

Integrative Genomic Analysis Reveals Cancer-Associated Gene Mutations in Chronic Myeloid Leukemia Patients with Resistance or Intolerance to Tyrosine Kinase Inhibitor

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Introduction: While the acquisition of mutations in the ABL1 kinase domain (KD) has been identified as a common mechanism behind tyrosine kinase inhibitor (TKI) resistance, recent genetic studies have revealed that patients with TKI resistance or intolerance frequently harbor one or more genetic alterations implicated in myeloid malignancies. This suggests that additional mutations other than ABL1 KD mutations might contribute to disease progression.

Methods: We performed targeted-capture sequencing of 127 known and putative cancer-related genes of 63 patients with CML using next-generation sequencing (NGS), including 42 patients with TKI resistance and 21 with TKI intolerance.

Results: The differences in the number of mutations between groups had no statistical significance. This could be explained in part by not all of the patients having achieved major molecular remission in the early period as expected. Overall, 66 mutations were identified in 96.8% of the patients, most frequently in the *KTM2C* (31.82%), *ABL1* (31.82%), *FAT1* (25.76%), and *ASXL1* (22.73%) genes. *CUX1*, *KIT*, and *GATA2* were associated with TKI intolerance, and two of them (*CUX1*, *GATA2*) are transcription factors in which mutations were identified in 82.61% of patients with TKI intolerance. *ASXL1* mutations were found more frequently in patients with *ABL1* KD mutations (38.1% vs 15.21%, $P=0.041$). Although the number of mutations was low, pairwise interaction between mutated genes showed that *ABL1* KD mutations cooccurred with *SH2B3* mutations ($P<0.05$). In Kaplan–Meier analyses, only *TET2* mutations were associated with shorter progression-free survival ($P=0.026$).

Conclusion: Our data suggested that the *CUX1*, *KIT*, and *GATA2* genes may play important roles in TKI intolerance. *ASXL1* and *TET2* mutations may be associated with poor patient prognosis. NGS helps improving the clinical risk stratification, which enables the identification of patients with TKI resistance or intolerance in the era of TKI therapy.

Keywords: chronic myeloid leukemia, mutations, tyrosine kinase inhibitor, intolerance, resistance

Introduction

The cytogenetic hallmark of chronic myeloid leukemia (CML) is the chromosomal translocation t(9;22)(q34;q11), yielding the Philadelphia (Ph) chromosome and generating a fusion gene that encodes BCR-ABL1, a constitutively active protein tyrosine kinase. Tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 have substantially improved the prognosis of patients with CML, from prolonging survival time to

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achieving treatment-free remission. However, some patients still exhibit resistance or intolerance to TKIs, leading to treatment failure. Although the acquisition of a point mutation in the BCR-ABL1 kinase domain (KD) has been noted as an important mechanism of drug resistance,¹ recent studies²⁻⁴ have reported patients with poor prognosis bearing one or more somatic mutations associated with myeloid malignancies, which means that additional mutations may contribute to disease progression. In the last decade, a variety of somatic mutations besides those in BCR-ABL1 have been found in myeloid malignancy, which have different frequencies and combinations and often overlap in different diseases.⁵ In studies on transcription factors and signal-transduction genes, mutations were found in genes conferring a growth advantage, which caused cancers.^{6,7} Over time, other pathways such as epigenetic modification, abnormalities in RNA splicing, and adhesion protein complexes have also been found to be associated with myeloid leukemia.⁸⁻¹² The purpose of this study was to identify the difference of additional CML-related mutations between patients resistant or intolerant to TKIs, and to understand the possible effects of these mutations on disease progression.

Materials and Methods

Study Design and Subjects

A total of 63 CML patients admitted to Nanfang Hospital from August 2018 to October 2019 who were resistant or intolerant to TKIs were analyzed retrospectively. All cases had clear evidence regarding bone marrow cytology and genetics and met the diagnostic criteria of CML in the hematopoietic and lymphoid tissue tumor classification revised by the WHO in 2016. The group consisted of 42 patients with TKI resistance and 21 with TKI intolerance. The clinical data of the patients are displayed in Table 1 (details in [Supplementary file 1](#)). There was no significant correlation between the age of all CML patients and the number of detected gene mutations (Spearman's rho correlation coefficient 0.047, $P = 0.703$).

Inclusion Criteria

TKI resistance¹³ was defined as the lack of a complete hematologic response (CHR) after 3 months of TKI treatment, the lack of any cytogenetic response after 6 months of treatment, the lack of minor cytogenetic response (MCyR) (Ph-positive cells >35%) after 12 months of treatment, an increase of white blood cell (WBC) count in at least two consecutive samplings (with a doubling of the count from the nadir to $\geq 20 \times 10^9/L$ or an absolute increase of $\geq 50 \times 10^9/L$), or

a relapse after a CHR or MCyR. TKI intolerance^{13,14} was defined as at least grade 3 nonhematologic toxicity or grade 4 hematologic toxicity persisting for more than 7 days, related to TKIs at any dose.

