis)	Mutation detected	3rd line TKI	Time of follow-up since TKI shift (mo)	BCR-ABL1 transcript level % (other symptom)	Other	Status (March 2020)
30	F	CML-CP	Imatinib	Not tested	–	Dasatinib	60	28.82	Yes (192)	M244V	Nilotinib	7	1.91 ~CCyR		Alive
29	M	CML-CP	Imatinib	24	Y235H	Imatinib 800 mg	6	Not tested *(leukocytosis)*	Not tested	Not tested	Dasatinib	3	No data		Alive
36	M	CML-CP	Imatinib	24	M388I	Imatinib 400 mg continued	6	0.07 MMR	Yes(6)	No mutation detected					Alive
26	M	CML-CP	Imatinib	24	No mutation detected	Nilotinib	7	No response	Yes (72)	T315I	Ponatinib	7	No response		Dead
37	M	CML-CP	Imatinib	Not tested	–	Nilotinib	3	Not tested *(T-lymphoid blast crisis)*	Yes (84)	T315I	Ponatinib	12	No response	ASCT	Dead
53	F	CML-CP	Imatinib	No data	T315I	Dasatinib	3	15.24	No	No	Ponatinib	8	0.04 MMR		Dead
32	M	CML-CP	Imatinib	Not tested	–	Nilotinib	60	Not tested	Yes (84)	T315I	Ponatinib	15	0.01 MMR4		Alive
52	M	CML-CP	Imatinib	6	T315I	Imatinib 800 mg	5	4.18 ~PCyR							Alive

Abbreviations: CCyR, complete cytogenetic response; CyR, cytogenetic response; CML, chronic myeloid leukemia; CP, chronic phase; DMR, deep molecular response; MMR, major molecular response; MR, molecular response; PCyR, partial cytogenetic response.

Table 3 Survival analysis of the patients harboring mutations in selected groups with criteria set as: three or greater than three patients carrying a mutation

Mutations	Rate of survival (%)	Median time to reach MMR since last TKI shift (mo)	Median time to reach CyR/MMR response since diagnosis (mo)	Survival vs. T315I group *p*-value (significant)
E255K	66.6	5	51.5	0.3 (No)
F317L	100	5	40	0.07 (No)
F359V	100	3	94	0.07 (No)
T315I	40%	11	85	

Abbreviations: CyR, cytogenetic response; MMR, major molecular response; TKI, tyrosine kinase inhibitor.

Tripathi et al in 2017, in contrast to previous studies, reported M351T as the most frequent mutation (43.5%) in their patient group and surprisingly no T315I, touting geographical variation as a possible reason. In our patient group M351T is found in a much lower frequency (4.3%) from patients Pan-India. A comparative analysis by Kim et al between Asians and non-Asian population cohorts, has stated the distinct pattern of mutations in Asians with highest prevalence of T3151 and P-loop mutations in Asians that has been reinstated this study.[36] It has been observed that patients who achieve molecular response are less likely to relapse to a high leukemia burden, hence achieving this milestone has become a primary objective during TKI therapy.[37] Among our patients who were followed up, each subject presented with BCR-ABL1 level > 10% (HR) before TKD mutation analysis. We observed MMR achievement after TKI shift in half of the patients in follow-up. Regular molecular quantitative monitoring of BCR-ABL1 plays a crucial role in identifying lack of response and must be adhered to for maximum benefits. Lack of testing, irregularities in follow-up, and economic burden are some of the factors behind treatment gaps in majority of patients. Evidently, the relatively small follow-up data available for this patient cohort is a limitation of this study and must be addressed in future studies.

It has been shown that sequential exposure to TKIs lead to accumulation of compound ($\geq$ 2 mutations in the same BCR-ABL1 clone) or polyclonal mutations ($\geq$ 2 mutations in different BCR-ABL1 clones).[38] Double/triple mutations were observed in 14% patients ($n = 33$) in our cohort. Compound mutations are known to arise under 2G- or 3GTKI pressure, partnering with preexisting mutation against first-line or second-line TKI and pose a clinical challenge. The combination of mutations is associated with particular TKI administered as dasatinib and nilotinib are known to give rise to distinct compound mutation profiles. Double mutations are the most common form detected as reiterated by our analysis along with T315I observed as the most frequent mutation partner.[39] Triple mutations found in this patient group characterized as E255K/T315I/F317L, E279K/G250E/M244V, and G250E/L248V/H396R have not been reported previously. Presence of compound mutations gives us insight into the complex interplay of TKIs and CML clonal behavior.

Sanger sequencing is currently the routine technique for mutation analysis with a marked specificity and relative affordability. Nevertheless, this technique is limited in sensitivity to detect mutations at a frequency of 20 to 30%.[13] This must be considered when patients are tested and detected with "no mutations" despite showing clinical signs of intolerance or resistance to therapy. The possibility of missing out on mutant clones at very low variant frequency (< 10%) can be addressed with advanced technologies such as the NGS. Therefore, NCCN recommends the use of NGS, if available.[5] Identifying the low burden mutant clones can be advantageous as a preemptive effort to reduce leukemia burden in the overall course of disease.

Conclusion

Knowledge on CML continues to evolve in context to molecular diagnostics and clinical management. It is advisable to perform BCR-ABL1 KD mutation analysis before administering a new TKI at any time point to attain desirable results. Novel mutations such as L298P as identified in this study can have distinct response to TKIs and thus, are recommended to be monitored for their clinical significance. Regular quantitation of BCR-ABL1 transcript according to the treatment guidelines is critical for evaluating appropriate response to TKIs as each TKI seems to show a distinct clinical profile. In addition, NGS-based mutation analysis could benefit patients at baseline to aid in the decision of the correct frontline TKI for the individual by identifying preexisting KD mutations at low frequencies independent of TKI selection pressure.

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Conflict of Interest

None declared.

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Table 4 Comparison of our study with the previous published literature from India.

Characteristics	Present study	Chandrasekhar et al 2019	Chaitanya et al 2017	Tripathi et al 2017	Patkar et al 2016	Rajappa et al 2013	Markose et al 2009
Cohort size (*n*)	791	41	269	46	385	90	76
Age range (median age)	10–85 (42)	29–68 (53.73)	18–66 (36)	15–72 (36.8)	7–73	18–65 (36)	(44)
Male-to-female ratio	1.8:1	1.1:1	1.7:1	1.3:1	3:1	2.4:1	No data
Phases of CML included	29 CP (follow-up)	25 CP,11 AP, and 5 BC	CP	45 CP and 1 AP	331 CP, 29 AP, and 25 BC	CP	54 CP, 14 AP, 8 BC
Frequency of mutations in KD	29.8%	51.2%	32.7%	40%	51.9%	32.2%	33%
Most common mutations	T315I (20%), E255K (14%), M244V (13%)	T315I (33.3%) F317L (33.3%), novel mutations (61.9%)	T315I (31.8%), E255V/K (5.7%)	M351T (43.75%), Y253H (18.75%), H396R (18.75%)	T315I (20.7%), Y253H (10.1%), M244V (7.2%), F317L (7.2%)	T315I (31%), G250E (27.6%), F359V (13.8%)	M244V (16%), T315I (16%), E255K (12%)

Abbreviations: AP, accelerated phase; BC, blast crisis; CML, chronic myeloid leukemia; CP, chronic phase; KD, kinase domain.

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