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MOLECULAR TESTING AND DIAGNOSTICS (J KHOURY, SECTION EDITOR)

Chronic Myeloid Leukemia: Beyond BCR-ABL1

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Abstract

Purpose of review In this review, we emphasize up-to-date practical cytogenetic and molecular aspects of chronic myeloid leukemia (CML) and summarize current knowledge on tyrosine kinase inhibitor (TKI) resistance and treatment response monitoring of CML.

Recent findings The introduction of TKIs has changed the natural course of CML and markedly improved patient survival. Over the past decades, many research efforts were devoted to elucidating the leukemogenic mechanisms of BCR-ABL1 and developing novel TKIs. More recent studies have attempted to answer new questions that have emerged in the TKI era, such as the cytogenetic and molecular bases of treatment failure and disease progression, the clinical impact of genetic aberrations in Philadelphia chromosome (Ph)-positive and Ph-negative cells, and the biological significance of Ph secondarily acquired during therapy of other hematological neoplasms.

Summary Recent progresses in the understanding of the cytogenetic and molecular mechanisms underlying therapeutic failure and disease progression have improved the risk stratification of CML and will be helpful in the design of novel therapeutic strategies.

Keywords Chronic myeloid leukemia · Philadelphia chromosome · BCR-ABL1 · Tyrosine kinase inhibitor · Additional chromosomal abnormality · TKI resistance

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) characterized by a biphasic or triphasic natural history: an indolent chronic phase (CP) followed by an accelerated phase (AP) and then an aggressive blast phase (BP), or both. The BCR-ABL1 fusion, produced as a result of the t(9;22)(q34;q11.2), is a constitutively active tyrosine kinase that activates a network of downstream pathways and leads to leukemogenesis. The introduction of tyrosine kinase inhibitors (TKIs) has changed the natural course of CML and markedly improved patient survival. Despite the highly promising results, TKI resistance develops in ~ 13% of patients.

Ever since the first description of the Philadelphia chromosome (Ph)/der(22)t(9;22)(q34;q11.2), this translocation has served as a paradigm for how a single genetic abnormality causes malignant transformation and, most importantly, how a targeted therapy alters the disease course and dramatically improves patient outcome. However, many important questions remain unanswered. What are the mechanisms underlying TKI resistance? What are the cytogenetic and molecular bases of disease progression? What is the biological significance of chromosomal alterations in Ph-positive (Ph+) and Ph-negative (Ph-) cells? What is the importance of Ph acquired during therapy of other hematological malignancies? Herein we will try to synthesize recent advances on these important issues.

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BCR-ABL1 Isoforms and Phenotypic Correlation

The normal *ABL1* and *BCR* genes have 11 and 23 exons, respectively. In *ABL1*, the breakpoints are distributed in the

intron between exons 1b and 1a, or in the intron between 1a and 2 (Fig. 1). Regardless of the *ABL1* breakpoints, the two alternative first exons (1a and 1b) are always spliced out. The common exons 2–11 of *ABL1* are then fused to different exon sets of *BCR*. In *BCR*, three breakpoint cluster regions are characterized: major (*M-BCR*), minor (*m-BCR*), and micro (*μ-BCR*), corresponding to three fusion proteins designated as P210, P190, and P230, respectively. All three BCR-ABL1 fusion proteins contain the common ABL1 domains. Depending on the *BCR* breakpoints, the fusion proteins may contain some or all of the BCR domains (Fig. 1) [1–3]. The e13a2 (b2a2) and e14a2 (b3a2) transcript subtypes, which encode P210, are seen in ~98% of all CML cases. The total frequency of other minor transcripts is ~2% [4, 5], including fusion transcripts lacking exon 2 of *ABL1* (ela3, e13a3, and e14a3), e1a2+e13a2, e1a2+e14a2, e6a2, e12a1, e19a2, and "bizarre" insertions or breakpoints within exons [6].

There is a clear association of different BCR-ABL1 proteins with distinct disease phenotypes. The P210 form is reported in ~98% of CML but in <20% of Ph+ B-lymphoblastic leukemia/lymphoma (B-ALL). In contrast, the P190 BCR-ABL1 is present in >80% of Ph+ B-ALL but only rarely (~1%) observed in CML. CML patients with P190 tend to be older, present with monocytosis, and have a higher frequency of BP at initial presentation. Those who are not in BP initially have a higher risk of subsequent blastic transformation [7]. The rare e19a2 (P230) has been described in a small fraction of CML patients who often present with prominent neutrophilic maturation or thrombocytosis. Because of their low frequency, the clinical importance of other rare transcripts is unclear.

