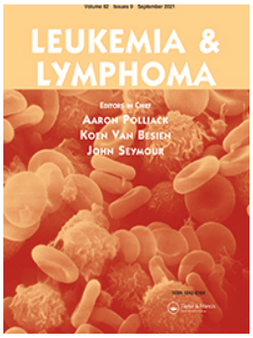

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REVIEW

Mutational landscape of chronic myeloid leukemia: more than a single oncogene leukemia

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ABSTRACT

The *BCR-ABL1* fusion gene, which causes aberrant kinase activity and uncontrolled cell proliferation, is the hallmark of chronic myeloid leukemia (CML). The development of tyrosine kinase inhibitors (TKI) that target the BCR-ABL oncoprotein has led to dramatic improvement in CML management. However, some challenges remain to be addressed in the TKI era, including patient stratification and the selection of frontline TKIs and CML progression. Additionally, with the emerging goal of treatment-free remission (TFR) in CML management, biomarkers that predict the outcomes of stopping TKI remain to be identified. Notably, recent reports have revealed the power of genome screening in understanding the role of genome aberrations other than *BCR-ABL1* in CML pathogenesis. These studies have discovered the presence of disease-phase specific mutations and linked certain mutations to inferior responses to TKI treatment and CML progression. A personalized approach that incorporates genetic data in tailoring treatment strategies has been successfully implemented in acute leukemia, and it represents a promising approach for the management of high-risk CML patients. In this article, we will review current knowledge about the mutational profile in different phases of CML as well as patterns of mutational dynamics in patients having different outcomes. We highlight the effects of somatic mutations involving certain genes (e.g. epigenetic modifiers) on the outcomes of TKI treatment. We also discuss the potential value of incorporating genetic data in treatment decisions and the routine care of CML patients as a future direction for optimizing CML management.

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Chronic myeloid leukemia; blast phase; somatic mutations; risk stratification; personalized medicine

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder that accounts for about 15% of adulthood leukemia with a median age at onset of 57 years [1,2]. The hallmark of CML is the Philadelphia chromosome (Ph), which emerges in the reciprocal translocation between chromosomes 9 and 22. This translocation results in the formation of the *BCR-ABL1* hybrid gene, which encodes a constitutively active oncokinase protein [3]. Tyrosine kinase inhibitors (TKI) have been developed as a targeted therapy inhibiting BCR-ABL1 kinase activity [4]. The implementation of TKIs in CML management has led to a dramatic improvement in the disease's outcomes and the nearly normal life expectancy of CML patients [5,6]. Furthermore, some patients with sustained deep molecular remission on TKI treatment have suspended therapy successfully without the incidence of disease relapse and have achieved treatment-free remission (TFR). Because of the improved survival rates, the achievement of TFR has been adopted as a new goal in CML management [7–9]. Understanding the biological factors that contribute to successful TFR would enable the optimization of CML management.

Historically, since the discovery of the *BCR-ABL1* fusion gene, CML has been considered a prototype of cancer evolution in which a single oncogene, *BCR-ABL1,* is capable of initiating and maintaining the cancer phenotype [10]. Additionally, the high response rates of TKIs in most CML patients have added to the evidence showing the principal role of *BCR-ABL1* in CML pathogenesis. Furthermore, the retroviral transduction of B*CR-ABL1* in murine stem cells successfully induces a CML-like phenotype [11,12]. However, the

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heterogeneity of the clinical outcomes of CML in TKI treatment suggests the contribution of BCR-ABL1-independent mechanisms to CML pathogenesis. A proportion of CML patients have shown either primary (5–10%) or secondary resistance (20–30%) to TKI treatment, even with the use of more potent second- and third-generation TKIs [2,13]. Acquired Abl-kinase domain (Abl-KD) mutations are the most common cause of secondary resistance where they are detectable in about 60% of cases; however, the majority of primary resistance mechanisms remain elusive [14,15]. Moreover, many patients with secondary resistance lack Abl-KD mutations and have been suggested to have BCR-ABL1-independent mechanisms [16,17]. Furthermore, about 5% of CML patients progress from the chronic phase (CP) to the aggressive blast phase (BP) with very poor outcomes and few treatment options [18,19]. Additionally, leukemic stem cells (LSC) are the main culprit in CML relapse in about half of patients who attempt TKI discontinuation [20,21]. CML LSCs have been suggested to have additional survival pathways in addition to BCR-ABL1-induced signaling [22,23]. These mechanisms contribute to the persistence of CML LSCs despite efficient TKI treatment and the achievement of durable deep molecular responses [24,25].

The evidence accumulated with the advances made in sequencing technologies suggests the contribution of additional genetic events to CML pathogenesis. *BCR-ABL1* has been detected in healthy individuals [26], which suggests that additional genetic events may be required for leukemia transformation [27]. Moreover, the genomic instability state induced by *BCR-ABL1* leads to the acquisition of additional genetic aberrations ranging from point mutations to chromosomal abnormalities [28–30]. Mechanisms that include enhanced DNA damage *via* induced reactive oxygen species (ROS) activity and the inhibition of DNA repair mechanisms [31,32] contribute to *BCR-ABL1*-induced mutagenesis. Furthermore, the acquisition of genetic aberrations involving known cancer genes has been linked to CML progression [33,34]. In elderly-onset leukemia, mutations related to clonal hematopoiesis of intermediate potential (CHIP) [35] have been suggested to play a role in CML pathogenesis [36]; however, this role remains elusive. Although genetic data have been incorporated in diagnosis, risk stratification, and treatment strategies for acute leukemia patients [37], they are still lacking in CML. Currently, the risk stratification of CML patients involves mainly clinically based scoring systems (e.g. Sokal score) and molecular monitoring of *BCR-ABL1* levels [1,2]. Systematic studies that investigate the mutational landscape of CML patients in different phases are highly warranted. The analysis of serial samples would also enable the in-depth examination of clonal evolution and mutational dynamics in CML patients under treatment. In addition, the role of genetic data in tailoring treatment strategies in high-risk CML patients remains to be investigated.

