Review article

Genetic landscape of chronic myeloid leukemia

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Abstract

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm caused by the *BCR::ABL1* fusion gene, which aberrantly activates ABL1 kinase and promotes the overproduction of leukemic cells. CML typically develops in the chronic phase (CP) and progresses to a blast crisis (BC) after years without effective treatment. Although prognosis has substantially improved after the development of tyrosine kinase inhibitors (TKIs) targeting the BCR::ABL1 oncoprotein, some patients still experience TKI resistance and poor prognosis. One of the mechanisms of TKI resistance is *ABL1* kinase domain mutations, which are found in approximately half of the cases, newly acquired during treatment. Moreover, genetic studies have revealed that CML patients carry additional mutations that are also observed in other myeloid neoplasms. *ASXL1* mutations are often found in both CP and BC, whereas other mutations, such as those in *RUNX1*, *IKZF1*, and *TP53*, are preferentially found in BC. The presence of additional mutations, such as *ASXL1* mutations, is a potential biomarker for predicting therapeutic efficacy. The mechanisms by which these additional mutations affect disease subtypes, drug resistance, and prognosis need to be elucidated. In this review, we have summarized and discussed the landscape and clinical impact of genetic abnormalities in CML.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm caused by the Philadelphia (Ph) chromosome der(22)t(9;22)(q34;q11.2). This translocation generates *BCR::ABL1* fusion gene, which aberrantly activates ABL1 kinase, causing the overproduction of leukemic cells. Most patients with CML are diagnosed in the chronic phase (CP) without any clear clinical symptoms, except for increased blood cell counts. Although the prognosis of CML has dramatically improved after the development of the tyrosine kinase inhibitor (TKI) imatinib, which targets the BCR::ABL1 oncoprotein, a minority of patients still experience blast crisis (BC), characterized by the proliferation of undifferentiated blasts and dismal outcome, due to the acquired resistance to TKI in leukemic cells [1–3]. Except for the *ABL1* kinase domain mutations found in about half of the patients, the mechanism of TKI resistance and clonal evolution of CML is not completely understood [4–7]. Cytogenetic studies and recent genetic studies using next-generation sequencing (NGS) have revealed not only *ABL1* kinase domain mutations, but also additional cytogenetic and genetic abnormalities in CML. Additional mutations are rarely found in CML-CP at diagnosis [8, 9]. In contrast, some driver mutations found in other myeloid neoplasms [10–15] are frequently acquired in CML-BC [16–24]. As these mutations confer CML resistance to TKI and drive leukemic transformation, a detailed genetic analysis is critical for understanding the pathogenesis and optimal clinical management of CML. Recent genetic studies have demonstrated the mutational landscape of CML, suggesting the clinical utility of genetic abnormalities at CML diagnosis to predict future TKI resistance and clinical outcomes [24–28].

The 5-year overall survival of patients with CML-BC has almost doubled from 16% during 2000–2004 to 33% during 2010–2016, which can be attributed to the increased use

of TKIs [29]. Despite the effectiveness of TKIs on certain patients with BC and their contribution to improved survival, the majority of patients with BC no longer respond to the inhibitors. Unfortunately, only a few clinical biomarkers are currently known to be associated with the clinical outcomes of BC patients treated with TKI-based regimens [29, 30]. As genetic data have been incorporated in the diagnosis, risk stratification, and treatment choice for acute myeloid leukemia [31], it is of considerable clinical importance to evaluate whether mutations other than *BCR::ABL1* can predict prognosis and guide clinical decisions in CML-BC [24].

In this review, we summarize and discuss the mutational landscape of CML and the clinical impact and utility of genetic abnormalities in distinct phases of CML.