How different BCR-ABL1 proteins differentially drive lymphoid and myeloid leukemogenesis has not yet been completely elucidated. The three forms of BCR-ABL1 have been shown to be equally potent in inducing a CML-like MPN in a murine bone marrow transduction/transplantation model [8]. However, the expression of these three oncoproteins might be largely restricted to different hematopoietic cell types. Most studies suggest that P210 originates at the level of hematopoietic stem cells (HSCs) whereas P190 has a B cell progenitor origin without myeloid involvement [9]. It is important to keep in mind that HSCs and lineage-committed progenitors differ in many biological activities, such as metabolism, replication stress, chromatin condensation, transcriptional activity, and DNA damage response. These differences might play a role in inducing DNA breaks in different regions and generating different mutagenic patterns. In addition, P190 has a more potent lymphoid leukemogenic activity than P210 [10–12]. This may be associated with the increased intrinsic tyrosine kinase activity of P190 that allows elevated tyrosine phosphorylation of substrates critical for proliferation or transformation of lymphoid cells [13–15]. One key substrate is STAT6, a transcription factor implicated in lymphoid proliferative responses [16]. Its DNA binding activity is prominently activated via tyrosine phosphorylation by P190 but not P210 [14, 15]. How different domain compositions and

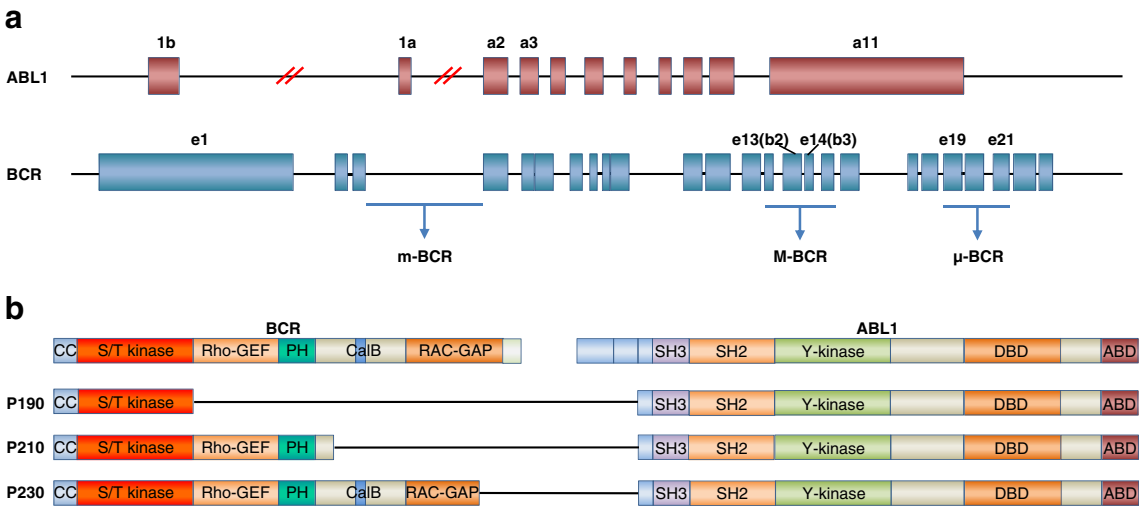

Fig. 1 Schematic representation of *BCR* and *ABL1* gene structures and essential protein domains of BCR-ABL1 fusion proteins. **a** Gene structure and breakpoints of *BCR* and *ABL1*. In *ABL1*, the breakpoints (designated by parallel oblique lines) are distributed in the intron between exons 1b and 1a, or in the intron between exons 1a and 2. In *BCR*, most breakpoints in CML occur within the *M-BCR* region which encompasses exons 12–15. The *m-BCR* is located in the 3′ half of the first *BCR* intron. The *μ-BCR* is located further downstream between exons 19 and 21. **b** Essential domains of BCR-ABL1 fusion proteins. All three main BCR-ABL1 fusion proteins contain the common ABL1 domains, including the SRC homology domains SH2 and SH3, tyrosine kinase (Y-kinase) domain SH1, and DNA- and actin-binding domains (DBD and ABD). Depending on the BCR breakpoints, the fusion proteins may contain some or all of the following BCR domains: a coiled-coil (CC) oligomerization domain, a serine/threonine kinase (S/T-kinase) domain, a Rho guanine nucleotide exchange factor (Rho-GEF, also designated DBL-like) domain, a pleckstrin homology (PH) domain, a calcium-dependent lipid-binding domain (Cal-B), and a truncated RAC-GAP domain