In this review, we discuss recent knowledge about the mutational landscape in different phases of CML, focusing on somatic single nucleotide variants (SNV), small insertions and deletions (indels), as well as focal deletions. We also highlight a potential association between non-*BCR-ABL1* mutations and poor outcomes, including treatment resistance and CML progression. The current evidence emphasizes the significance of genetic data in CML management and supports the incorporation of genetic investigations in routine CML work-ups.

Genetic events in CP-CML

Initial studies that investigated genetic events beyond *BCR-ABL1* focused on BP-CML rather than CP-CML, which has long been considered a genetically uniform disease. Early studies employed Sanger sequencing to analyze a limited number of selected genes with known cancer associations. One of the earliest studies reported a missense *RUNX1* mutation in the diagnostic sample of a CP-CML patient with trisomy 21, secondary resistance to imatinib, and later progression to BP-CML [38]. Another study screened the *RUNX1* gene in 14 CML patients with trisomy 21 and identified *RUNX1* mutations in six patients (one CP-CML patient and five myeloid BP-CML patients) [39]. In the following studies [40–42], *ASXL1* was the most frequently mutated gene in CP-CML patients, while mutations in other leukemia-associated genes that were selected for screening, including the *TET2* and *IDH1/2* genes, have rarely been identified [40,43].

With the application of high-throughput sequencing techniques, a greater number of CML diagnosis samples have been sequenced using a targeted sequencing panel [44–48], whole genome sequencing (WGS), or whole exome sequencing (WES) [48–54] (Table 1). Data in high-throughput sequencing studies have suggested that CP-CML is a genetically heterogenic leukemia although it shows less heterogeneity compared with acute leukemia at diagnosis [55]. Non-silent mutations affecting cancer-associated genes were detected in about 35% of CP-CML patients (range: 29%–50%) by either targeted sequencing or

Table 1. Studies of the genetic events at different phases of CML in the TKI era.

	Patients						Sequencing			
		BP		Matched samples						
Study	CP	No.	Phenotype*	CP/ follow-up	CP/BP	Total	Method	No. of patients**	No. of genes***	Targeted genes****
Corm et al. [38]	1				1	1	Sanger	1	1	*RUNX1*
Roche-Lestienne et al. [39]	1	12	My-BP:8 Ly-BP:1 AP:3	–	–	13	Sanger	13	1	*RUNX1*
Roche-Lestienne et al. [40]	91			71	20	91	Sanger	91	4	*ASXL1, TET2, IDH1/2*
Menezes et al. [41]	14			8	6	14	WES	1	–	*ASXL1, TP53, IKZF3*
							Sanger	13	3	
Valikhani et al. [42]	66					66	Sanger	66	2	*ASXL1, JAK2*
Soverini et al. [43]	50	120	My-BP:75 Ly-BP:31 Amb-BP:9 AP:5		1	170	RNA seq Sanger	1 169	– 2	*IDH1/2*
Schmidt et al. [44]	29			15		29	Targeted	29	25	
Kim et al.† [45]	100			92	8	100	Targeted	100	92	
Nteliopoulos et al.† [46]	124			11	3	124	Targeted	124	71	
Ernst et al. [47]	21			1		21	Targeted	21	30	
Adnan Awad et al.† [48]	43	19	My-BP:10 Ly-BP:4 AP:3	25	3	62	WES RNA Targeted	17 4 41	– – 578	
Togasaki et al.† [49]	24					24	WES	24		
Mitani et al. [50]	20			20		20	WES	20		
Mologni et al.† [51]	19					19	WES	19		
Branford et al.† [52]	51	39	My-BP:19 Ly-BP:20		25	65	WES RNA	38 59		
Ko et al. [53]	13	52	My-BP: 27 Ly-BP:23 AP: 2		13	52	WGS WES	13 39		
Kim et al. [54]	5	8	BP:4 AP:4			13	WES			
Makishima et al. [61]	14	40	My-BP:20 Ly-BP/ Amb-BP:6 AP:14			54	Sanger	54	7	*JAK2, CBL, CBLB, ASXL1, TET2, IDH1/2*
Zhang et al. [63]		85	BP:57 AP:28			85	Sanger	85	2	*RUNX1, GATA2*
Yamamoto et al. [64]		13				13	Sanger	13	1	*RUNX1*
Boultwood et al. [65]	32	21	My-BP:10 Ly-BP:2		12	41	Sanger SNP array	41		*ASXL1, TP53, IKZF1*
Magistroni et al. [66]	41	24	My-BP:5 Ly-BP:4 ns:1		10	55	WES Amplicon	10 45	– 1	*UBE2A*
Grossmann et al. [67]		39	My-BP:24 Ly-BP:10 ns:5			39	Amplicon	39	11	
Sklarz et al. [68]	1	1	My-BP		1	1	WES	1		
Huang et al. [69]	1	1			1	1	WES	1		
Adnan Awad et al. [70]	2	8	My-BP:5 Ly-BP:2 Amb-BP:1		2	8	WES	1		
Mullighan et al. [71]	19	20	My-BP:12 Ly-BP:3 AP:5		16	23	SNP array	22		

†Study that primarily investigated the clinical significance of mutations on treatment outcomes.
*Phenotype of blasts from BP patients as reported in the included studies. My: myeloid, Ly: lymphoid, Amb: ambiguous, ns: not specified.
**Refers to the number of patients sequenced with the specified sequencing method.
***Refers to the number of genes the panel sequenced by Sanger, amplicon or targeted sequencing.
****Only genes in panels including less than 10 genes are listed.

