Chronic myelogenous leukemia in the age of imatinib: assessing response, acceleration, and blast phase

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Abstract Chronic myelogenous leukemia, *BCR-ABL1*+ (CML) is a myeloproliferative neoplasm defined by the presence of the *BCR-ABL* fusion gene and a t(9;22)(q34;q11) cytogenetic abnormality. Its natural history is that of inexorable progression to an acute leukemia (blast crisis) after a prolonged chronic phase. The recent development of imatinib and other inhibitors of *BCR-ABL* tyrosine kinase activity has dramatically altered the clinical course of CML, with long-term remissions in most patients treated early in the course of the disease. Monitoring of resistance to therapy and progression on therapy are critical in the current management of the disease. Problems facing the diagnostic pathologist in this “Age of Imatinib” include accurate diagnosis and determination of CML disease phase prior to therapy, evaluating response to therapy, and assessing for disease progression on therapy.

Keywords Chronic myelogenous leukemia · Philadelphia chromosome · *BCR-ABL* · Imatinib · Tyrosine kinase inhibitors · Myeloproliferative neoplasms

Background

Chronic myelogenous leukemia, *BCR-ABL1*+ (CML) is a myeloproliferative neoplasm defined by the presence of the

BCR-ABL fusion gene that is usually associated with a t(9;22)(q34;q11) cytogenetic abnormality (the Philadelphia chromosome). This fusion of the *ABL* tyrosine kinase gene with the breakpoint cluster region gene (*BCR*, encoding a serine–threonine kinase) produces a constitutively active chimeric protein that promotes neoplastic transformation via dysregulated phosphorylation of various substrates and activates the RAS, PI3K, and JAK-STAT pathways, among others [1, 2]. In the vast majority of CML cases, the *BCR* breakpoints occur in the relatively small (5.8 kb) major breakpoint cluster region [3] and result in a 210-kDa fusion protein (p210). Rare CML cases (about 0.3%) have a breakpoint in the minor breakpoint cluster region, resulting in a p190 fusion protein [4]. The p190 fusion protein is far more common in Philadelphia chromosome-positive B acute lymphoblastic leukemia (Ph+ B-ALL) than in CML [5]. In very rare CML cases, the breakpoint occurs at the micro-breakpoint cluster region, generating a p230 fusion protein.

The characteristic features of CML hematopoiesis include a differentiation pattern that favors the granulocytic lineage over the erythroid lineage, deregulated proliferation, growth factor independence, defective adhesion to bone marrow stromal cells, and defective apoptosis. This abnormal hematopoiesis results in bone marrow hypercellularity and the inappropriate entry of immature myeloid cells into the peripheral blood (Fig. 1a) and their colonization of extramedullary tissues such as the spleen [6, 7]. The main clinical manifestations are leukocytosis with increased circulating immature granulocytic precursors, basophilia, and splenomegaly (Table 1). Patients typically present with clinical symptoms related to the increased white cell mass, including fatigue, weight loss, and/or abdominal discomfort due to splenomegaly, but the disease is discovered incidentally in asymptomatic patients in up to 50% of cases [8, 9]. When untreated, the natural history of CML is that of inexorable progression to an acute leukemia (blast

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Fig. 1 Typical findings of CML in chronic phase at diagnosis. **a** Peripheral blood smear shows marked leukocytosis with a spectrum of maturing granulocytic elements and basophilia. **b** The bone marrow trephine biopsy is markedly hypercellular with an increased myeloid/erythroid ratio and numerous band and segmented granulocytes; megakaryocytes are small with simplified nuclear contours. **c** The bone marrow aspirate shows a spectrum of nondysplastic maturing granulocytic elements and increased basophils. **d** Karyotype of blood or bone marrow typically shows a t(9;22)(q34;q11) abnormality (arrows)

crisis) after a prolonged chronic phase. Earlier therapies used to treat CML, mainly hydroxyurea and interferon, were generally ineffective at prolonging survival or delaying disease progression in most patients. However, the development of pharmacologic inhibitors of BCR-ABL (the tyrosine kinase inhibitors (TKIs) imatinib, dasatinib, nilotinib, and bosutinib) has dramatically improved the survival of CML patients since the beginning of the twenty-first century and has radically altered the disease course.