Next-Generation Sequencing

All the patients in our group had recent q-PCR quantitative results of BCR-ABL1 fusion gene before the second-generation sequencing, and the enrollment standard was BCR-ABL1 fusion gene (IS) $\geq 10\%$, which make sure the proportion of Ph-positive cells will not be too low.

Using next-generation sequencing (NGS), we analyzed 127 genes (Table 2). After collecting peripheral blood of the patients, we extracted gDNA from the qualified samples using an automatic nucleic acid extractor for quality inspection. After passing the quality inspection, an Illumina standard library (Illumina, Inc.), was constructed to create a library suitable for high-throughput sequencing. The library peak map was detected by Agilent2100 (Agilent, Inc.), and the main peak size of the library was required to be about 350 bp. After qualification, the Roche NimbleGen hybridization capture chip (Roche, Inc.) was used to capture the target sequence of 127 genes related or putatively related to hematological disease. After the captured exon library passed the library quality inspection and achieved accurate qPCR quantification, PE75 sequencing was carried out on Illumina NextSeq 550AR (Illumina, Inc.). Bioinformatic analysis was carried out on the off-machine data qualified for quality control, including point mutations, insertions and deletions, internal tandem duplication, partial tandem duplication, and other types of variation. The Burrows-Wheeler Alignment algorithm version 0.7.12¹⁵ was used to compare the sequence data with the human genome (version: GRCh37), and Picard version 1.115 (<https://github.com/broadinstitute/picard>) was used to mark the polymerase chain reaction duplicates. We corrected the quality value of the sequence alignment results by means of BaseRecalibrator in Genome Analysis Toolkit version 3.5.¹⁶ The MuTect2 version 3.5 software¹⁶ was employed for mutation detection, and all test results were annotated in the Annovar version 0722 software.¹⁷

Statistical Analysis

The Kaplan-Meier survival curve was analyzed using a Log rank test to compare progression-free survival between the *TET2*-positive and *TET2*-negative groups (GraphPad Prism; GraphPad Software, Inc.). For all other analyses, significant differences between two groups were examined using two-sample Student's t-tests, Chi-square

Table I Clinical Data of the Patients

		TKIs-Resistant	TKIs-Intolerant	P value
Number		42	21	
Gender	(Male/Female)	26/16	11/10	$\chi^2=0.524$ P=0.589 ^a
Age		38.5 (19, 66)	49.0 (31, 65)	P=0.068 ^b
Disease staging	CP AP/BC	29 13	16 5	$\chi^2=0.554$ P=0.768 ^a
Clinical data at diagnosis	WBC ($\times 10^9/L$) HGB(g/L) PLT ($\times 10^9/L$) BM blast (%) Spleen size (subcostal length/cm)	201 (32.95, 406.54) 100.1(66, 140) 572.84(49, 1197) 1.0(0, 17.0) 4.8(0, 23)	232.03 (37.02, 508) 82.36(225, 1790) 732.18(225, 1790) 3.0(0.5, 7.5) 4(0, 23)	P=0.212 ^b F=6.426 P=0.017 ^c F=1.272 P=0.269 ^c P=0.085 ^b P=0.506 ^b
Sokal	Low Medium High	0.98(0.22, 1.56) 16(38.1%) 16(38.1%) 10(23.8%)	0.98(0.11, 2.32) 5(23.8%) 12(57.1%) 4(19%)	P=0.484 ^b
EUTOS	Low High	66.5(7, 112) 25(59.5%) 15(35.7%)	56.4(11.4, 101) 16(76.2%) 5(23.8%)	P=0.743 ^b
Clinical data at detection	WBC ($\times 10^9/L$) HGB(g/L) PLT ($\times 10^9/L$) EOS (%) BASO (%)	5.54(1.93; 149.87) 111(49; 165) 156(12; 1126) 0.4(0; 9.7) 1.2(0; 18)	4.6(1.48; 11.8) 108.5(43; 131) 34(6; 75) 0.35(0; 2.9) 0.75(0; 8.9)	P=0.035 ^b P=0.236 ^b P=0.000 ^b P=0.704 ^b P=0.470 ^b
Additional chromosomal aberrations*				P=1.000 ^d
Yes	Major route Minor route	4(12.12%) 4(12.12%)	3(20%) 1(6.67%)	
No		25(75.76%)	11(73.33%)	
Achieved MMR				
	Yes	1(2.94%)	1(5.00%)	
	No	33(97.06%)	19(95.00%)	

Notes: ^aChi-square test. ^bWilcoxon rank sum test. ^ct-test. ^dFisher's exact test. *Only 48 patients with karyotype analysis.