overall structures of the two isoforms of BCR-ABL1 interact with different protein complexes and drive leukemogenesis remain elusive. One hypothesis is that differential subcellular localization causes the two isoforms to encounter different subsets of the proteome and consequently activates different signaling pathways [17–19].

Leukemogenic Effects of BCR-ABL1

The proto-oncoprotein ABL1 is a ubiquitously expressed non-receptor tyrosine kinase whereas BCR is a kinase with poorly understood functions. ABL1 shuttles continuously between the nucleus and cytoplasm but is predominantly localized in the nucleus in normal cells. However, fusion with BCR relocates ABL1 to the cytoplasm where it has the opportunities to interact with a number of proteins and exert its leukemogenic effect. Most importantly, the oligomerization domain encoded by the first exon of BCR constitutively activates the tyrosine kinase activity of ABL1 by promoting dimerization or tetramerization, which in turn autophosphorylates other sites on ABL1 and generates more binding sites for SH2 domain-containing proteins. Thus, BCR-ABL1 can recruit and activate multiple downstream signal transducers through the SH2 domains on these proteins.

The most clinically relevant and extensively-studied pathways include RAS/RAF1/MEK/ERK, PI3K/AKT, and SFKs/STAT1/STAT5 [15, 20–24]. In physiological conditions, these pathways are turned on by binding of growth factors or cytokines to their specific receptors. BCR-ABL1 abrogates this growth factor and cytokine dependence by activating essential downstream molecules through direct interaction or via the GRB2/SOS/GAB2 complex. Constitutive activation of these pathways induces endogenous reactive oxygen species and DNA double-strand breaks, causes unchecked cell cycle progression, impairs DNA repair, dysregulates cell adhesion, and inhibits apoptosis and autophagy [25–27]. BCR-ABL1 also activates MYC, which is related to drug resistance, aberrant DNA synthesis, and genomic instability [28]. The level of MYC in CML at diagnosis may predict treatment response and progression to BP [28]. Furthermore, the abovementioned events contribute to genomic instability and make CML cells more susceptible to developing further genomic abnormalities, likely a major driver of disease progression.

CML evolves through a partially understood multistep process. A hypothetical model considers the initiation and progression of CML being caused by a stepwise accumulation of cytogenetic or molecular aberrations. In this model, BCR-ABL1 is crucial in transforming HSCs and initiating CML. Further secondary cytogenetic, molecular, and epigenetic alterations, cooperating with increased expression levels of BCR-ABL1, provide a significant proliferative and survival advantage to a committed myeloid progenitor and lead to blastic transformation. The Ph is typically the sole cytogenetic abnormality in patients with CML-CP. With disease progression, additional chromosomal abnormalities (ACAs) increase (30% in AP and 70–80% in BP) [29••, 30]. The acquisition and accumulation of these abnormalities is due, at least in part, to genomic instability caused by BCR-ABL1. Both in vitro and in vivo studies have indicated a direct causal role of BCR-ABL1 in the development of karyotypic abnormalities and point mutations [31].

Monitoring CML Treatment Response

Ongoing assessment is required for early identification of patients who are not responding optimally so that alternative treatment strategies can be considered. Based on the time to reach various levels of responses, treatment responses can be categorized into three groups: optimal, failure, and warning (Table 1) [32••]. Optimal response is associated with the best long-term outcome, and patients are expected to live a similar lifespan to that of the general population. Continuing the current therapy is appropriate in this group. In contrast, "failure" means that a different treatment should be initiated to limit the risk of progression and death. "Warning" implies that although the current treatment may still be beneficial, the long-term outcome of treatment is less likely to be favorable. More frequent monitoring is required to permit timely changes in therapy in case of treatment failure.