The first step: accurate diagnosis of CML is still critical in the imatinib era

The bone marrow biopsy in CML patients at diagnosis is markedly hypercellular, usually approaching 100%, with a markedly elevated myeloid/erythroid ratio (Table 1; Fig. 1b). Megakaryocytes are often increased and are usually smaller than normal, with hypolobated nuclei, a feature that

distinguishes them from the typical megakaryocyte morphology of other myeloproliferative neoplasms such as essential thrombocythemia and primary myelofibrosis. This is an important distinction, because the bone marrow in CML may exhibit varying degrees of reticulin fibrosis either at diagnosis or upon progression, potentially mimicking primary myelofibrosis. The bone marrow aspirate also shows an elevated myeloid/erythroid ratio with a spectrum of all stages of granulocytic maturation represented and increased basophils and eosinophils (Fig. 1c). Unless in an advanced phase of the disease, blasts comprise <10% of the marrow and peripheral blood cells and there is no significant morphologic dysplasia in erythroid and myeloid cells. The *BCR-ABL* translocation can be readily detected in peripheral blood by cytogenetics, fluorescence in situ hybridization (FISH), and/or reverse transcription PCR (RT-PCR) and thus a diagnosis of CML usually can be presumptively made prior to a bone marrow biopsy [10] (Fig. 1d). However, a pre-therapy bone marrow sample is desirable for the Bone

Table 1 Typical features of chronic myelogenous leukemia in chronic phase

Peripheral blood findings	Bone marrow findings	Cytogenetic and molecular genetic findings
Leukocytosis (median, $100 \times 10^9/L$)	Hypercellularity (approaching 100%)	Karyotype: t(9;22)(q34;q11) translocation or complex translocation involving 9q34 and 22q11 loci
Increased immature myeloid precursors	Increased myeloid/erythroid ratio (usually at least 10:1)	FISH: fusion of <i>BCR</i> and <i>ABL1</i> loci
Eosinophilia	Increased myelocytes	RT-PCR: fusion transcript of <i>BCR</i> and <i>ABL1</i>
Basophilia	Basophilia	
Often thrombocytosis	Small, hypolobated megakaryocytes	
	Lack of morphologic dysplasia of erythroid and myeloid elements	

marrow blast count is an important determinant of CML phase and may be higher than in the peripheral blood. (2) Adverse cytogenetic markers of clonal evolution are more readily detected in bone marrow than in peripheral blood samples. (3) The bone marrow biopsy may show reticulin fibrosis, providing both prognostic information as well as a baseline to measure the reduction in reticulin fibrosis that occurs in most patients responding to TKIs [11]. A pre-therapy bone marrow examination, including biopsy (with reticulin stain), aspiration, and cytogenetics, is thus recommended in all newly diagnosed CML cases. Flow cytometry has no current role in the diagnosis of CML in chronic phase, but is useful in determining the blast phenotype (lymphoid, myeloid, or biphenotypic) in blast crisis (see below).

Importantly for both the diagnostic pathologist and hematologist, CML may present with atypical features that can lead to its misdiagnosis as other myeloproliferative neoplasms. Given the highly effective targeted therapies available to treat CML, molecular genetic testing should be performed to exclude CML in any suspected myeloproliferative process. The absence of *BCR-ABL* effectively excludes CML. However, in cases that are highly suspicious for CML and are unexpectedly negative for *BCR-ABL* by a single testing modality (cytogenetics, FISH, or RT-PCR), an additional test should be performed to exclude the possibility of occasional false negative results that can occur with any of these tests; for example, about 5% of cases of CML cases lack a classic t(9;22) due to a variant translocation or a cryptic *BCR-ABL* fusion. Long-template qualitative RT-PCR can detect most variants and thus represents a highly sensitive method of detecting *BCR-ABL* in putative CML cases at diagnosis [12, 13]. However, some variant translocations may escape detection by even long-template RT-PCR [14–16]. The pathologist should be aware of this possibility and request confirmatory multiplex PCR testing and/or FISH in rare cases of CML that are falsely negative by RT-PCR [17].