Abbreviations: TKIs, tyrosine kinase inhibitors; WBC, white blood cell; HGB, hemoglobin; PLT, platelets; BM, bone marrow; EOS, eosinophil; BASO, basophil; MMR, major molecular remission.

test or Fisher's exact test depending on types of data. The odds ratio was calculated as the ratio of mutation frequency between two different groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Result

The results of the analysis of the 127 genes of CML patients with intolerance or resistance to TKIs confirmed that there

were 66 individual somatic mutations in 63 patients. The number of mutations per patient ranged from 0 to 13, with a median of 3. There was no significant correlation between the number of mutations and the Sokal score or EUTOS score of the patients (Spearman's rho correlation coefficient between Sokal and the number of mutations 0.174, $P = 0.171$; Spearman's rho correlation coefficient between EUTOS and the number of mutations 0.149, $P = 0.245$).

Table 2 Detected Genes

Pathway	Genes
Methylation	<i>DNMT3A, TET2, IDH1, IDH2, TPMT, MLL, EZH2, SETD2, NPM1</i>
Tumor suppressor	<i>WT1, TP53, MPL, APC, CBLB, CBLC, MDM2, CDKN2A</i>
Transcription factor	<i>CEBPA, AML1, RUNX1, GATA1, GATA2, GATA3, ETV6, IKZF1, CREBBP, CUX1, ERG, MEF2B, RBI, STAT5B, STAT5A, TCF3, GFI1</i>
Spliceosome and RNA metabolism	<i>SF3B1, SRSF2, U2AF1, ZRSR2, PRPF8, DDX41</i>
Chromatin modifiers	<i>ASXL1, KDM6A, BCORL1, BCOR, KMT2C, ID3, SETBP1, PHF6, EP300, BLM</i>
Activated Signaling	<i>CBL, NRAS, KRAS, HRAS, PTPN11, KIT, CSF1R, CSF3R, JAK1, JAK2, JAK3, SH2B3, NTRK1, NTRK2, ABL, CRLF2, AKT2, AKT3, AMER1, ATM, ATRX, BRAF, EGFR, IL7R, MAP2K4, MAP3K7, NOTCH1, NOTCH2, PIK3CA, PTEN, PDGFRA, SMAD4, SYK, TRAF3, NFI, ETNK1, STAT3, MYD88, FLT3-ITD, FLT3</i>
Cell Metabolism	<i>GSTP1, NT5C2, NQO1, PIGA, MTHFR, ELA2, CYP2C19, CYP3A4, GSTM1</i>
Adhesion and cohesion complex	<i>RAD21, STAG2, SMC1A, SMC3, FAT1</i>
Others	<i>ABCB1, ABCC3, BCL2, CACNA1E, CARD11, CCND1, CD79B, CDA, DIS3, ERCC1, CTLA4, FAM46C, FBXW7, GNAS, MLH1, NF2, TERC, XRCC1, CALR, BIRC3, SRP72, HAX1, TERT, DNAH9, DKC1</i>

Summary of Mutations in 127 Genes Detected

Among the total mutation rates, *ABL1* KD showed the highest value (31%), followed by *KMT2C*, *FAT1*, and *ASXL1* (Figure 1A). The coexistence relationship among genes is complicated (Figure 1B). In addition, we identified de novo mutations in CML patients with TKI intolerance, including in *KIT1*, *CUX1*, and *GATA2* (Figure 1C, details in [Supplementary file 2](#)).

There was no significant difference in the number of mutations between the TKI-resistant group and the TKI-intolerant group (3.10 vs. 3.79, $P=0.123$). There was also no significant difference in the *ABL1* KD mutation rate between the TKI-resistant group and the TKI-intolerant group (34.09% vs. 26.09%, $P=0.351$).

Mutational Heterogeneity Between TKI-Resistant and TKI-Intolerant Groups

Myelodysplastic Syndrome (MDS)-Related Mutations Found in TKI-Intolerant Group

We analyzed 20 driving genes related to MDS in 63 patients and found that *CUX1*, *GATA2*, and *KIT* had higher mutation rates in patients with TKI intolerance, while *AMER1* mutations were only found in drug-resistant patients (Table 3). Although the mutation rates of *ASXL1* and *RUNX1* did not show any difference between the two

groups, the mutation rates of these two genes remained high in the two groups, indicating that their existence may lead to a poor response to TKIs.

The distribution of mutations in these 20 genes was also different in the two groups (Figure 2). The median number of mutations of these 20 genes in the TKI-intolerant group was higher than that in the TKI-resistant group (2.0 vs. 1.0, $P=0.029$). This suggests that MDS-related genes may play an important role in TKI intolerance.

Heterogeneity of Mutant Genes' Function in TKI-Resistant or -Intolerant Patients

The number of mutations in genes encoding transcription factors was significantly higher in patients with TKI intolerance than that in patients with TKI resistance (0.83 vs. 0.27, $P=0.014$). *GATA2* and *CUX1*, both of which encode transcription factors, have higher mutation rates in patients intolerant to TKIs (Figure 3).

Effect of Additional Gene Mutations on CML Progression

We also analyzed the effect of additional gene mutations on the progression of CML. In this paper, the term "additional gene mutations" is used to indicate the existence of somatic mutations besides those in *ABL1* KD in patients with CML.