Per the European LeukemiaNet (ELN) recommendations, response can be assessed with either cytogenetic analysis or quantitative reverse-transcription polymerase chain reaction alone; when local laboratory facilities permit, both cytogenetic and molecular tests are recommended until a complete cytogenetic remission (CCyR) and major molecular remission (MMR) are achieved [32••]. Following these landmarks, molecular tests alone may be sufficient (Table 2). However, in case of suspicion of warning, failure, or progression, cytogenetic analysis is mandated. In addition, mutation analysis is recommended in certain situations as following imatinib failure, up to 48% of cases with primary resistance and 68% of cases with secondary resistance show mutations in the BCR-ABL1 kinase domain [34]. Noteworthily, a single measurement of the *BCR-ABL1* transcript level is not sufficient to define failure. Two tests at 3 and 6 months and supplementary tests in between should be performed before a decision is made to change the treatment. Furthermore, failures must be distinguished as either primary (a lack of initial response) or secondary (loss of an established response).

Patients with CML-CP who achieve CCyR have a favorable survival similar to that of the general population, and deeper treatment responses beyond CCyR provide no additional survival benefit [35]. However, a deeper response, such as MR4.5, may serve as an indicator of being one step closer to successful discontinuation of treatment [36]. In contrast,

Table 1 Recommendations for cytogenetic and molecular monitoring of CML

At diagnosis	• CBA of bone marrow metaphases
	• FISH in case of Ph negativity to identify variant translocations or cryptic *BCR-ABL1* rearrangement
	• qRT-PCR to identify the fusion transcript type and quantify the baseline level of *BCR-ABL1* expression on the international scale
During treatment	• qRT-PCR of *BCR-ABL1* on the international scale
	- Every 3 months until MMR achieved
	- After MMR, every 3–6 months
	• CBA of bone marrow metaphases
	- At 3, 6, and 12 months until CCyR achieved
	- After CCyR, every 12 months[a]
	• FISH on blood cells
	- After CCyR achieved
Failure, progression	• qRT-PCR, mutation analysis, and CBA of bone marrow metaphases
	• Immunophenotyping in BP
Warning	Molecular and cytogenetic analyses performed more frequently; CBA of bone marrow cells in case of myelodysplasia or clonal cytogenetic changes in Ph-negative cells with chromosome 7 abnormalities

Data adapted from the ELN recommendations [32••] and Cancer Cytogenetics [33]

CBA chromosome banding analysis, *CCyR* complete cytogenetic remission, *MMR* major molecular remission, *qRT-PCR* quantitative reverse-transcription polymerase chain reaction

[a] After CCyR is achieved, if adequate qRT-PCR can be ensured, cytogenetic analysis can be omitted

patients with CML-BP who achieve CCyR have a dismal survival similar to that of patients with CML-BP who achieve hematologic remission only [37]. Achieving molecularly undetectable leukemia is required for an optimal outcome, making precise and timely monitoring of *BCR-ABL* level essential in the management of CML-BP.

Mechanisms of TKI Resistance

The tyrosine kinase activity of BCR-ABL1 depends on the conformation of four highly conserved regions: (1) the ATP-binding loop (P-loop), which admits ATP that donates terminal phosphates to tyrosine residues on BCR-ABL1 and its substrates; (2) the direct binding site, which serves as a docking site for adapter proteins such as GRB2, CBL, and CRKL that further activate multiple downstream pathways; (3) the activation loop (A-loop), which switches between active (phosphorylated) and inactive (unphosphorylated) conformations. The inactive A-loop functions as a "pseudo-substrate" and is folded into and blocks the substrate binding site; and (4) the SH2 and SH3 domains, which assemble onto the catalytic domain of ABL1 and allosterically clamp it in an inactive stage. Importantly, imatinib only binds to ABL1 in the inactive state. Imatinib acts as a competitive inhibitor for ATPs. It binds to the catalytic domain locating close to the ATP binding pocket, displaces ATPs, and freezes the BCR-ABL1 kinase in the inactive non-ATP-binding conformation.