Atypical presentations of CML that can challenge the diagnostician include cases with blunted leukocytosis due to concomitant metabolic deficiencies, marked eosinophilia, prominent thrombocytosis that may be more impressive than the leukocytosis, or monocytosis. These atypical presentations can mimic other myeloid neoplasms, leading to a missed diagnosis of CML and a missed opportunity to provide effective TKI therapy. CML cases with an increased absolute monocyte count can be mistaken for chronic myelomonocytic leukemia (CMML). This is an important distinction, as CMML (as defined by the WHO 2008 Classification) does not respond to TKI therapy. Monocytes only rarely comprise >10% of all peripheral blood leukocytes in CML, and cases with >10% monocytes are often associated with a rare p190 (e1a2) *BCR-ABL* (Fig. 2a, b). Atypical CML, *BCR-ABL* negative (often referred to in earlier publications as “Philadelphia-negative CML”) also presents with leukocytosis and immature myeloid elements, but lacks basophilia and often has dysplastic morphology. Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative neoplasm that also presents with leukocytosis, but lacks significant circulating immature granulocytic elements or basophilia. CML patients with p230 *BCR-ABL* may present with a mature neutrophilia resembling CNL or with prominent thrombocytosis mimicking essential thrombocythemia [18]. (Fig. 2c, d). Most importantly, atypical CML, *BCR-ABL* negative, CMML, and CNL all lack *BCR-ABL* by definition; thus molecular genetic testing is the most reliable method to distinguish these entities from CML. The pathologist must be astute to these atypical presentations and should suggest *BCL-ABL* testing if CML is even a remote possibility.

Rarely, CML patients may present in blast crisis (CML-BC), mimicking a de novo Ph⁺ ALL or acute myeloid leukemia (AML). In contrast to Ph⁺ ALL that usually bears the e1a2 p190 *BCR-ABL* variant, only very rare cases of

Fig. 2 CML presenting with unusual features mimicking other neoplasms. **a** Patient with moderate leukocytosis and absolute monocytosis (absolute monocyte count $5.6 \times 10^9/L$); although the marked myeloid hyperplasia and small, hypolobated megakaryocytes in the bone marrow aspirate suggest CML, RT-PCR failed to detect *BCR-ABL* translocation. Cytogenetics showed a t(9;22) and repeat RT-PCR using different primers revealed a variant e1a2 *BCR-ABL*

fusion (p190 protein). **b** Patient presenting with a platelet count of $1,093 \times 10^9/L$ and only minimal leukocytosis. The bone marrow trephine is markedly hypercellular with small, hypolobated megakaryocytes unlike the enlarged megakaryocytes with complex nuclear lobation seen in essential thrombocythemia. Cytogenetics revealed a t(9;22), establishing the diagnosis of CML

CML show the *e1a2* variant [4]. Distinguishing rare cases of de novo Ph⁺ AML from CML-BC may be more difficult, as p210 BCR-ABL characterizes both. Unlike CML-BC, Ph⁺ AML usually lacks basophilia and is less likely to manifest with massive splenomegaly [19]. Cases of presumed acute leukemia treated with induction chemotherapy in which the post-induction bone marrow shows no acute leukemia, but morphologically resembles chronic phase of disease (CML-CP) and continues to show a t(9;22) by conventional cytogenetics, likely represent cases of CML presenting in blast crisis.

Prognosis of CML at diagnosis

The natural history of CML is characterized by evolution through three clinical phases of disease. Patients usually present in the chronic phase of disease (CML-CP), characterized by indolent and clinically stable features. However, about 10% of CML patients present in advanced phases, termed accelerated phase (CML-AP) or blast crisis (CML-BC), without a clinically evident chronic phase [20]. Even with the advent of TKI therapy, the phase of disease remains an important prognostic factor in CML [21, 22]. According to the 2008 WHO Classification, bone marrow and peripheral blood blast count, peripheral blood basophil and platelet counts, unresponsiveness to cytoreductive therapy, and cytogenetics are the parameters used to define CML-AP [10] (see Table 2). One recent study suggests that imatinib-treated CML patients with 20–29% blasts (currently classified as CML-BC according to WHO Classification criteria) have a prognosis similar to CML-AP rather than other CML-BC patients [23]. Although not a defining feature of CML-AP in the WHO Classification, marked reticulin and/or collagen fibrosis in the bone marrow accompanied by a prominent increase in megakaryocytes is also associated with disease progression, and is used as a

defining feature of CML-AP according to International Bone Marrow Transplant Registry criteria [24].