Landscape of genetic abnormalities in CML-CP

CML is a disease caused by the Philadelphia (Ph) chromosome, and in some cases, additional genetic abnormalities. Additional cytogenetic and genetic abnormalities in CML-CP have been analyzed using various technologies, including Sanger sequencing, SNP array, and NGS [8, 9, 21, 24, 25, 32–38]. Most previous studies have analyzed relatively small genes and patients with CML using different methodologies. A meta-analysis revealed that only *ASXL1* and *IKZF1* mutated in more than 5% of CML-CP cases [32]. Other recurrent mutations were found in *RUNX1, TET2, DNMT3A, TP53, SETD1B, JAK2, KMT2D, CBL*, and *EZH2*. More recently, somatic mutations in 148 adult CML-CP cases were comprehensively analyzed by whole-exome sequencing or targeted-capture sequencing, which demonstrated that additional genetic alterations were found in approximately 25% of patients with CP at diagnosis [24]. *ASXL1* mutations were most frequently observed in CML-CP at diagnosis, with a frequency of approximately 20 %, followed by recurrent mutations,

such as *TET2, KMT2D, PTPN11, RUNX1,* and *WT1,* with a frequency of less than 5% **(Fig. 1)** [24]. These previous studies analyzed mutations in CP using different analytical approaches; a common finding is frequent mutations in epigenetic regulators, including *ASXL1* [24, 32].

Mutations in *ASXL1* are frequently observed in several types of myeloid malignancies, such as acute myeloid leukemia, myelodysplastic syndromes, and myeloproliferative neoplasms [10–14]. Functional studies have shown that mutated *ASXL1* alters epigenetic regulation and promotes myeloid transformation [39]. Although *ASXL1* is one of the most frequent targets of mutations associated with age-related clonal hematopoiesis [40–42], many CML patients with *ASXL1* mutations are younger than 60 years at the time of diagnosis [21, 24, 25, 38]. In particular, *ASXL1* mutations were identified in 6/21 (29%) children and young adult CML patients [38], suggesting that the acquisition of *ASXL1* mutations is not an age-related phenomenon in terms of CML development. Deep sequencing of driver mutations in paired CP and BC samples obtained from 52 patients revealed that *ASXL1* mutations were already present in the CP samples, whereas other driver mutations, such as *RUNX1*, *ABL1*, and *TP53* mutations, were initially absent in CP and newly emerged in BC **(Fig. 2)** [24]. Almost all patients with *ASXL1* mutations acquire other additional driver mutations during progression to BC, including *RUNX1*, *TP53*, *BCOR*, and *SETD1B* mutations. Thus, *ASXL1*-mutated CP clones preferentially evolve by acquiring other driver mutations during clonal development.

Clinical relevance of genetic abnormalities in CML-CP

Approximately half the patients with CML suffering from TKI resistance acquire *ABL1* kinase domain mutations. To understand the clinical impact of additional mutations, several

studies have examined possible relations between genomic abnormalities and clinical outcomes in CML-CP [24–28, 43]. The sequencing of a panel of mutations in 300 serial samples from 100 CML patients demonstrated that patterns of mutation acquisition, persistence, and clearance were diverse and had some correlation with clinical outcomes [25]. The acquisition of driver mutations during TKI treatment is associated with a poor response [25]. In a study where 85 samples were genotyped by whole-exome sequencing, the mutation burden differed between the CP and accelerated phase or BC, and non-optimal responders had more mutations than optimal responders at the time of diagnosis and in follow-ups [43]. As reported by a comprehensive sequencing study involving 148 CML-CP cases, patients who received TKI and later experienced BC progression, carried driver mutations, such as *ASXL1* mutations, and copy number abnormalities (CNAs) more frequently than those without BC [24]. Consistent with aforementioned results, in a study involving 71 CML-CP cases, *ASXL1* mutations in CML-CP were shown to be the only risk factors associated with worse outcomes [28]. In terms of treatment choice, a recent study analyzed mutations in patients with newly diagnosed CML-CP treated with imatinib (n=62) or second-generation TKIs (n=62) and demonstrated that the presence of mutations such as *ASXL1* mutation predicted progression-free survival in the imatinib cohort but not in the second-generation TKI cohort [27]. This suggests that the earlier use of second-generation TKIs in the course of treatment may overcome the negative impact of mutations on disease progression.