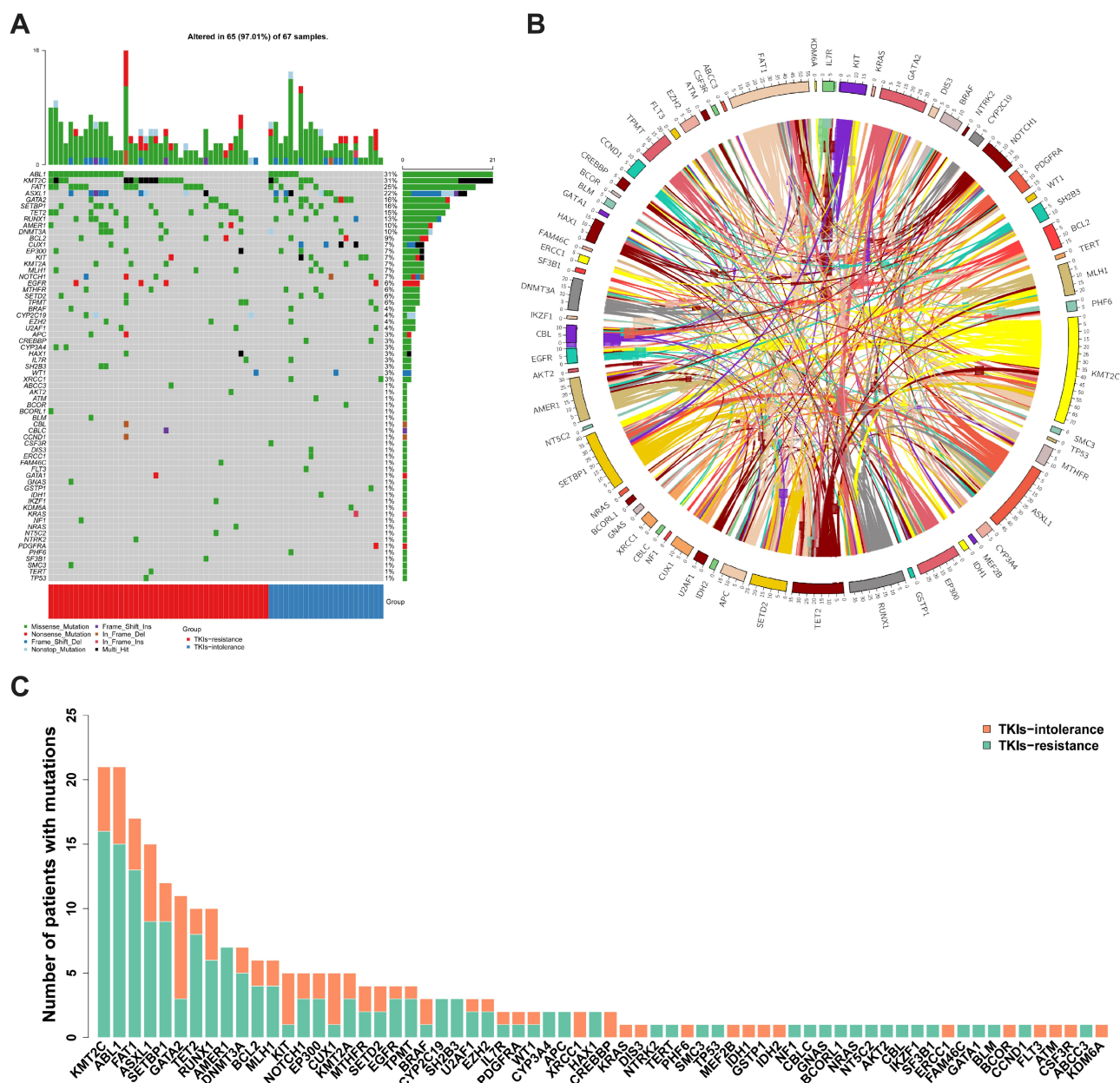


Figure 1 Summary of mutations **(A)** Most of the 68 individual somatic mutations were missense mutations, while multiple hit mutations accounted for 38% of *KMT2C* mutations, and frameshift mutations also accounted for 46.67% of *ASXL1*. Among the total mutation rates, the highest was *ABL1* KD (31%), followed by that of *KMT2C*, *FAT1* and *ASXL1*, which were 31%, 25% and 22%, respectively. **(B)** The coexistence relationship among genes is complicated. **(C)** The TKIs-resistant group accounted for the majority of the mutations in most genes, while the number of mutations in the TKIs-intolerant group in the *CUX1*, *GATA2* and *KIT* genes was higher than those in the TKIs resistant group.

Abbreviations: KD, kinase domain; TKIs, tyrosine kinase inhibitors.

Coexistence of Additional Gene Mutations and *ABL1* KD Mutations

The patients in this study were divided into two groups: those positive and negative for *ABL1* KD mutation. Although the mutation rates of *ASXL1*, *FAT1*, and *RUNX1* in patients with *ABL1* KD mutation were higher than in those without it, this was statistically significant only for the *ASXL1* gene mutation rate (Table 4). Moreover, the mutation rate of *KIT* in patients without

ABL1 KD mutation was higher, but the difference did not reach statistical significance, which may have been due to the insufficient number of patients in this study.