WES. In the above-mentioned studies, *ASXL1* was the most frequently mutated gene in about 10% of CP-CML patients (Figure 1). Other frequently mutated genes included *IKZF1* mutations and deletions (4%), *RUNX1* (2%), *TET2* (2%), and *DNMT3A* (2%). Other mutated genes reported by more than one study included the *KMT2D, TP53, KIA1594, CREBBP,* and *EP300* genes. Interestingly, typical AML-related mutations were seldom identified in CP-CML, including *IDH1/2, FLT3, EZH2,* and *NRAS* mutations.

Preleukemic and CHIP-related mutations contribute to the complexity of CP-CML genetics. CHIP refers to

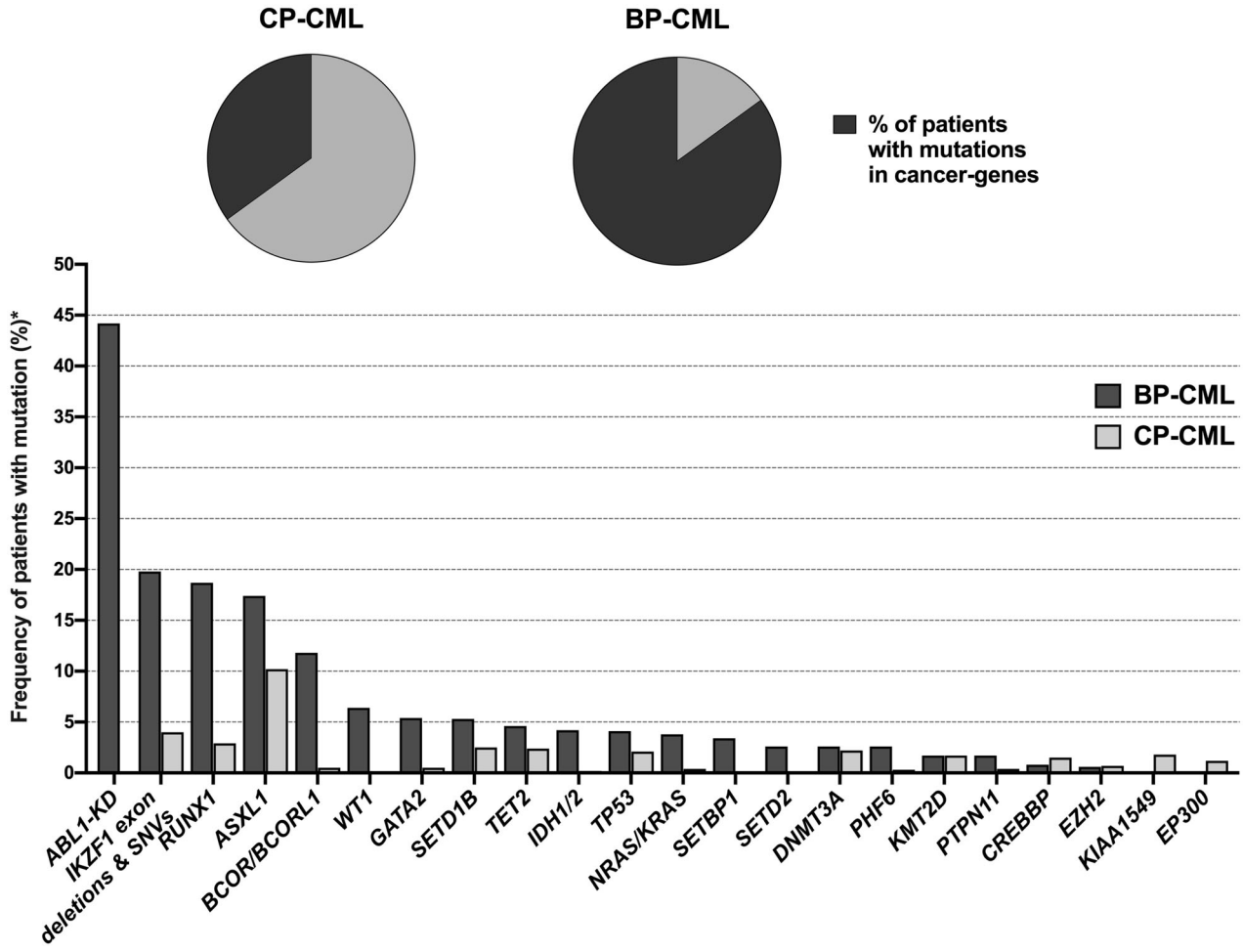

Figure 1. Frequency of mutations in cancer-associated genes at diagnosis (CP) and BP-CML. The data used to build the figure were derived from 27 studies of CP and/or BP [38–54,61,63–71] and included genes that were reported to be mutated in more than one patient and in more than one study. *The frequency of patients with mutated genes was calculated in relation to the number of patients screened for each individual gene, which was highly variable between different genes.

the acquisition of somatic mutations in hematopoietic stem cells (HSC) in healthy individuals as a part of the aging process, which is associated with an increased risk of hematological malignancies, including AML, MDS, and MPN [56,57]; however, its role in CML remains unclear [36]. Many of the genes reported to be frequently mutated in CP-CML are also known to be CHIP-associated genes, including the *ASXL1, TET2,* and *DNMT3A* genes. Two CML studies [50,51] reported a weak correlation between age and the number of somatic mutations, suggesting that some mutations may be passenger age-related mutations. Sequencing Ph-negative (Ph-neg) remission samples [44] or T-cell samples [45,46,48] from CP-CML patients enabled the identification of preleukemic mutations in many CHIP-related genes, including *DNMT3A, TP53, TET2, ASXL1, BCOR,* and *CREBBP*, which were detected in both leukemic and non-leukemic cells. However, somatic *ASXL1* mutations were identified in 6/21 (29%) of children and young adult CML patients [47], suggesting that the high frequency of *ASXL1* mutations in CP-CML is not an age-related phenomenon. Another interesting observation is the low frequency of *DNMT3A* mutations in CML patients compared with CHIP and other myeloid leukemias (Figure 2), which suggests that *DNMT3A* mutations might not greatly contribute to CML pathogenesis [58]. The contribution of CHIP-related mutations to the mutational landscape of CP-CML remains to be addressed.