Aside from disease phase, several other clinicopathologic parameters have been identified at the time of diagnosis that correlate with time to progression and survival. The Sokal score incorporates age spleen size, platelet count, and blast percentage in the peripheral blood, which are combined into a hazard ratio. The Sokal scoring system was developed at the time of earlier CML therapies, but it retains its prognostic significance even in TKI-treated CML patients [25]. Increased bone marrow reticulin fibrosis represents an independent adverse prognostic risk factor in interferon and hydroxyurea-treated CML patients [26] and pre-treatment increased reticulin has also been associated with a lower rate of cytogenetic remission in imatinib-treated CML patients [27].

While the development of additional chromosomal abnormalities represents progression to at least CML-AP, the significance of chromosomal abnormalities additional to t(9;22) at the time of diagnosis is uncertain. Additional chromosomal abnormalities present at diagnosis were associated with a poor prognosis in earlier studies, but not in more recent studies examining imatinib-treated patients [28–30]. Gene expression profiling shows promise in risk-stratifying CML patients treated with TKI. In one study, microarray analysis of CD34⁺ progenitor cells in CML-CP cases revealed differential gene expression in patients with indolent disease from those who rapidly progressed to CML-BC; specifically, lower expression of CD7 and higher expression of proteinase 3 or elastase were associated with a more indolent course [31]. CD7 expression in CD34⁺ CML blasts detected by flow cytometry has also been independently associated with adverse prognosis in CML-CP [32, 33]. Several studies have also shown differential gene expression between peripheral blood leukocytes from CML cases that subsequently respond or do not respond to imatinib [34–36].

Table 2 WHO classification 2008 staging system for chronic myelogenous leukemia [10]

Feature	Chronic phase	Accelerated phase	Blast crisis
Blast % in blood or bone marrow	<10%	10–19%	≥20%
Basophil % in blood	<20%	≥20%	NA
Platelet count (×10 ⁹ /L)	≤1,000 or responsive to therapy if >1,000 ≥100 or related to therapy if <100	>1,000, unresponsive to therapy <100, unrelated to therapy	NA
White blood count (×10 ⁹ /L)	≤10 or responsive to therapy if >10	>10, unresponsive to therapy	NA
Splenomegaly	Responsive to therapy	Persistent or increasing, unresponsive to therapy	NA
Extramedullary blast tumor (chloroma)	Absent	Absent	Present
New cytogenetic changes after initial karyotype ^a	Absent	Present	NA

NA not applicable

^a Most frequently observed changes of cytogenetic evolution are additional Philadelphia chromosome, isochromosome 17q, trisomy 8, and/or trisomy 19

One recent study showed differential expression of a single drug metabolism gene, *PTGS1/COX1*, in CML cases that had primary resistance to TKI therapy; in contrast, the signature of secondary TKI resistance that did not involve the common *BCR-ABL* KD mutations was more complex, including up to 11 different genes [37]. These studies suggest that gene expression profiling in the future may obviate the current “trial-and-error” practice of using molecular monitoring (see below) to guide the use of TKI versus alternative therapies for CML.

Evaluating response to therapy

Imatinib therapy is highly effective in treating CML, but its effectiveness is limited in some patients by the development of disease resistance. Primary resistance to TKIs is infrequent and is usually related to drug-efflux mechanisms or *BCR-ABL* amplification that overcome the inhibitory effects of the drug [38, 39]. Secondary resistance, occurring after an initial response to TKI, is more common and is frequently due to mutations in the *BCR-ABL* kinase domain (KD) that abrogate the inhibitory activity of imatinib and/or other TKIs [40]. These KD mutations develop as a result of selection pressure and correlate with progression to advanced phases and shortened survival. Thus, early identification of imatinib resistance is critical to allow the use of other, potentially effective therapies, such as second-generation TKIs and/or stem cell transplantation [41]. Cytogenetic analysis and molecular genetic testing to quantify the disease burden are the cornerstones of disease monitoring of CML patients on TKI therapy.