The clinical features of patients with *ASXL1* mutation also differed from those in patients without such mutation. Patients with *ASXL1* mutation had a higher proportion of basophils in peripheral blood and a larger spleen at initial diagnosis (Table 5). Although Sokal score and EUTOS score did not show a difference between the two groups,

Table 3 Mutations Rates of Genes Related to MDS

	<i>AMER1</i>	<i>CUX1</i>	<i>KIT</i>	<i>GATA2</i>	<i>ASXL1</i>	<i>RUNX1</i>
TKIs-resistant	7/44 (15.90%)	1/44 (2.27%)	1/44 (2.27%)	3/44 (6.82%)	9/44 (20.45%)	6/44 (13.64%)
TKIs-intolerant	0/23 (0.000%)	4/23 (17.39%)	4/23 (17.39%)	8/23 (34.78%)	6/23 (26.09%)	4/23 (17.39%)
P value	0.044	0.044	0.044	0.006	0.408	0.470

Abbreviation: MDS, myelodysplastic syndrome.

ASXL1 mutation may increase the risk of disease progression. Analysis of the other mutated genes and clinical features at initial diagnosis was presented in [Table S1](#). In the analysis of the existence of other mutations in combination with *ABL1* KD mutation, the results showed that *SH2B3* gene mutation significantly coexisted with it ([Figure S1](#)).

Additional Gene Mutations May Play an Important Role in CML Progression

The 63 patients were divided into a chronic phase (CP) group and an accelerated phase (AP)/blast crisis (BC) group. There was no difference in the number of gene mutations between the two groups (3.0 vs. 3.0). This may have been due to the fact that the patients in the CP group did not achieve early molecular remission or the optimal therapeutic response, in which additional gene mutations may play an important role. However, the rate of *ABL1* KD mutations in the AP/BC group was higher than that in the CP group (38.89% vs. 28.57%, $P=0.301$), and similar trends were found for *TET2* (38.89% vs. 6.12%, $P=0.003$) and *FAT1* (33.33% vs. 22.45%, $P=0.272$). Similarly, patients with mutations in *TET2* showed

a poorer prognosis and had a shorter PFS than those without them ($P=0.026$) ([Figure 4](#)). Analysis of PFS for the other mutated genes was presented in [Table S2](#). It was shown that patients with *TET2* mutation had higher ETOUS score than those without, while there was no statistical difference in Sokal score between the two groups, which may because the number of patients with *TET2* mutation was not enough ([Table S3](#)).

We defined disease-related events as *ABL1* KD mutations or development to accelerated phase or blast crisis in CML patients. There were 35 patients (35/63) with disease-related events and 28 (28/63) in a relatively stable condition. It was found that patients with disease-related events carried more mutations than those in a relatively stable condition (3.5 vs. 2.0, $P=0.024$).

Discussion

TKI drug resistance and intolerance have remained major clinical challenges in the TKIs era. Although ponatinib, a third-generation TKI, improves outcomes for patients with BCR-ABL1-dependent mechanisms of resistance, including

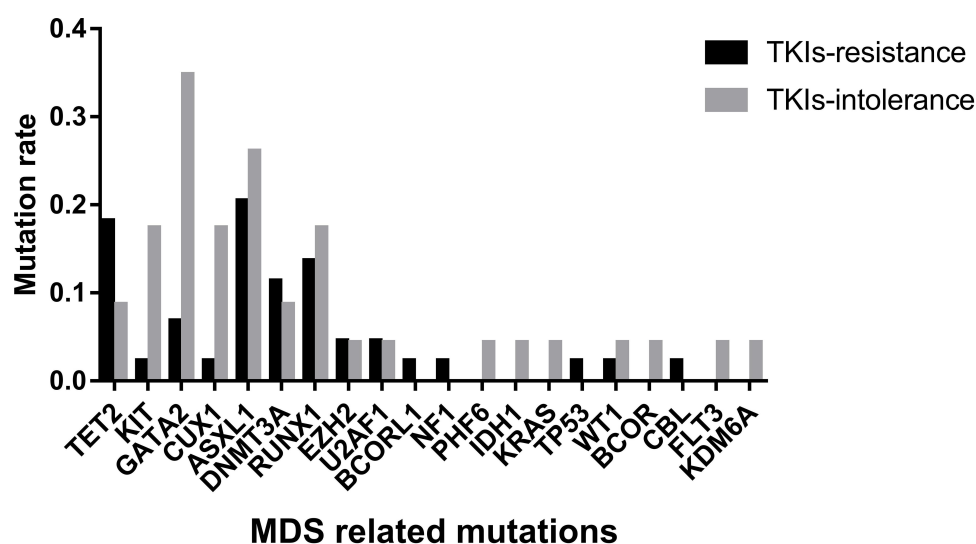


Figure 2 Mutation rates of MDS related genes. Mutation rates of 20 genes related to MDS; Mutation rates of *KIT*, *GATA2* and *CUX1* in patients with TKIs intolerance were significantly higher than in TKIs-resistant group.