It is noteworthy that the above-mentioned studies showed considerable variations regarding (i) the selection of patients as random, consecutive, or response-based, (ii) the phenotype of the sample as diagnostic unsorted MNCs or sorted CD34+ cells, (iii) the availability and source of germline control as T-lymphocytes, mesenchymal cells, buccal swabs, or skin biopsy, and (iv) the number of screened genes in targeted

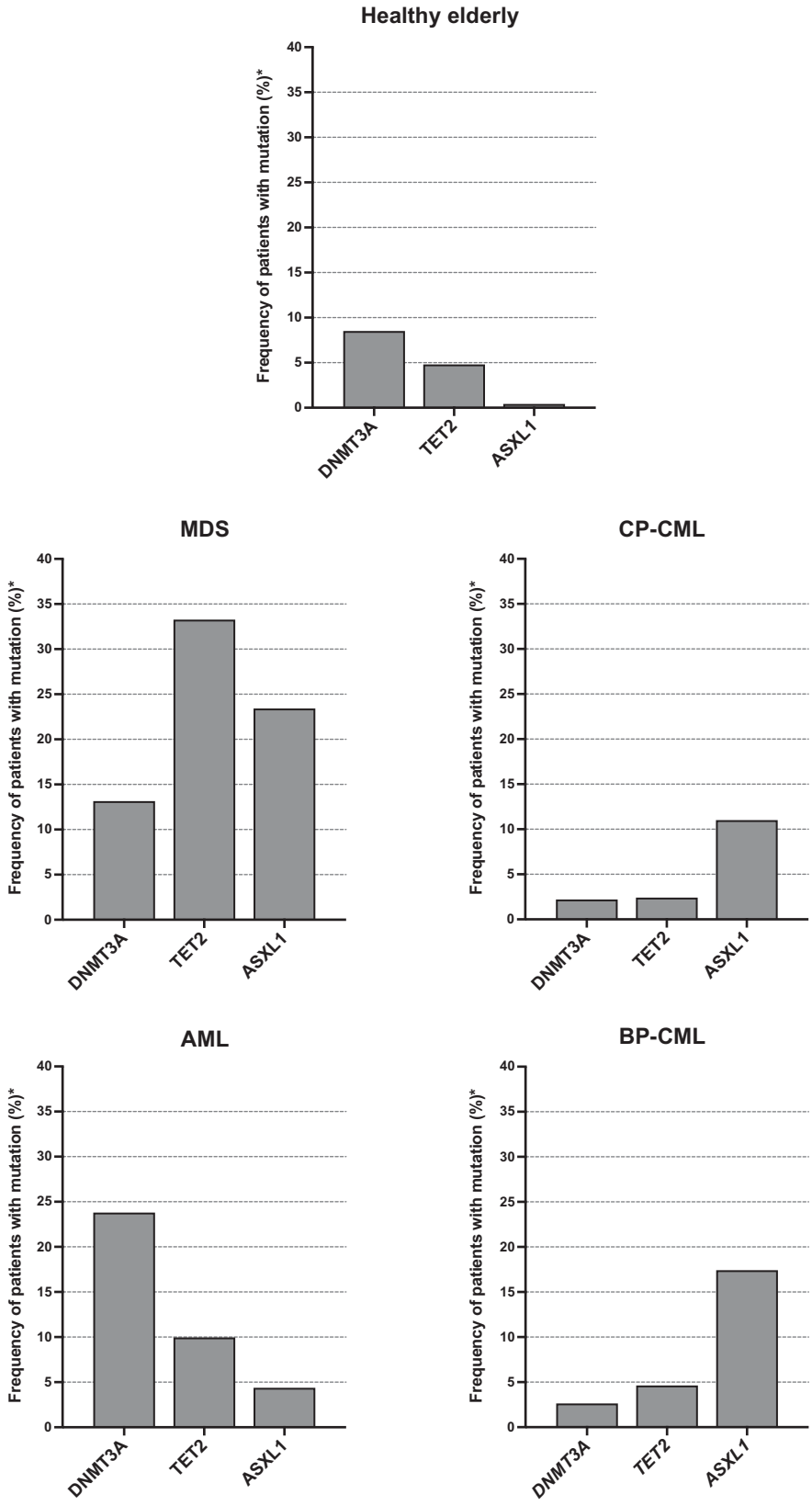

Figure 2. Frequency of the most common CHIP mutations in elderly healthy individuals and different leukemias. The frequency of DNMT3A, TET2, and ASXL1 mutations were calculated based on pivotal studies of elderly healthy individuals (>55 years) [143], AML [144,145], and MDS [146].

sequencing panel studies. Such variations, in addition to the small sizes of the studied cohorts, preclude definitive conclusions and warrant further standardized screenings of larger cohorts.