Accumulating evidence suggests that additional genomic abnormalities, such as *ASXL1* mutations, found at diagnosis and during the clinical course, may affect sensitivity to TKIs and predict clinical outcomes. Prospective studies can elucidate the utility of additional

mutations as biomarkers to predict TKI response and encourage early treatment of high-risk patients with second-generation TKI.

Landscape of genetic abnormalities in CML-BC

In CML-BC, additional genetic and cytogenetic abnormalities are found in more than 90% of the cases, which is much higher than that in CP, where additional mutations are found in approximately 25% of cases [24, 32]. Although differences in target genes and analysis pipelines make interpretation difficult, a meta-analysis study revealed that somatic mutations recurrently reported in CML-BC included *ABL1*, *RUNX1*, *IKZF1*, *ASXL1*, *GATA2*, *TET2*, *TP53*, *SETBP1*, *PTPN11*, *IDH1*, *IDH2*, *NRAS*, *JAK2*, and *CBL* mutations [32]. Among these, *ABL1*, *RUNX1*, *IKZF1*, and *ASXL1* were mutated at a frequency of >15%. Large-scale studies using NGS have provided more comprehensive and unbiased results. A recent analysis demonstrated the landscape of mutations in 108 BC cases **(Fig. 3)** [24]. Mutations in *RUNX1, ASXL1, ABL1,* and *IKZF1* were observed at a frequency of >10%, which is consistent with a previous meta-analysis [32]. *RUNX1,* the most frequently mutated gene in CML-BC, encodes a master transcription factor of hematopoiesis and is frequently mutated in various hematological malignancies [44]. Other recurrent mutations found at a frequency of 3-10% included *CDKN2A/B, BCORL1, TP53, BCOR, UBE2A, WT1, GATA2, KMT2D, SETD1B*, and *SETD2*. Among these, the ubiquitin-conjugating enzyme E2A gene, *UBE2A*, was recently identified as a unique target of mutations in CML, found in approximately 5% of BC cases [21, 22, 24, 45]. These studies show that *UBE2A* mutations may be specific to myeloid BC. Sequencing-based copy number analysis identified CNAs, including an extra Ph chromosome (+Ph), +8, -7/del(7p), del(17p), +21, -9/del(9p), +19, +6, and isochromosome 17q (i17q, resulting in one copy of 17p and three copies of 17q) [24]. This

is consistent with previous studies reporting frequent cytogenetic abnormalities in CML-BC by cytogenetic analysis [30].

While the *BCR::ABL1* fusion gene is a well-known hallmark of CML, other concurrent gene fusions have also been described. Cytogenetic studies reported several translocations in CML, including those involving *RUNX1, MECOM,* and *MLL* (*KMT2A*) [34, 46–49]. Furthermore, recent RNA-seq studies have provided a more comprehensive view of gene fusion in CML patients. In an RNA-seq analysis of 33 CML-BC patients, 14 had known or novel gene fusions that were not associated with Ph translocation [21]. *MLL* fusions were most frequent (n = 5), two of which were cytogenetically cryptic. Other known fusions included *CBFB-MYH11* and *PAX5-ZCCHC7* and novel fusions involved cancer genes implicated in hematological malignancies, such as *RUNX1, IKZF1*, and *MECOM*. Another whole-exome sequencing study also identified an inversion event resulting in a novel *RUNX1-ETS2* fusion [24]. At several time points, quantitative RT-PCR showed that this fusion was present at the time of CP diagnosis, and the burden was correlated with that of *BCR::ABL1*, indicating that gene fusion may play a role in the progression from CP to BC.