Table 2 Definition of response to TKIs as first-line treatment

	Optimal	Warning	Failure
Baseline/diagnosis	N/A	High risk or major route ACAs	N/A
3 months	*BCR-ABL1* ≤ 10% and/or Ph+ ≤ 35%	*BCR-ABL1* > 10% and/or Ph+ 36–95%	Non-CHR and/or Ph+ > 95%
6 months	*BCR-ABL1* < 1% and/or Ph+ 0%	*BCR-ABL1* 1–10% and/or Ph+ 1–35%	*BCR-ABL1* > 10% and/or Ph+ > 35%
12 months	*BCR-ABL1* ≤ 0.1% (MMR)	*BCR-ABL1* 0.1–1%	*BCR-ABL1* > 1% and/or Ph+ > 0%
After 12 months	*BCR-ABL1* ≤ 0.1%	Cytogenetic changes in Ph-negative cells (−7 or 7q-)	Loss of CCyR Confirmed loss of MMR Mutations ACA in Ph+ cells

Data adapted from the ELN recommendations [32••] and Cancer Cytogenetics [33]

ACAs additional chromosomal abnormalities, *CHR* complete hematologic remission (WBC < 10×10^9/L, platelet count < 450×10^9/L, no immature granulocytes in differential, and spleen non-palpable), *CCyR* complete cytogenetic remission (no Ph+ metaphase), *MMR* major molecular response (*BCR-ABL1* expression of ≤ 0.1%), *TKI* tyrosine kinase inhibitor

This interaction blocks the tyrosine kinase activity and prevents BCR-ABL1-mediated autophosphorylation, and in turn substrate phosphorylation, thereby switching off downstream signaling pathways.

TKI resistance (~13% of patients) [38] can develop as a result of mechanisms involving BCR-ABL1-dependent and BCR-ABL1-independent pathways. The BCR-ABL1-dependent pathways include mutations and amplification of *BCR-ABL1*; the BCR-ABL1-independent pathways include resistance of CML stem cells, clonal evolution, activation of other tyrosine kinases, pharmacokinetic variability, and drug transport mechanisms.

Over 100 mutations in *ABL1* have been identified that confer TKI resistance [39]. As the disease progresses, their frequency increases, with mutations detected in 27%, 52%, and 75% of patients with CML in CP, AP, and myeloid BP, respectively. Additionally, they are more common in acquired than primary resistance [40, 41]. These mutations contribute to TKI resistance through the following mechanisms: (1) interference with TKI binding by altering the three-dimensional structure of the TKI binding site (T315I mutation). This mutation is highly resistant to imatinib, nilotinib, dasatinib, and bosutinib, although ponatinib remains effective [32••, 42]; (2) preventing BCR-ABL1 from assuming the inactive conformation required for imatinib binding (e.g., mutations in position M351 and A-loop) or stabilizing the active conformation of BCR-ABL1 (e.g., P-loop mutations) [43]; and (3) changing the auto-inhibitory conformation by disrupting the SH3-SH2 domain clamp through destabilizing intramolecular interactions (SH2 domain mutations) [44]. Amplification of *BCR-ABL1* can be caused by extra copies of Ph or ring chromosomes harboring multiple copies of *BCR-ABL1*. The level of *BCR-ABL1* expression correlates with the speed at which resistance to imatinib develops, which provides further evidence that the *BCR-ABL1* level serves as an excellent marker for TKI resistance [45].

TKIs eradicate CML progenitor cells but do not effectively target CML stem cells. Several possible mechanisms have been proposed to explain how CML stem cells escape the effects of TKIs, including low intracellular imatinib levels due to either inadequate active uptake or excessive drug efflux, elevated *BCR-ABL1* expression, and a quiescent cell cycle status [46, 47]. Additionally, CML stem cell survival may not actually depend on BCR-ABL1 kinase [48–50]. Acquisition of ACAs in Ph+ cells is thought to drive disease progression. As will be discussed later, certain ACAs are associated with poor TKI response, particularly, 3q26.2 rearrangement [51, 52••]. Resistance can also be mediated through overexpression of other tyrosine kinases such as the SRC family kinases. Two SRC family kinases, LYN and HCK, are highly activated and are not suppressed by imatinib [53]. Moreover, TKI resistance can develop from constitutive activation of downstream signaling pathways such as the mTOR pathway [54]. Decreased responses to imatinib therapy might also be related to pharmacokinetic variability. These mechanisms may involve poor patient compliance, variation in metabolizing enzyme activity (most importantly CYP3A4), and drug-drug interaction [55]. Recent studies have shown that a lower level of expression or activity of hOCT1, an active influx transporter, is associated with a lower probability of achieving a cytogenetic or molecular remission [56].