Genetic events at advanced phase CML (BP-CML)

CML progression has been attributed to *BCR-ABL1*-induced genomic instability and the acquisition of genetic aberrations. However, the exact molecular mechanisms underlying CML progression are still not well characterized. Illegitimate activity of the RAG enzyme has been reported to contribute to the acquisition of structural aberrations, especially in lymphoid BP patients [48,59]. Recent studies have also suggested that a unique CML mutational signature is implicated in the mutagenesis process [53]. Furthermore, mutations enriched for signatures of deficient DNA double-strand repair by homologous recombination and DNA mismatch repair system were identified in BP patients [48]. Because of the prognostic and therapeutic implications of genetic events in acute leukemia [60], further research is required to gain a comprehensive understanding of the mutational landscape of BP-CML, which would provide insights into BP-CML pathogenesis and enable the better management of this aggressive disease.

Genetic studies have shifted from using the Sanger sequencing of selected genes [41,43,61–65] to more extensive high-throughput sequencing approaches that have revealed the genetic heterogeneity of BP-CML patients [45,48,52–54,66–70] (Table 1). Aggregated data have demonstrated that mutations involving several known leukemia-associated genes are frequently encountered in BP-CML patients. In the TKI era, Abl-KD mutations are the most common mutations, and they are detected in about 44% of BP-CML patients (Figure 1). In addition to Abl-KD mutations, *RUNX1* and *ASXL1* are the most frequently mutated genes in 19% and 17% of BP-CML patients, respectively. *IKZF1* deletions are commonly encountered in 20% of BP-CML patients. Two WES studies have provided insights into the mutation profile in different BP phenotypes [48,52] and showed that *IKZF1* deletions are associated with a lymphoid phenotype, while *ASXL1* mutations are more frequently encountered in patients with a myeloid phenotype. Other mutations that have been reported in BP-CML involve the *BCORL1, BCOR, SETD1B, SETD2, TP53, IDH1/2, GATA2, TET2, EZH2, WT1, PHF6, SETBP1, CBL, PTPN11,* and *NRAS* genes (Figure 1).

The frequency of mutations in certain genes has shown considerable variation among BP-CML studies. This discrepancy could be explained by methodological and technical differences as well as the small cohort sizes used in most previous studies. WES-based studies [48,52–54,70] have reported a higher frequency of *IKZF1* deletions than earlier SNP array-based studies reported [65,67,71] (27% and 11%, respectively). *IKZF1* deletions are also common events in acute lymphoblastic leukemia (ALL), where they are associated with an inferior prognosis [72,73]. Another example is *WT1*, a recurrently mutated gene in AML [74]. Two studies have reported high mutation frequencies (10% and 15%) in BP-CML [53,67], whereas other studies have shown much lower frequencies [48,52]. *TP53* mutations were reported at higher frequencies in very early pre-TKI studies [75,76] compared with the low frequency (4%) reported in high-throughput sequencing studies. Recurrent mutations of *BRCA2* in solid tumors [77] were reported in 4/39 BP patients in a single recent study [53]. Similarly, *JAK3* and *BRD3* mutations were reported to be recurrent in BP patients in another recent study [48].

In addition to recurrent mutations in established leukemia-associated genes, recent genomic studies on BP-CML have identified recurrent novel mutations with a potential pathogenic role in CML progression. *SETD2* mutations, which are frequent in solid tumors and, to a lesser degree, in acute leukemia [78,79], have been detected in recent studies [48,52,53]. One study also reported *SETD2* loss of function by post-translational mechanism was recurrent in BP-CML patients [80]. Another epigenetic modifier, *SETD1B* [81], was reported in several studies to be mutated in about 5% of patients [48,52,53,82]. *BCOR/BCORL1* mutations common in MDS and MDS/MPN [83–85] have been reported in about 13% of BP patients [48,52,53]. Mutations in the ubiquitin-related gene, *UBE2A* [86], were described by Magistroni et al. [66] in 17% of BP-CML patients and at lower frequencies in other studies [48,52,82]. *CDKN2A* deletions, known as leukemia-initiating events [87], were identified in 5% of BP-CML patients and typically associated with lymphoid phenotype [52,53]. Because of the increasing number of WES studies in BP-CML, the synchronization and combination of the data are warranted to enable a better overview of the mutational landscape of BP-CML and overcome the limiting factor of small cohort sizes in individual studies.

Fusion genes represent another class of somatic mutations that have an established driver role in leukemia [88]. Early cytogenetic studies described

translocations, including cryptic translocations, that resulted in fusion genes involving known leukemia-associated genes, including *CBFB-MYH11* [89], as well as *RUNX1* [39,90–93] and *MLL* [94] fusions with various partners. RNA sequencing is a powerful tool for identifying clinically relevant fusion genes [52,95]. Recent studies that applied RNA sequencing have highlighted the potential role of fusion genes in BP-CML [48,52]. Branford et al. [52] reported fusion genes in 14/33 BP-CML patients, including *KMT2A* (*MLL)* rearrangements (five patients) and *CBFB-MYH11* (two patients). Known leukemia-associated genes, including *RUNX1*, *MECOM, PAX5,* and *IKZF1,* were involved in many fusions reported in the literature. Fusion genes occurred as the sole genetic event in 15% of BP-CML patients. Similarly, Adnan-Awad et al. [48] reported fusion genes in 5/7 BP-CML patients, including *CBFB-MYH11* and *RUNX1* fusion. However, further RNA sequencing studies are needed to investigate the clinical value of fusion genes in CML progression.

Mutational dynamics and clonal evolution in CML

The analysis of serial samples of CML patients provides insights into the dynamics of the mutational profile in CP patients under TKI treatment, as well as during CML progression. Only a few studies have performed longitudinal analyses of CP-CML under TKI treatment. Mitani et al. [50] analyzed matched diagnosis-remission samples from 20 CP patients and found that successful TKI treatment was associated with the elimination of almost all mutations identified at diagnosis. A handful of mutations, including a *TET2* mutation, were identified in remission samples from 30% of patients. Interestingly, mutations were also detected at much lower variant allele frequency (VAF) in respective diagnosis samples, suggesting the expansion of Ph-neg clones. Similarly, Nteliopoulos et al. [46] reported the clearance of mutations detected at diagnosis in remission samples drawn from TKI-treated CP patients. This finding was associated with the emergence of a few low-VAF mutations, including a *DNMT3A* mutation.