Clinical relevance of genetic abnormalities in CML-BC

Leukemic blasts in CML-BC exhibit either myeloid or lymphoid immunophenotypes. Certain genetic abnormalities, such as +21, +8, +19, and *ASXL1* and *TP53* mutations, are enriched in myeloid crisis, while others are enriched in lymphoid crisis, including *CDKN2A/B* and *IKZF1* deletions, −7/del(7p), and −9/del(9p) [24]. In contrast, *RUNX1* mutations and +Ph were equally found in both crises. Immunoglobulin and/or T-cell receptor gene rearrangements are observed not only in lymphoid BC cases, but also in a few myeloid BC cases [24]. This suggests that leukemic cells in BC show lineage plasticity, which is also

seen in other types of hematological neoplasms, such as acute leukemias and histiocytic/dendritic cell neoplasms [50–53]. Collectively, myeloid and lymphoid BC showing distinct lineage commitment, have both common and unique molecular features, and the lineage of leukemia cells is occasionally ambiguous.

Examining the influence of TKI therapy on the mutational profile may be useful for understanding the mechanism of TKI resistance and clonal evolution of CML. In whole-exome sequencing analysis, *ABL1* mutations were, as expected, almost exclusively found in patients receiving TKIs and represented the most frequent mutation in 56 BC patients with a prior history of TKI therapy [24]. Notably, *ABL1* mutations are typically accompanied by other genetic alterations, such as *RUNX1*, *ASXL1*, and *BCORL1* mutations; focal deletions in *CDKN2A/B* and *IKZF1*; and cytogenetic abnormalities, such as −7/del(7p), −9/del(9p), and +8. These findings suggest that *ABL1* mutations confer therapeutic resistance, while leukemic transformation may be driven by additional mutations. Moreover, BC patients with a history of TKI treatment carried −7/del(7p), −9/del(9p), and complex CNA (defined as ≥3 CNAs) more frequently than those who had not undergone TKI treatment, whereas *BCOR*, *TP53*, and *RUNX1* mutations were less frequent in TKI-treated patients. These mutations may affect TKI response or resistance, which requires further experimental validation.

The prognosis of CML-BC has improved since the development of TKIs; however, many patients still experience a refractory disease course. While several clinical factors and additional chromosomal abnormalities have been reported as prognostic factors [29, 30], few studies have comprehensively investigated the prognostic impact of genomic abnormalities, such as gene mutations. In a whole-exome sequencing study, the prognostic impact of clinical factors and genetic abnormalities was comprehensively evaluated in patients with CML-BC [24]. In terms of clinical factors, treatment for BC with TKI-containing regimens

and lymphoid lineage of blasts were good prognostic factors, whereas other factors, such as age and blood cell counts, were not significant. In contrast, genomic abnormalities, such as *ASXL1* mutations, *TP53* mutations, del(17p), i(17q), +19, +21, and complex CNAs, were associated with poor prognosis, indicating that genomic abnormalities may be useful in predicting the outcome of BC treated with TKI-containing regimens. Notably, cases with biallelic *TP53* mutations showed an extremely poor prognosis, which is consistent with that of other myeloid tumors [54, 55]. In the multivariate analysis of 59 TKI-treated patients, *ASXL1* mutations, complex CNAs, i(17q), and +21 were independent predictors of a worse prognosis. These findings were largely recapitulated in an independent cohort, although the number of patients was limited [21, 24]. Therefore, heterogeneity in the genetic profile of CML-BC results in diverse clinical manifestations, including poor prognosis.

Conclusion and future perspectives

CML is mainly characterized by the *BCR::ABL1* fusion gene, but other additional mutations cause clonal evolution and disease progression, as summarized in **Fig. 4**. Additional mutations, which are also frequently observed in other myeloid neoplasms, are typically found in genes involved in epigenetic regulation. As already discussed for other hematological malignancies, it is necessary to consider the possibility of prognostic stratification based on genetic mutations in CML [11, 13]. In this regard, *ASXL1* mutation is a potential biomarker for therapeutic efficacy in both CML-CP and BC [24, 28]. Furthermore, as the mutational profile in patients with acute myeloid leukemia is shown to be associated with sensitivity to various anticancer drugs [15, 31], adjustment of the treatment depending on the mutational status is a promising approach in patients with CML.