The clinical and genetic definitions of responses to TKI therapy are shown in Table 3. Imatinib and other

TKIs induce a complete hematologic remission (CHR; defined by normalization of blood counts and spleen size) in nearly all patients treated in chronic phase. CHR is usually accompanied by normalization of bone marrow cellularity, myeloid/erythroid ratio, megakaryocyte number and morphology, and reduction of any reticulin fibrosis that was present prior to therapy [11] (Fig. 3a). These changes are accompanied by increased apoptosis of CML cells and are maintained with long-term imatinib therapy, provided that a cytogenetic response persists [42]. Cytogenetic responses are measured by the percentage of at least 20 bone marrow metaphases that bear the t(9;22) translocation (Ph⁺ metaphases): a major cytogenetic response (MCR) represents $\leq 35\%$ (<7/20) Ph⁺ metaphases, with a partial response being 1–35% Ph⁺ metaphases and a complete response (CCR) being 0% Ph⁺ metaphases. CML patients showing cytogenetic response to imatinib will almost always exhibit CHR and morphologic normalization of bone marrow appearance. However, in the first year of TKI therapy, complete hematologic responses and morphologic normalization of marrow may occur even in patients lacking a cytogenetic response, underscoring the need to monitor residual disease using genetic and/or molecular genetic techniques [11] (Fig. 3b). However, a lack of cytogenetic response may be suspected on the basis of bone marrow morphology if small megakaryocytes are prevalent, patches of increased reticulin fibrosis persist, or bone marrow basophilia is present [43] (Fig. 3c–f). Indeed, small megakaryocytes present in CML that persists on imatinib therapy have been shown to be *BCR-ABL* positive by FISH analysis, indicating their derivation from the persistent CML clone [44].

Most imatinib-treated CML-CP patients achieve CCR and failure to achieve at least a MCR is associated with

Table 3 Definitions of response of CML on imatinib and therapeutic goals [46, 49, 50, 86]

Hematologic parameters: CHR expected within 3 months of imatinib therapy ^a		
CHR	Complete hematologic response	White blood count of $<10 \times 10^9/L$, basophils of $<5\%$, no myeloid precursors in blood, platelets of $<450 \times 10^9/L$, and normalization of spleen size
Cytogenetic parameters: at least a minor CR expected within 3 months, MCR expected within 6 months, and CCR expected within 12 months of imatinib therapy ^a		
Minor CR	Minor cytogenetic response	36–65% Ph ⁺ metaphases on bone marrow cytogenetics
MCR	Major cytogenetic response	$\leq 35\%$ Ph ⁺ metaphases on bone marrow cytogenetics
CCR	Complete cytogenetic response	0% Ph ⁺ metaphases (at least 20 counted) on bone marrow cytogenetics
Molecular genetic parameters (RQ-PCR): MMR expected within 18 months of therapy ^a		
MMR	Major molecular response	≥ 3 -log reduction in <i>BCR-ABL</i> transcript ratio ^b in blood compared with pre-treatment reference level by RQ-PCR assay
CMR	Complete molecular response	Negativity for <i>BCR-ABL</i> by sensitive RQ-PCR assay

^a Failure to meet expected therapeutic targets indicates a suboptimal response, and should elicit TKI-resistance mutation analysis and/or switch of therapy

^b *BCR-ABL* transcript ratio = ratio of *BCR-ABL* transcript level to transcript level of a reference gene, as assessed by real-time quantitative RT-PCR (RQ-PCR)

Fig. 3 Morphologic features of CML treated with TKIs. **a** Mildly hypercellular bone marrow trephine in a 66-year-old woman treated with imatinib for 1 year: megakaryocytes and myeloid/erythroid ratio appear normal and cytogenetics shows complete cytogenetic remission. **b** Similar-appearing bone marrow trephine in a 61-year-old woman treated with dasatinib for 6 months; however, cytogenetics reveals persistent t(9;22) in all 20 metaphases, indicating treatment failure with discordant “normalization” of bone marrow appearance. **c** Reticulin staining of bone marrow trephine from (**b**) shows focal fiber increase, which can be a clue to persistent disease. **d** Even in normocellular marrow, frequent hypolobated megakaryocytes in the bone marrow trephine also correlate with treatment failure or relapse. **e, f** Small hypolobated megakaryocytes with rounded nuclei can also be seen in the aspirate smears

increased risk of progression to advanced phases of disease and shortened survival [45]. Expected responses for newer TKIs (nilotinib and dasatinib) are more rapid than for imatinib [46]. Although cytogenetic analysis of bone marrow is considered to be the “gold standard” of cytogenetic responsiveness, interphase FISH analysis of peripheral blood to detect *BCR-ABL* appears to correlate well with cytogenetic responsiveness and is used in lieu of bone marrow cytogenetic analysis at some centers [47]. However, periodic bone marrow cytogenetic analysis to confirm CCR and to assess for clonal evolution is still indicated during disease monitoring on therapy [48, 49].