Kim et al. [45] systematically analyzed matched diagnosis-follow-up (FU) samples from 100 patients and their sorted T-cell fractions. The results provided novel insights into the mutational profiles of TKI-treated CP patients, identifying five patterns of mutational dynamics associated with TKI treatment. In pattern 1, the mutations were presented at a stable VAF in both diagnosis and follow-up samples, despite a significant decline in *BCR-ABL1* levels, which suggested the preleukemic nature of mutations. Pattern 2 demonstrated the acquisition of mutations in genes, including *ABL1* and *TP53*, that were associated with poor responses and treatment resistance. Pattern 3 showed the elimination of diagnosis mutations in the FU samples, and it was associated with mixed outcomes. Patterns 4 and 5 included a few mutations that were detected in T-cell fractions, which suggested their preleukemic/Ph-neg origin. Notably, some of these patterns, such as the acquisition and clearance of diagnosis mutations on TKI treatment, were also described by Branford et al. [52]. In a recent study by Adnan-Awad et al. [48], a longitudinal analysis was performed on matched samples drawn from 28 CP patients. Similar to Kim et al. [45], the persistence of truncal mutations and/or the acquisition of leukemia-associated mutations were associated with poor responses [48]. Preleukemic and Ph-neg mutations involving *TET2* and *DNMT3A* genes were identified and associated with mixed outcomes.

The analysis of matched diagnosis progression samples enables the identification of BP-specific mutations with a potential role in CML progression. Early studies performed WES on matched samples of individual cases [41,68,69], which precluded the identification of recurrent mutations. One of the most comprehensive studies that addressed genetic events in CML progression was by Branford et al. [52], which matched diagnosis progression samples from 25 patients. Abl-KD mutations were the most frequently acquired event associated with disease progression. *IKZF1* deletions, in addition to *RUNX1* and *BCORL1* mutations, were frequently acquired during disease progression. Other progression-associated mutations involved *BCOR, SETD1B, IDH1,* and *UBE2A* genes, which were detected in individual cases. *ASXL1* mutations showed variable progression-related patterns, which were acquired during progression in 3 of 25 patients, persistent in CP samples from 3 of 25 patients and lost during progression in 4 of 25 patients. Similar progression-associated mutational profiles were reported in abstract form [82,96], where Abl-KD and RUNX1 mutations were frequent progression-associated events. Magistroni et al. [66] analyzed matched samples from 10 patients and found acquired mutations in the *ABL1* (30%), *UBE2A* (20%), *RUNX1* (10%), *ASXL1* (10%), and *NRAS* (10%) genes. In a recent study by Ko et al. [53] WES was performed on matched samples from 13 patients. In agreement with previous data, Abl-KD mutations were the most common, affecting 6 of 13 patients. Other progression-associated events were involved in *RUNX1* (2/13), *EZH2* (1/13) mutations, and *IKZF1* deletion (1/

13). Similarly, a study by Adnan-Awad et al. [48] highlighted progression-specific mutations, including Abl-KD (2/3 patients), *RUNX1,* and *ASXL1* (in each 1/3 patients) mutations. The study also investigated patterns of clonal dynamics in two BP-CML patients with matched BP-relapse samples using WES. A lymphoid BP patient showed clonal drift with the eradication, in treatment, of the diagnosed dominant clone with *ABL1* (T315I) and *RUNX1* mutations and the emergence of a new clone with an *EZH2* mutation. Data on another patient demonstrated a linear evolution pattern in which the diagnosed *DOT1L* mutated clone expanded despite treatment after the acquisition of *ABL1, MSH6,* and *SETD1B* mutations.

Somatic mutations and TKI treatment in CP-CML

Several clinical scoring systems have been used for the risk assessment of CML patients at diagnosis, including the Sokal [97], Hasford [98], EUTOS [99], and ELTS [100] scoring systems. Despite their wide clinical use, these scoring systems have limited specificity and sensitivity [101,102]. Additional risk factors, including marrow fibrosis and high-risk additional chromosomal abnormalities (ACA), can predict inferior responses to TKI and a higher risk of progression [103–105]. The integration of genetic data in risk stratification has been successfully implemented in the management of acute leukemia patients [106,107]. However, similar efforts are still pending in CML despite the increasing number of investigations of the prognostic value of genetic data in CML management. For example, germline mutations of the *BIM* gene have been reported to be associated with TKI resistance [108], and they constitute an independent risk factor for inferior imatinib responses that can complement clinical risk scores in CP-CML patients [109,110]. Moreover, the polymorphism of the *HMGCLL1* gene was suggested as a novel biomarker for predicting the achievement of deep molecular response in imatinib-treated patients [111].

Recent high-throughput sequencing studies have suggested that somatic mutations at diagnosis are potential biomarkers for predicting TKI treatment outcomes. In a recent study [48], the burden of somatic mutations was identified as an independent prognostic marker in CP-CML patients. Patients with poor responses showed a higher mutational burden, especially when the calculation was restricted to mutations in cancer-associated genes. Another study applied a scoring system to evaluate the oncogenic potential of the variants and reported a significant association between a high mutational burden and imatinib resistance [51]. Mutational burden has been shown to correlate weakly with age in some studies [49–51], suggesting that some variants are passenger mutations, which warrants further refinement of the data.