ACAs in Disease Progression

Studies in the pre-TKI era stratified ACAs into "major" and "minor" routes based on their frequency. The abnormalities with a frequency of $\geq$ 10% (of all cases with ACAs) were designated as major-route ACAs, including +Ph, +8, i(17q), and +19 [29••]. All others with a frequency of < 10% were designated as minor-route ACAs. In the 2017 update of the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue [57], the major-route but not minor-route ACAs, complex karyotype, 3q26.2 abnormalities at initial diagnosis, and ACAs emerging during therapy are considered defining criteria for CML-AP. Per ELN recommendations, major-route ACAs emerging during therapy, but not minor-route ACAs emerging during TKI therapy nor any ACAs detected at initial diagnosis define TKI failure and mandate a change of treatment [32••].

Most recent studies have established a four-tier risk stratification model based on the presence or absence of ACAs and the types of ACAs [58••, 59••]. These four different subgroups confer different risk of blastic transformation and patient outcome. This four-tier stratification remains somewhat valid for ACAs detected at initial diagnosis of CML, particularly for the high-risk ACAs, as follows:

- High risk: 3q26.2 rearrangement, −7/7q- or i(17q), either as an isolated single ACA or as a component of a complex karyotype;
- Intermediate risk-2: complex karyotypes without any of the three high-risk components;
- Intermediate risk-1: single ACAs other than the three high-risk single ACAs;
- Standard risk: no ACAs.

The 5-year cumulative probability of blastic transformation from initial diagnosis of CML for these four subgroups is 67%, 42%, 28%, and 10%, respectively, and the 8-year survival rate is 31%, 47%, 58%, and 80%, respectively. The pre-BP disease course in the high-risk patients treated with TKIs resembles that observed in CML patients treated in the pre-TKI era. These patients may benefit from timely hematopoietic stem cell transplantation before the onset of blastic transformation. Regardless of the risk of ACAs, interestingly, the

three ACA subgroups have a similar latency from initial diagnosis of CML to their emergence (median: 13 months). In addition, the prognostic difference among the three ACA subgroups is minimized once the disease progresses to BP [52••, 59••].

The type of treatment received during CP seemingly influences the patterns of secondary abnormalities acquired in progression [29••]. Trisomy 8 is more commonly associated with treatment of busulfan than hydroxyurea. In patients treated with IFN-α, there is increased occurrence of unusual secondary abnormalities, such as del(7p) and del(13q), and cytogenetically divergent subclones and a higher incidence of cytogenetic evolution [60]. While aberrations following autologous stem cell transplantation seem to be similar to the ones seen in non-transplanted patients, the cytogenetic evolution after allogeneic transplant seems to be random, structurally complex, and sometimes transient. There are fewer major-route abnormalities but a significantly higher frequency of balanced translocations and divergent subclones. Cytogenetic changes in TKI-treated patients were originally thought to follow the same genetic evolution pattern as before the introduction of TKIs [61]. However, a recent study of a large cohort of CML-BP patients treated in the TKI era reveals a significant shift of ACA pattern, particularly the marked increase in the incidence of 3q26.2 rearrangement in the TKI era [52••]. The emergence of 3q26.2 rearrangement as a major-route change in the TKI era correlates with a high frequency of ABL1 mutations in patients harboring this rearrangement, supporting a role of TKI resistance in the changing cytogenetic landscape.