Several questions regarding the understanding of CML remain unanswered. As few studies with a large cohort have prospectively evaluated the genetic and epigenetic features of CML, future studies need to comprehensively identify the landscape and clinical relevance of genetic and epigenetic alterations in CML, which can be used to develop novel therapeutic strategies. Furthermore, the molecular mechanisms by which distinct additional mutations induce TKI resistance and disease progression remain elusive. Although the functional roles of mutations in epigenetic regulators have been evaluated in several mouse models [14, 56], most studies have been conducted in a genetic context without the *BCR::ABL1* fusion, a hallmark of CML. Further studies are necessary to elucidate the mechanism of clonal evolution and acquired drug sensitivity in CML through the functional interaction between *BCR::ABL1* fusion and additional genetic alterations.

Figure legends

Fig. 1. Driver mutations in chronic phase (CP).

Frequencies of mutations in 148 CP patients.

Fig. 2. Clonal evolution of *ASXL1* and other driver mutations.

Tumor cell fractions (TCFs) of mutations in the corresponding CP and BC samples from each patient with *ASXL1* mutations. The black dashed lines indicate the TCF of 0%.

Fig. 3. Driver mutations in blast crisis (BC).

Frequencies of mutations in 136 BC patients.

Fig. 4. Overview of clonal evolution in Chronic myeloid leukemia (CML).

Representative mutations found in each stage of CML are indicated below.

KD, kinase domain; +Ph, extra Philadelphia chromosome; i(17q), isochromosome 17q.