Mutations in the epigenetic modifiers are common events in leukemia, and they have been suggested to play a role in pathogenesis and treatment [112,113]. Recent studies have reported frequent mutations of epigenetic modifiers in 20–30% of CP-CML patients, and they have been associated with inferior responses to TKI treatment [45,48], especially imatinib [46]. Kim et al. [45] reported that patients carrying mutations in epigenetic modifiers had significantly inferior outcomes at the 12-, 24-, and 36-month milestones. Similarly, another study [48] demonstrated an increased frequency of epigenetic mutation in poor responders compared with suboptimal and optimal responders. A study of 124 CP-CML patients [46] reported that mutations in epigenetic modifiers at diagnosis could efficiently predict the achievement of major molecular responses and survival rates in imatinib-treated patients but not in the second-generation TKI cohort. Moreover, *ASXL1* germline mutations were also reported to be strong biomarkers of imatinib responses in CP-CML [114]. *ASXL1* mutations were associated with TKI resistance and the increased risk of disease progression in a recent study [115].

The potential effects of CHIP on treatment outcomes in CML patients remain elusive. CHIP-related mutations (i.e. *ASXL1, PTPN11, ATM,* and *DNMT3A*) have been reported at a high frequency in remission samples drawn from patients with Ph-neg clonal abnormalities and associated with reduced survival rates [116]. Another interesting aspect is the association of CHIP mutations with the risk of cardiovascular disease [117]. Cardiovascular events are also known as adverse effects of some TKIs [118]. The frequency of CHIP mutations (especially *DNMT3A, TET2,* and *ASXL1*) was significantly higher in remission samples of patients who developed arterial occlusive disease (AOD) compared with patients with no AOD (65% vs. 32%, respectively) in a recent study involving 36 nilotinib-treated patients [119].

In conclusion, there is an increasing amount of evidence of the predictive value of the mutational status of CP-CML patients at diagnosis regarding TKI treatment responses. However, further systematic studies involving larger patient cohorts are warranted to overcome the limiting factors of small cohort sizes and response-based patient selection in previous studies.

Genetic data in BP-CML management

The use of TKIs has dramatically improved survival rates in CP-CML patients [120], whereas TKIs have only modestly improved the survival rates of BP-CML patients, even with the use of the more potent second-and third-generation TKIs [33]. The current treatment of choice in BP-CML is a combination of TKIs with chemotherapy followed by allogenic stem cell transplantation [19]. BP-CML remains the main clinical challenge in CML management in the TKI era, and there is still a major need to identify better treatment options for BP-CML patients.

Regarding genetic data, *ex vivo* high-throughput drug testing represents a promising complementary method in the personalized treatment of leukemia [121–125]. High-throughput drug sensitivity and resistance testing (DSRT) of BP-CML cell lines and patient samples have identified novel candidate drugs, including VEGFR-, MEK-, and NAMPT inhibitors [126]. Interestingly, *ex vivo* drug testing has led to the groundbreaking discovery of the selective and efficient inhibitory activity of axitinib against primary *ABL1*-T315I mutated cells, which highlights the potential benefits of repurposing approved targeted drugs in BP-CML management [127]. A recent study emphasized the role of a personalized approach in tailoring treatment for BP-CML patients [48]. The integrated approach of genetic, transcriptional, and drug sensitivity profiling was used to guide the treatment of two BP-CML patients with DSRT-based axitinib treatment, inducing significant clinical responses in both cases. Additionally, genetic data could indicate druggable targets and activated transcriptional pathways that underlie CML progression and relapse.

Many of the recurrently mutated genes in BP-CML are potential targets for targeted therapy. *RUNX1*, the most frequently mutated gene in BP-CML, is a transcription factor that is commonly mutated in other leukemias, including AML. Several studies have investigated potential targeted therapies for *RUNX1*-mutated AML, reporting specific sensitivity to glucocorticoids [128], mTOR- [129], VEGFR- [130], and, recently, BET inhibitors [131]. A recent study integrated genetic and DSRT profiling to characterize *RUNX1*-mutated BP-CML patients and identify a targeted therapy [70]. The study identified distinct phenotypic and transcriptional criteria in *RUNX1*-mutated BP-CML patients, including the frequent expression of lymphoid markers in myeloid BP patients, enhanced off-target activity of the mutagenic AID/RAG axis, as well as the dysregulation of stem cell, lymphoid, and immune-related pathways. These genomic findings were translated into the sensitivity of *RUNX1*-mutated blasts to glucocorticoid, mTOR- and BCL2-inhibitor targeted therapy, suggesting the presence of a common *RUNX1* signature in CML and AML. Furthermore, *RUNX1*-mutated blasts were sensitive to CD19-CAR T-cell immunotherapy not only in lymphoid BP patients but also in a myeloid patient with aberrant CD19 expression, which was in line with recent reports on *RUNX1-mutated AML* [132].

IKZF1 is another potential target for targeted therapy in BP-CML. *IKZF1* is a tumor suppressor that is commonly affected by focal deletions in ALL and lymphoid BP-CML [72,133]. In Ph + ALL, retinoids were reported to enhance dasatinib activity in IKZF1-mutated patients [134]. Furthermore, the targeting of truncating mutations of the *ASXL1* gene, which is another recurrently mutated gene in BP-CML, by BET inhibitors was recently reported [135]. The known cancer-related genes, *TP53* and *EZH2*, have been targeted by many specific drugs [136–139]; however, they were mutated in only a minority of BP-CML. Rare BP-CML cases with *IDH1/2* mutations could benefit from clinically approved drugs for IDH-mutated AML [140]. In addition to the *RUNX1*-associated aberrant expression of CD19 in myeloid patients, *WT1* represents another attractive target for immunotherapy [141,142].

In summary, the integration of genetic and drug sensitivity data provides an intriguing means of personalizing and improving BP-CML management. Only a very few of all recurrently mutated genes in BP-CML have been investigated in a limited number of cases. Thus, further systematic high-throughput drug testing of a greater number of BP-CML samples is required. This approach may allow for the identification of new potential biomarkers and treatment modalities for BP-CML.