Molecular Genetic Changes in Disease Progression

The molecular changes in CML can be categorized, somewhat simplistically, into the following groups based on their functions:

1. Increased level of *BCR-ABL1* expression. Qualitative analyses have shown increased levels of the *BCR-ABL1* mRNA and protein in CML-AP and BP compared with CP [62–64]. Furthermore, studies have supported a dose-dependent relationship for many of the leukemogenic effects of BCR-ABL1, including uncontrolled proliferation and escape from apoptosis [64, 65].
2. Oncogenic activation or amplification of pro-oncogenes. These genes include mutation or amplification of *RUNX1* [66], increased translation or amplification of *MYC* [67, 68], mutations of *NRAS*, *KRAS* [66], and the Wnt/beta-catenin pathway [69, 70], and gain-of-function mutation of *GATA2* [71].
3. Loss-of-function mutations of tumor suppressor genes. Mutations in the *TP53* have been detected in approximately 20–30% of CML-BP [66, 72]. Deletions of *CDKN2A* (~50%) and *RB1* (18%) are frequent in lymphoid BP [31, 73]. Mutations of *IKZF1* are not detected in CML-CP [74], but are observed in 18–27% of CML-BP samples [66, 74], mainly of lymphoid BP.
4. Suppressed expression or function of genes important for differentiation. Mutation of *C/EPB alpha* [75], t(3;21)(3q26.2;q22)/*RUNX1-MECOM* [76, 77], and translocations involving *HOX* genes are reported in myeloid BP [78, 79].
5. Mutations in genes important for epigenetic regulation, such as *ASXL* (20%) and *TET2* (8%), are seen in CML-BP [66]. CpG site methylation is significantly increased in CML-BP compared with CP [80]. Aberrant DNA methylation of multiple genes, such as the calcitonin gene, *CDKN2B*, *PDLIM4*, *OSCP1*, and the *ABL1* promotor region, is associated with disease progression or TKI resistance [79–82].

As demonstrated in a recent study, the dynamics of additional mutations are associated with treatment outcomes [83]. Unsurprisingly, mutations acquired during TKI treatment are strongly correlated with treatment failure. *ABL1* tyrosine kinase domain mutations exclusively follow this pattern. However, caution should be taken when interpreting clearance or persistence of pre-existing mutations as the prognostic impact depends on the timing of acquisition and cellular origin of these mutations. When mutations originate from a Ph− preleukemic clone, a durable satisfactory TKI response is usually achieved despite persistence of these mutations. Mutations acquired in preleukemic HSCs implied by the presence of these mutations in T cells at diagnosis and both Ph+ and Ph− clones may show persistence, significant reduction, or clearance following TKI therapy. No obvious association between treatment outcome and dynamics of these mutations following treatment is observed. These mutations are frequently seen in the genes associated with chromatin modification and DNA methylation. Although not currently widely used, assessment of the baseline mutational profile at diagnosis and determination of involvement of the T cell lineage may be of value in the future.

Clonal Cytogenetic Abnormalities in Ph-Negative Cells

About 3–9% of patients who receive imatinib develop clonal cytogenetic abnormalities in Ph− (CCA/Ph−) cells, most commonly −7, +8, −5, and −Y [84–87]. However, only a small subset of these patients (2–5%) develops clinically evident myelodysplastic syndrome (MDS) or acute myeloid leukemia

(AML) [88, 89]. In the absence of dysplasia, the presence of CCA/Ph− does not adversely affect patient outcome, with the exception of −7. About 30% of patients with −7 develop MDS or AML [90]. According to the ELN recommendations, −7 and del(7q) detected during TKI treatment is a "warning" sign and requires more frequent cytogenetic and molecular genetic monitoring and long-term follow-up with the performance of bone marrow assessment [32••]. However, other CCA/Ph− do not necessitate immediate therapeutic intervention or more frequent monitoring. Bone marrow examination is reserved for cases with cytopenia or dysplastic morphology.

Ph Acquired Secondarily During Therapy of AML, MDS, and ALL

The acquisition of a secondary Ph during therapy of myeloid or lymphoid neoplasms is rare (summarized in [91•]). It has been reported in AML, MDS, B-ALL, and T-ALL. The emergence of secondary Ph is always associated with advanced stage of disease: during relapse or in refractory stage of de novo acute leukemia or after acute transformation from MDS. The transcript subtype is of overwhelmingly the e1a2 (P190) subtype (~ 74% of all cases). In the vast majority of patients, the diseases before and after the emergence of the Ph are clonally related and usually show similar morphological and immunophenotypic features. Features of CML are rare regardless of the size of Ph+ clones.