Abbreviation: MDS, myelodysplastic syndrome.

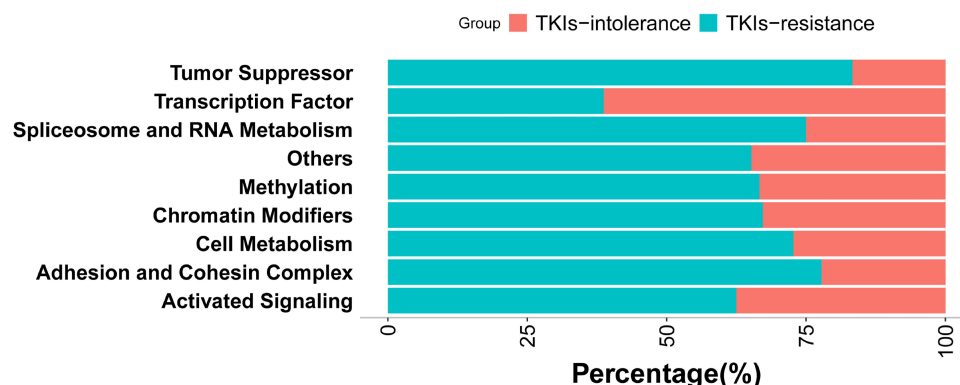


Figure 3 Mutation rates of genes with different functions. According to the function of genes, they were divided into chromatin modification (86.57%, 58/67), activated signaling (58.70%, 40/67), transcription factor (47.76%, 32/67), methylation (44.78%, 30/67), other (34.33%, 23/67), adhesion and cohesin complex (26.87%, 18/67), cell metabolism (16.42%, 11/67), tumor suppressor (8.96%, 6/67), spliceosome and RNA metabolism (5.97%, 4/67). It can be seen that the mutations rate in transcription factor is higher in patients intolerant to TKIs (82.60% vs 27.27%, $P=0.016$).

T315I mutation, a proportion of patients may have or develop BCR-ABL1-independent resistance and suffer from ponatinib treatment failure.¹⁸ The most frequent mechanism of BCR-ABL1-dependent resistance is point mutation in the *ABL1* KD, which directly or indirectly impairs appropriate TKI binding to the kinase pocket either by altering BCR-ABL1 conformation with reduced affinity to the specific inhibitor or by interfering with a TKI-binding site.¹⁸ The causes of BCR-ABL1-independent resistance are complex, including an insufficient concentration of TKIs caused by their pharmacokinetics,^{19,20} activation of alternative signal pathways,²¹ and persistence of leukemia stem cells independent of BCR-ABL1 activity.^{22,23}

In recent years, research on CML has greatly benefited from the development of NGS technology, which enables the profiling of patients with poor TKI response at the genetic level, so that we can better understand the potential mechanisms of drug resistance.

Relationship Between *ABL1* KD Mutation and Additional Mutations

At the 2019 European Hematology Association, Branford et al² mentioned that frequently mutated genes of CML patients with a poor prognosis at diagnosis were *ASXL1*, *IKZF1*, and *RUNX1* and that the methyltransferase

SETD1B was a novel repeatedly mutated gene. They also found that no patient with *MLL* fusion had an *ABL1* KD mutation. Conversely, *ABL1* KD mutations were frequently observed in patients with *IKZF1* variants. In our research, *ABL1* KD mutations were often observed in patients with *ASXL1* variants.

However, in the analysis of gene interaction with *ABL1* KD mutation, *SH2B3* gene mutation was found to significantly coexist with it. It has been proven that LNK, a lymphocyte adapter protein encoded by the *SH2B3* gene, restricts the expansion of hematopoietic stem cells and progenitor cells by binding to *JAK2*, which inhibits the activity of *JAK2* kinase. In mice, LNK dysfunction may promote the development of hematological malignant tumors, and LNK deficiency can cooperate with BCR-ABL1 oncogenes in the development of CML.²⁴

MDS-Related Gene Mutations are Common in Patients with TKI Intolerance

Schmidt et al²⁵ found that 43% of CML patients with Ph-negative clonal cytogenetic abnormalities (CCAs) bear gene mutations, and most of these mutations are similar to those found in MDS patients. Bejar et al²⁶ conducted a comprehensive analysis of 439 patients with MDS based on NGS and determined that three genes (*TET2*, *DMNT3A*,

Table 4 Statistics on Mutation Rates of Additional Genes Associated with *ABL* KD

	<i>ASXL1</i>	<i>FAT1</i>	<i>RUNX1</i>	<i>KIT</i>	<i>SETP1B</i>	<i>IKZF1</i>
<i>ABL1</i> KD positive	8/21(38.1%)	8/21(38.1%)	5/21(23.81%)	0/21(0%)	5/21(23.81%)	0/21(0.00%)
<i>ABL1</i> KD negative	7/46(15.21%)	9/46(19.56%)	5/46(10.87%)	5/46(10.87%)	7/46(15.22%)	1/46(2.17%)
P value	0.041	0.096	0.156	0.142	0.299	0.687

Abbreviation: KD, kinase domain.