While the achievement of a CCR is a desirable goal with imatinib therapy, real-time quantitative RT-PCR (RQ-PCR) currently represents the “gold standard” to detect and quantify low levels of persistent CML. Molecular response by RQ-PCR is defined as the detectable *BCR-ABL* transcript ratio (ratio of the *BCR-ABL* transcript level to the level of a reference gene transcript, usually either *BCR*, *ABL*, or *GUSB*) as a percentage of the average *BCR-ABL* transcript ratio of a control group of pre-therapy CML cases, established at each laboratory [50]. *BCR-ABL* transcript levels can be determined on blood or bone

marrow. Although a quantitative technique, RQ-PCR results may be variable between and within laboratories [51]. Ideally, RQ-PCR analyses on a given patient should be done consistently on the same type of sample (usually blood) and in the same laboratory over the course of his/her treatment [51]. Major molecular response (MMR) represents a decrease in the *BCR-ABL* transcript ratio of at least 3 logs from the baseline (i.e., 0.1% of the transcript level present at initial diagnosis). Achieving MMR is the goal of imatinib therapy and is achieved in 50–60% of CML patients by 12 months of therapy and in up to 80% of patients after 4 years of therapy [1]. Patients achieving MMR have excellent progression-free survival with continued imatinib therapy and rapid attainment of MMR is particularly associated with long-term responsiveness to imatinib [52]. Surprisingly, low-level detectable *BCR-ABL* transcripts persist in the vast majority of CML patients with MMR, in spite of continued imatinib therapy and long-term disease free progression and only 10–40% of patients achieve a complete molecular response (CMR), which is defined as undetectable *BCR-ABL* transcript in a RQ-PCR assay that has at least 4–5 log dynamic range [25, 48]. The reasons for this persistent low-level disease are uncertain;

however, the durability of MMR in most patients indicates that imatinib can keep a small residual CML population in a quiescent state with low likelihood of relapse and very low incidence of progression to advanced phase disease. Importantly, even CMR cannot be equated to “cure,” as 61% of imatinib-treated CML patients achieving a CMR for at least 2 years relapsed after stopping imatinib therapy; most relapses occurred rapidly, within 6 months of stopping therapy [53].

Although there is some variability in practice, most current centers monitor disease by RQ-PCR in blood every 3 months during the first 12 months of TKI therapy, then every 6 months once CCR is attained [49]. Bone marrow cytogenetic studies should be performed prior to initiating TKI therapy and every 6 to 12 months until CCR, then every 1–2 years. These frequent periodic cytogenetic and molecular response assessments are performed in order to reveal patients that respond suboptimally to imatinib therapy (see Table 3). Suboptimal response, defined by a failure to achieve specific cytogenetic and/or molecular response targets at the designated timepoints in Table 3, correlates with resistance to imatinib and warrants an investigation for KD mutations and/or a switch to another therapy. Loss of CCR or hematologic relapse in a previously responding patient should also elicit resistance mutation analysis. A sustained or increasing rise in transcript level of 2- to 5-fold even at very low disease levels has been associated with an increased likelihood of detecting TKI-resistance mutations [54, 55], but most centers use a 10-fold increase in transcript levels above MMR to trigger mutation analysis or to consider a change in therapy [56]. Frequent monitoring by the above methods is important, since CML failing initial TKI therapy is more likely to respond to a second-line therapy if the therapeutic switch is instituted rapidly [57].

The most common mechanism of secondary resistance to TKI is mutation in the KD of *BCR-ABL*. KD mutations can be detected by several methods, including pyrosequencing, mutation-specific RQ-PCR, liquid bead arrays, and denaturing high performance liquid chromatography, but direct sequencing is by far the most common [58]. Although it is the least sensitive method, direct sequencing is the most routinely available and reliable method to cover all possible mutation sites and has been recommended for KD mutation analysis by an international consensus panel [59]. Mutations that confer TKI-resistance cluster in four critical regions in the *BCR-ABL* KD, involving ATP binding (P-loop), imatinib binding, catalytic activity, and kinase activation (A-loop). Mutations vary in their ability to inhibit the currently available TKIs and thus the specific mutation type can help guide the use of a TKI that is not inhibited by that particular mutation. The mutation at amino acid 315 in the imatinib-binding

site (T315I mutation) confers resistance to imatinib, dasatinib, and nilotinib by preventing access of these drugs to the ATP-binding pocket.