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Fig. 1

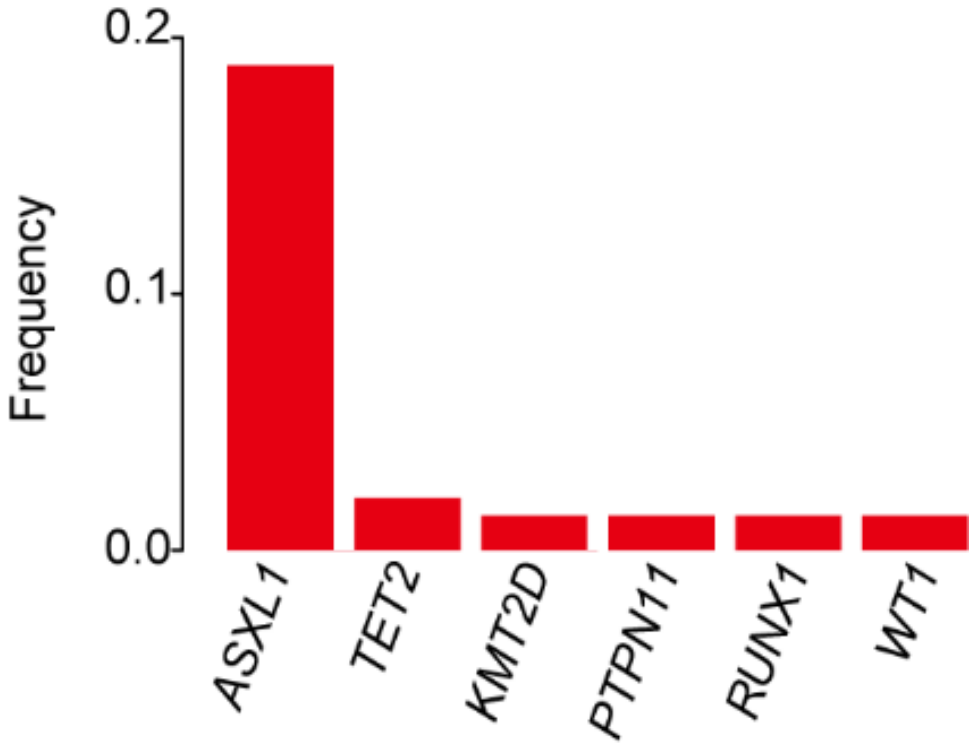


Fig. 2

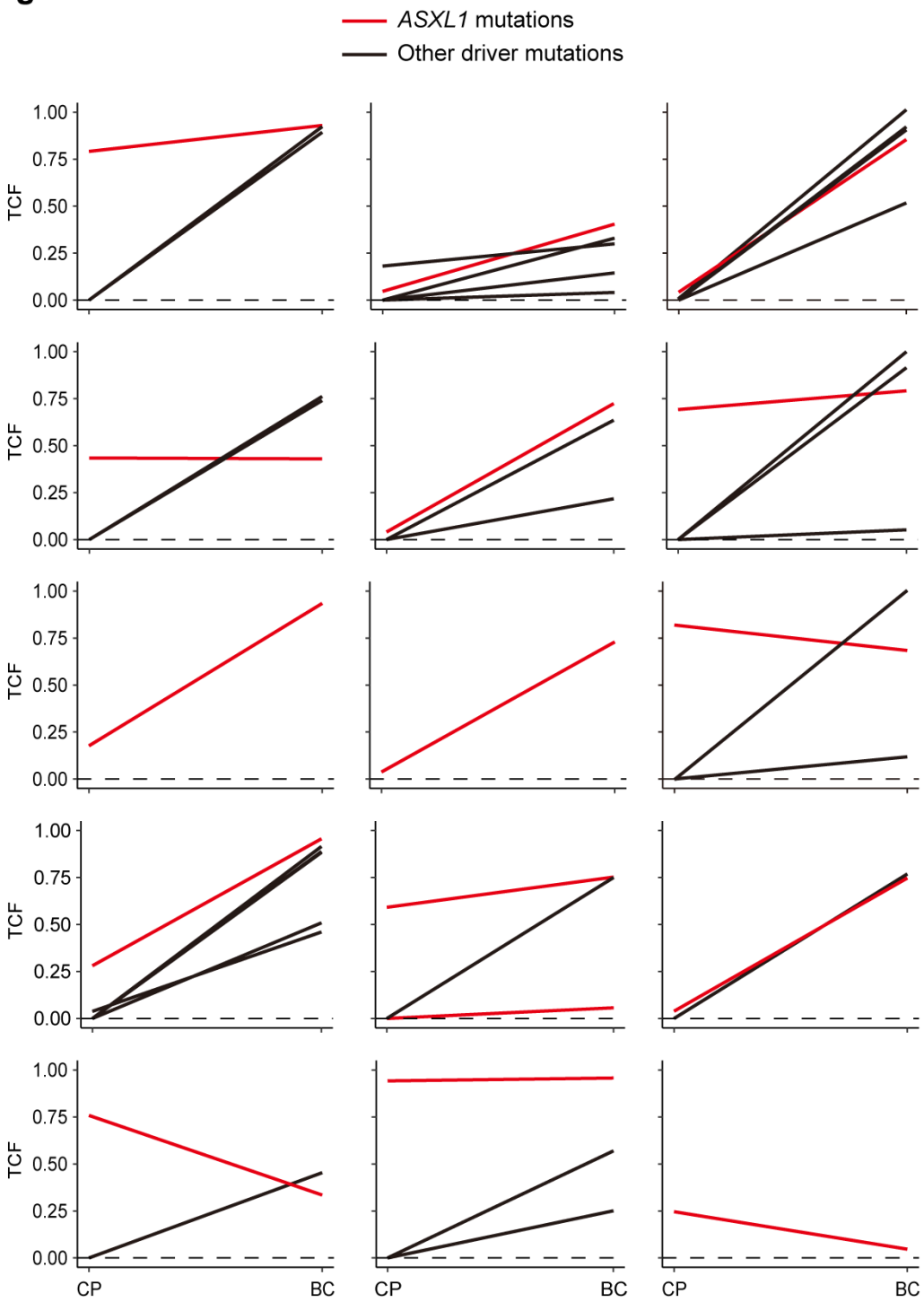

ASXL1 mutations
Other driver mutations
TCF
1.00
0.75
0.50
0.25
0.00
CP
BC

Fig. 3

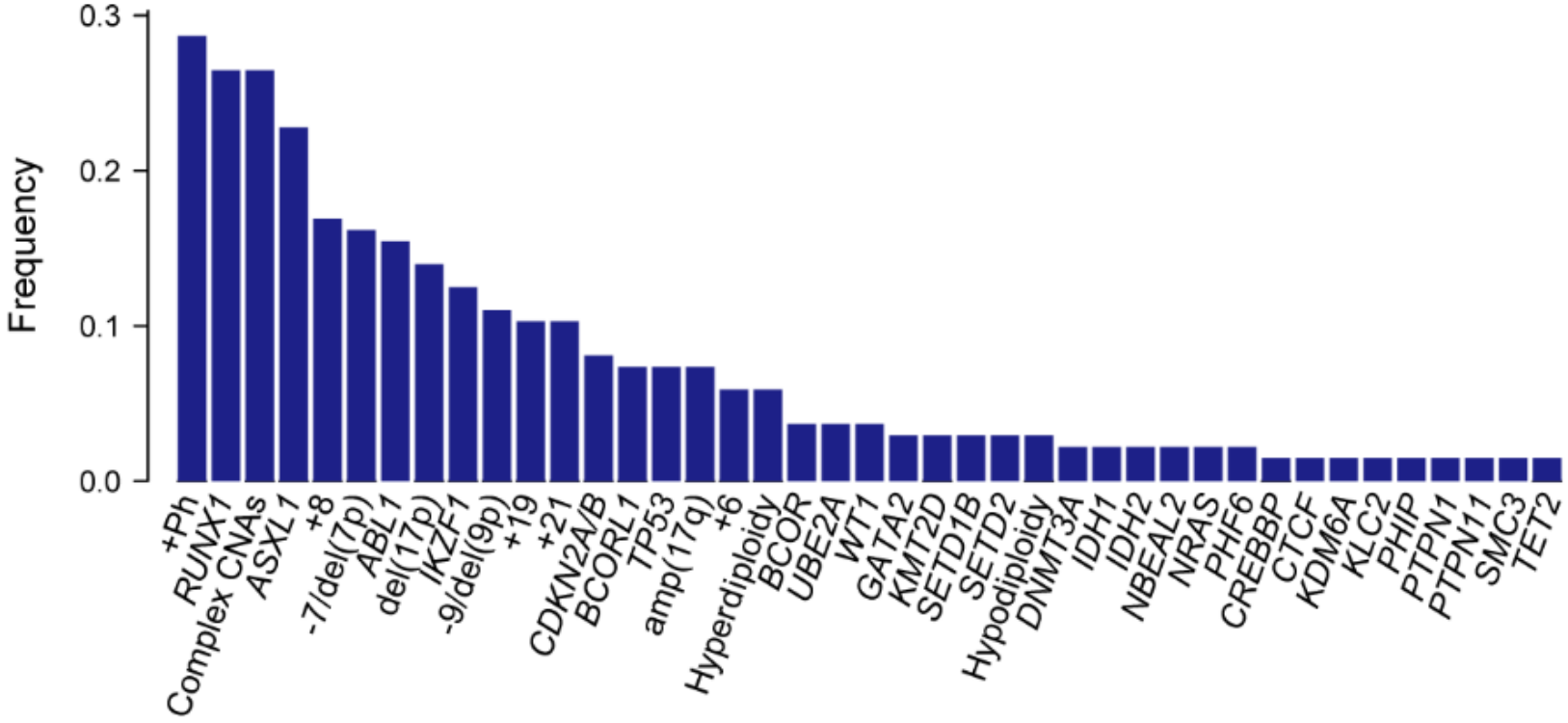