Table 5 Relationship Between *ASXL1* Mutation and Clinical Features at Initial Diagnosis

	HB (g/L)	WBC ($\times 10^9$ /L)	PLT ($\times 10^9$ /L)	BASO(%)	Spleen Size (Subcostal Length/cm)	Sokal	EUTOS
<i>ASXL1</i> positive	91 (80, 100)	232.0 (108.4;406.5)	663(616;845)	4.6 (3.5;15.25)	7.3(5.1;23)	0.98 (0.82;1.17)	67.5 (45.9;91.7)
<i>ASXL1</i> negative	97(72;105)	203.2 (110.9;273.0)	538(307;813)	3 (1.75;4.2)	3.95(0;6.67)	0.98(0.63;1.2)	61.85(42;88.2)
P value	0.777	0.914	0.065	0.031	0.041	0.99	0.995

TP53) were independently associated with a decline in overall survival. Among the additional gene mutations that we detected, the mutation rates of additional genes related to MDS were different in the two groups of CML patients. The number of mutations in the TKI-intolerant patients was significantly higher than that in the TKI-resistant patients. In addition, the mutation rates of *CUX1*, *GATA2*, and *KIT* were all increased in the TKI-intolerant group, while the rate of BCR-ABL1 KD mutation was lower than that in the TKI-resistant group (26.09% vs. 34.09%), indicating that the acquisition of BCR-ABL1 KD mutation plays a major role in drug resistance to TKIs. However, the mutation of additional MDS-related genes mainly affects patients' intolerance to TKIs.

In our data, 17.39% *CUX1* mutation detected in patients who were intolerance to TKIs, two patients with *CUX1* mutation accompanied with ACAs, including one with loss of chromosome 7. In the study of *CUX1* in leukemia, it was found that *CUX1* mutation was the most differentially expressed gene among the common deletion gene fragments,²⁷ and it was haploinsufficient in leukemia patients with -7q or -7.^{28,29} The role of *CUX1* gene in maintaining hematopoietic stem cell (HSC) quiescence through the PI3K/AKT signaling pathway has been confirmed in human CD34⁺ cells. Knockdown of the *CUX1* gene promotes PI3K signal transduction and induces HSCs to shift from a quiescent to a proliferative state, leading to HSC exhaustion.²⁹ In our patient cohort, mutations in the *CUX1* gene were mainly missense and frameshift mutations (p.P1271fs). According to InterPro's prediction, there are many DNA binding sites in the segment of 1256 to 1313 amino acids of the protein translated by the *CUX1* gene, while frameshift mutation of the *CUX1* gene in our patient cohort causes the 1271st and subsequent amino acids to change, which may change the original DNA binding site of the protein, leading to abnormal function.

We found 80% patients with *KIT* gene mutation intolerance to first- and second-generation TKIs. c-KIT is one

of the targets of dasatinib,³⁰ which can induce the death of acute myeloid leukemia cells (HEL92.1.7, Kasumi-1, and bone marrow cells of patients with c-KIT⁺).³¹ In our cohort, five patients carried *KIT* gene mutations, three of whom intolerance to dasatinib. Repeated dose reduction or even withdrawal of treatment due to thrombocytopenia caused treatment failure, and two patients progressed to blast crisis. We think that *KIT* gene mutation may attribute to the patients' intolerance to dasatinib, but need larger sample to verify.

In addition, in this study the mutation rate of *GATA2* was also increased in patients with TKI intolerance. Previous reports have shown that *GATA2* haploinsufficiency causes hemocytopenia and immunodeficiency.^{32,33} This is consistent with the observation of decreased proliferation and increased apoptosis of hematopoietic stem cells in mouse models of *GATA2* haploinsufficiency and conditioned *GATA2* inactivation.³⁴ In contrast, the over-expression of *GATA2* inhibited the output and erythroid differentiation of HSCs,³⁵ indicating that irrespective of whether the expression of *GATA2* was decreased or increased, it would affect the proliferation and differentiation of HSCs. However, the mechanism of its role in CML is not completely clear. In our cohort, the mutation rate in patients with TKI intolerance is increased, which may be related to the decreased hemogram in patients with TKI intolerance during TKI treatment.

Among the three genes (*CUX1*, *GATA2*, *KIT*) closely related to TKI intolerance identified in this study, two of them (*CUX1*, *GATA2*) encode transcription factors. After analyzing the functions of 66 somatic mutations detected, it was shown that the number of transcription factor gene mutations in patients with TKI intolerance is greater than that in patients with TKI resistance (82.60% vs. 27.27%, $P=0.016$). Single HSC dysplasia can lead to the occurrence of malignant tumors such as leukemia, and transcription factors are essential for regulating nodes in the genetic map of cell development. Hamey et al³⁶ described the

genetic map of mouse LT-HSCs developing to progenitor cells by single-cell sequencing, simulated the transcriptional regulatory network, and confirmed that *GATA2* regulates the transcription of many key factors. It can be seen that the distribution of mutations in transcription factor genes is very different in patients with drug resistance or intolerance to TKIs. In addition, the imbalance of the transcriptional regulatory network is severer in patients with TKI intolerance, which may suggest that it plays an important role in the mechanism of TKI intolerance.