The emergence of a secondary Ph likely signifies terminal illness with profound genomic instability, in which the Ph is acquired randomly as a passenger event in the founder clone. Alternatively, in a subset of patients, the emergence of Ph could be due to the expansion of a minute Ph+ subclone that initially is not detectable by cytogenetic methods. Nonetheless, the emergence of Ph clones is not essential for the maintenance of the leukemic process. Incorporating TKIs into treatment may eradicate the Ph+ clones and potentially change the disease prognosis in a small subset of patients, but most patients have refractory disease despite the disappearance of the Ph+ clones. The outcome in these patients is extremely poor with a median survival of 4 months after the emergence of the Ph.

Ph Acquired During Therapy of MPN

JAK2 V617F (or rarely *MPL* W515L/K) and *BCR-ABL1* were previously thought to be mutually exclusive. However, an increasing number of cases with coexistence of these two mutations have been described, acquired either simultaneously or sequentially. Herein, we only discuss the Ph arising in patients with a previous diagnosis of Ph− MPN. To date, < 40 cases have been reported in the literature [92–95], predominantly in patients with a history of polycythemia vera (~ 70%), followed by essential thrombocythemia and primary myelofibrosis. However, the mutational status of *JAK2* and *MPL* was not examined in all cases of Ph− MPN. The Ph acquired after Ph− MPN is often the e13a2/e14a2 (P210) subtype (~ 70%) [93]. Additionally, the disease in most patients, if not all, is bona fide CML. These two features are in sharp contrast to the Ph acquired during therapy in MDS and acute leukemia, where the transcript subtype is of overwhelmingly the e1a2 (P190) subtype and features of CML are rare.

De Novo Ph+ Acute Leukemia

The Ph can also be seen in other types of leukemia, including de novo ALL, AML, and mixed phenotype acute leukemia (MPAL). Among all Ph+ acute leukemias, the overall frequencies of Ph+ ALL, Ph+ MPAL, and Ph+ AML are ~ 65%, ~ 30%, and < 5%, respectively [96]. The Ph is the most commonly detected cytogenetic abnormality in B-ALL patients: ~ 3% of pediatric patients, ~ 25% of adults, and up to 50% of patients older than 50 years [97]. The P190 form is documented in ~ 85% of cases. Patients with Ph+ B-ALL tend to have a high leukocyte count and a high frequency of central nervous system involvement. Microscopically, Ph+ B-ALL is similar to Ph− B-ALL. Ph+ B-ALL frequently expresses myeloid-associated antigens including CD13 and CD33, and CD25 expression is highly characteristic [98]. Very rare cases of Ph+ T-ALL have been also reported [99].

As is the case in ALL, the Ph is the most common recurrent genetic abnormality in MPAL and is found in ~ 30% of cases. The P190 form is detected in ~ 60% of cases. Patients with Ph+ MPAL tend to present with a high leukocyte count but are otherwise clinically similar to patients with Ph− MPAL. Most cases of MPAL have a B/myeloid phenotype, with few cases reported to show T/myeloid phenotype.

In the 2017 revised WHO classification [57], Ph+ AML is included as a new provisional entity. Ph+ AML is rare, comprising < 1% of all cases of AML. Although most Ph+ AML cases express the P210 form of BCR-ABL1, P190 is not uncommon and detected in 17–41% of cases [100–102]. Ph+ AML occurs primarily in adults and shows distinct hematological, morphological, and genetic characteristics from CML-BP, such as absence of basophilia, a slightly lower bone marrow cellularity, a lower myeloid-to-erythroid ratio, more prominent dysplasia with lack of significant myeloid maturation, and a significantly lower prevalence of major ACAs [100].

Conclusions

Although TKIs have revolutionized the treatment of CML patients and dramatically improved their outcome, therapy resistance remains a major problem. Ongoing assessment is

required for early identification of patients who are not responding optimally so that alternative treatment strategies can be considered. Potential novel therapeutic strategies may include eradicating CML stem cells, inhibiting other tyrosine kinases that are overexpressed during progression, and simultaneously targeting both BCR-ABL1 and constitutively activated downstream molecules. Progress has been achieved in investigating the cytogenetic and molecular aberrations associated with disease progression. Based on the ACA-associated risk of blastic transformation, a four-tier risk stratification model has been established that would help identify high-risk patients who may need timely hematopoietic stem cell transplantation. Additionally, with the introduction of novel sequencing technology, patterns of molecular evolution will also likely be established, analogous with the identification of cytogenetic evolutionary routes in CML.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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