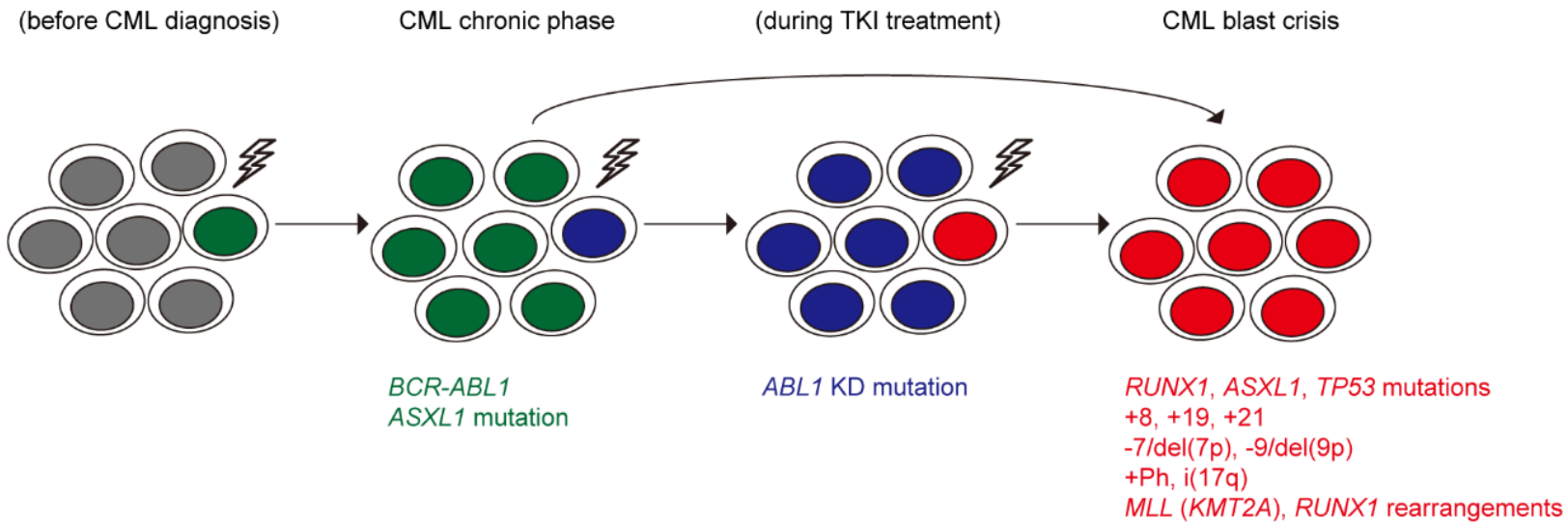

Frequency
0.3
0.2
0.1
0.0
+Ph
RUNX1
Complex CNAs
ASXL1
+8
-7/del(7p)
ABL1
del(17p)
IKZF1
-9/del(9p)
+19
+21
CDKN2A/B
BCORL1
TP53
amp(17q)
+6
Hyperdiploidy
BCOR
UBE2A
WT1
GATA2
KMT2D
SETD1B
SETD2
Hypodiploidy
DNMT3A
IDH1
IDH2
NBEAL2
NRAS
PHF6
CREBBP
CTCF
KDM6A
KLC2
PHIP
PTPN1
PTPN11
SMC3
TET2

Fig. 4


(before CML diagnosis)
CML chronic phase
(during TKI treatment)
CML blast crisis
BCR-ABL1
ASXL1 mutation
ABL1 KD mutation
RUNX1, ASXL1, TP53 mutations
+8, +19, +21
-7/del(7p), -9/del(9p)
+Ph, i(17q)
MLL (KMT2A), RUNX1 rearrangements