Relationship Between Age-Related Clonal Hematopoiesis and CML Progression

In our study, patients with mutations in *TET2* showed a poorer prognosis and had a shorter PFS than those without them ($P=0.026$). However, this may have been due to the insufficient number of patients included here, as its variant allele frequency (VAF) does not show a correlation with the progression of CML disease.

ASXL1 gene mutation showed an unusual relationship with CML in our study. First of all, these mutations were more common in *ABL1* KD-mutant patients. Additionally, patients with *ASXL1* mutation had a higher proportion of basophils in peripheral blood and a larger spleen at initial diagnosis. Although there were no differences in Sokal and EUTOS scores, *ASXL1* mutation with increased the risk of disease progression. In MDS/MPN patients, *ASXL1* mutation concentrated in exon 12. It has been reported³⁷ that frame-shift or nonsense mutation in exon 12 of *ASXL1* leads to truncation and removal of the C-terminal of the protein, which contains a PHD finger, a structural motif found in nuclear proteins related to transcriptional regulation, chromatin modification, and histone demethylation. In this study,

besides those in the *ASXL1* gene, the main other gene mutations were missense mutations, but frameshift mutations in exon 12 of the *ASXL1* gene accounted for 46.67% of the total, resulting in protein truncation, loss of PHD finger, and altered function of downstream proteins.

DNMT3A and *TET2* genes were previously considered to be drivers of myeloid malignancies,^{38,39} but recent studies⁴⁰ have shown that these two genes were most commonly mutated in age-related clonal hematopoiesis (ARCH). ARCH increases not only the risk of hematological malignancies in humans,⁴⁰ but also the risk of non-hematological diseases,^{41–43} for which age is the main risk factor. However, Sasaki⁴⁴ suggested that the VAFs of *ASXL1*, *DNMT3A*, *JAK2*, *TET2*, and *TP53* mutations are closely related to the progression of AML and can be used as prognostic factors for overall survival. At present, there are different opinions on the correlation between such mutations and the disease progression of hematological malignancies. Stronger evidence is needed to explain their relationships.

Conclusion

In summary, we found that *ABL1* KD mutations play dominant roles in drug resistance to TKIs, while *CUX1*, *KIT*, and *GATA2* mutations may contributors to TKI intolerance. Although some mutations associated with ARCH, their associations with poor prognosis of CML patients cannot be ignored. We believe that genomic risk assessment can improve the risk stratification in clinical diagnosis of CML, which enables us to accurately identify CML patients who are resistant or intolerant to TKIs at an early stage in the era of TKI treatment.

Abbreviations

TKIs, tyrosine kinase inhibitors; CML, chronic myeloid leukemia; KD, kinase domain; NGS, next-generation sequencing; Ph chromosome, Philadelphia chromosome; CHR, complete hematologic response; MCyR, major cytogenetic response; WBC, white blood cell; MDS, myelodysplastic syndrome; CP, chronic phase; AP, accelerated phase; BC, blast crisis; CCAs, clonal cytogenetic abnormalities; HSC, hematopoietic stem cell; ARCH, age-related clonal hematopoiesis; VAF, variant allele frequency.

Data Sharing Statement

All data and materials analyzed during the current study are included in this published article.

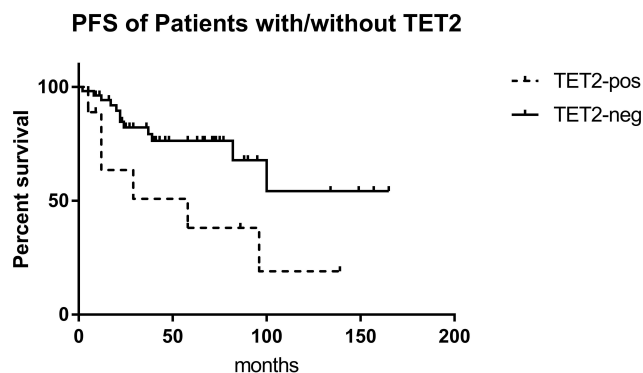


Figure 4 EFS of patients with/without *TET2* mutation. In Kaplan-Meier analysis, the EFS of patients with *TET2* mutation was significantly lower than those without ($P=0.039$). **Abbreviation:** EFS, event-free survival.

Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of Nanfang Hospital and carried out in according to the Declaration of Helsinki. All patients provided written informed consent.

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Waner Wu and Na Xu contributed equally to this work and should be considered as co-first authors.

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Disclosure

The authors report no conflicts of interest in this work.

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