Conclusion and further considerations

The concept of CML as a genetically uniform disease has changed as the number of studies has increased, which suggests the genetic heterogeneity of CML. Although *BCR-ABL1* is the principal event in CML pathogenesis, mutations involving other genes play important roles in different phases of CML. Somatic mutations, especially those affecting epigenetic modifiers, have been suggested as affecting TKI treatment outcomes in CP-CML. Additionally, because somatic mutations have particularly pronounced effects in BP-CML, the integration of genetic and drug sensitivity data in a personalized approach represents a promising strategy for disease management. However, further studies are needed to reach definite conclusions.

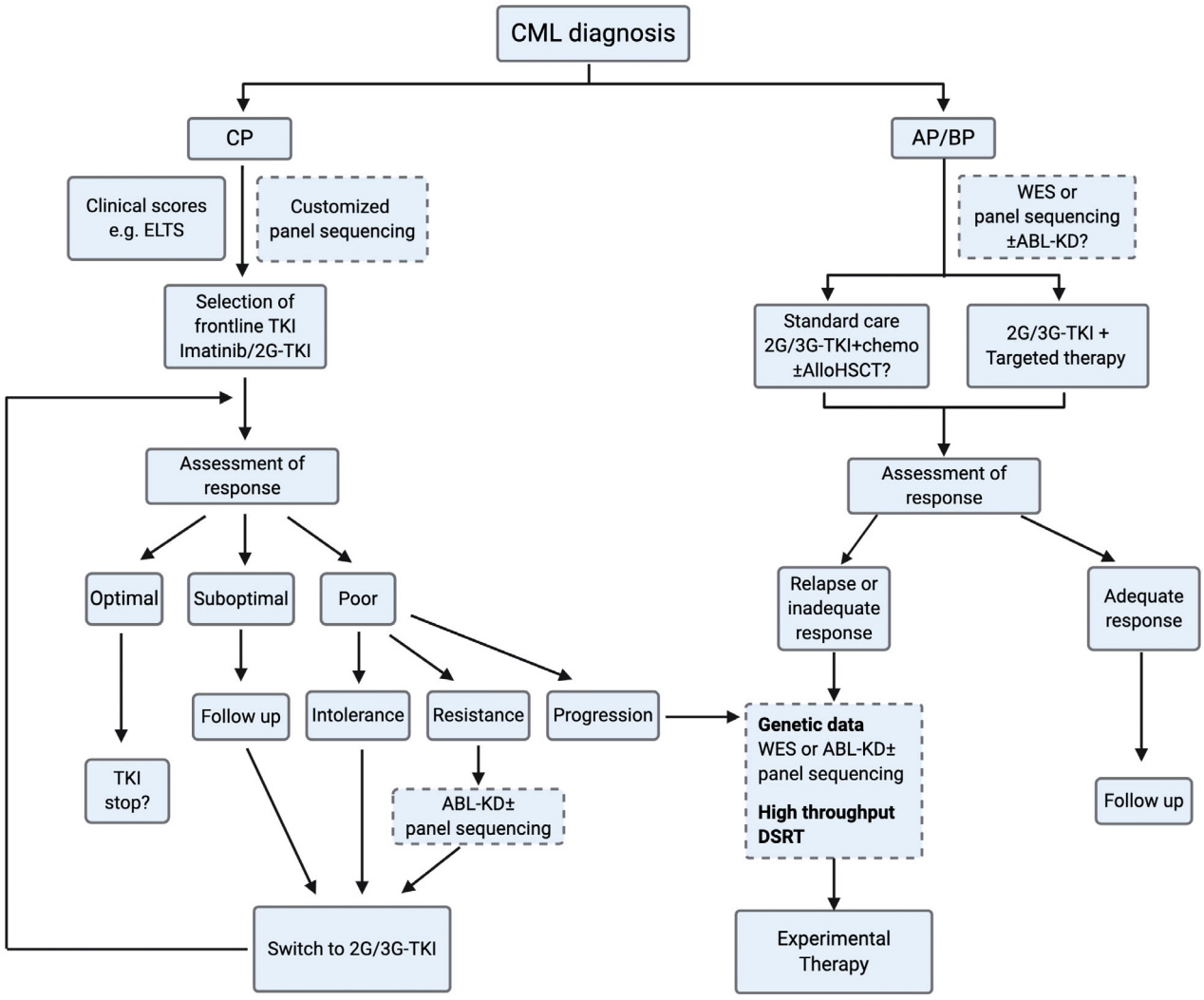

Figure 3. Algorithm of suggested future directions of CML management integrating genetic screening in risk stratification and drug selection. In BP, in case of non-fit patients ineligible for chemotherapy or allogenic hematopoietic stem cell transplantation (allo-HSCT), targeted therapy based on mutation profile can be considered.

Additionally, the role of CHIP and preleukemic mutations in CML pathogenesis remains to be addressed. Clinically-based scores are the only scoring system in current CML practice, however; screening for additional cancer-associated mutations can provide clinically relevant information and may be incorporated in CML patients' routine care in the future. Mutation profiling may be needed both at diagnosis and in the case of resistance or progression, as proposed in the algorithm shown in Figure 3. Notably, there are still many open questions to be addressed, such as the selection of the sequencing method (i.e. WGS, WES, or pre-designed targeted panel sequencing), required sequencing depth (i.e. the detection of subclonal mutations), sample type (i.e. sorted vs. whole blood), and germline control samples. Nevertheless, we believe that further studies of CML genetics will enable the adoption of better personalized treatment strategies that will significantly improve the management of high-risk CML patients and provide a means of enhancing TFR rates in CML.

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