The mechanisms by which these mutations develop are poorly understood. KD mutations can be present at low-levels in CML cases prior to exposure to TKI therapy. However, these small mutant clones do not necessarily confer an adverse prognosis and resistant clones may not expand even under the selection pressure of TKI therapy [60, 61]. For these reasons, up-front assessment for KD mutations is not recommended prior to the institution of TKI therapy, as it may not predict resistance to TKI therapy. Secondary resistance to TKIs due to KD mutations usually occurs within the first 2 years of TKI therapy and is much more common in CML-AP and CML-BC than CML-CP [25]. Although KD mutations are a common cause of secondary resistance to TKI therapy, about half of the resistance occurrences are due to other mechanisms [21]. These include *BCR-ABL* overexpression (due to amplification at the genomic level or other mechanisms) and rare mutations outside the KD. Mechanisms of resistance not directly involving *BCR-ABL* are often associated with cytogenetic evolution that results in the activation of other oncogenes and/or loss of tumor suppression genes. Gene expression profiling may be helpful in identifying target genes associated with KD-mutation-independent TKI resistance [37]. Alteration in drug metabolic kinetics is usually a mechanism of primary TKI resistance to TKI and only rarely a cause of secondary TKI resistance.

Determining progression on therapy

In the absence of effective therapy, CML patients inexorably progress to blast crisis. This blast crisis may exhibit a myeloid (about 60% of cases), lymphoid (20–30% of cases), or mixed myeloid–lymphoid phenotype (10–25% of cases), and may manifest in extramedullary organs as a myeloid sarcoma [62–64]. The median time to progression from CML-CP to CML-BC in untreated patients or in patients treated with older therapies such as hydroxyurea ranges from 3 to 5 years [65]. Blast crisis is typically preceded by an intermediate accelerated phase of disease (CML-AP) by a period of 4–6 months [66], but up to 25% of CML-BC cases develop without any clinically evident preceding accelerated phase [67]. Disease progression is uncommon currently in CML patients treated in chronic phase with TKIs. When it occurs, progression usually occurs early during treatment: in one series, 3.3% of CML-CP patients progressed to CML-AP/CML-BC within the first 18 months of therapy, while <1% progressed after 4 years of therapy [68]. Rare cases may abruptly progress to

Fig. 4 Progression of CML on TKI therapy. **a** Progression to myeloid blast crisis after 6 months of imatinib therapy: the bone marrow trephine cellularity is replaced by sheets of myeloid blasts interspersed with small megakaryocytes typical of CML. **b** Rapid development of lymphoid blast crisis after only 4 months of imatinib therapy, with sheets of small lymphoid blasts predominating in the bone marrow aspirate smear. **c, d** Patient treated in chronic phase with imatinib but tolerated therapy poorly and developed splenic rupture after 2 months. Splenectomy specimen shows red pulp replacement by sheets of myeloid blasts, representing extramedullary myeloid blast crisis. **e** Patient treated with imatinib, but not monitored and presented after 2 years with pancytopenia. Bone marrow trephine is markedly fibrotic with focal sheets of blasts (*upper right*, adjacent to bone); 30% myeloid blasts were present in the aspirate smear (not shown), confirming myeloid blast crisis. **f** CML in blast crisis with numerous blasts and prominent immature basophils

blast crisis, although this is uncommon if rigorous clinical, cytogenetic, and molecular monitoring is performed [69] (Fig. 4). In particular, central nervous system relapse has

been reported to occur as in CML patients treated with imatinib and diagnosticians must be vigilant to this presentation [70, 71].

Fig. 5 Development of cytogenetic abnormalities in non-CML cells during TKI therapy. **a** A 27-year-old woman with complete molecular response to imatinib developed pancytopenia with a hypocellular bone marrow trephine. **b** The bone marrow aspirate shows left-shifted myeloid maturation with abnormal retention of primary granules and dyserythropoiesis. **c** Abnormal pseudo-Pelger–Huet granulocytes are seen in the peripheral blood. **d** Conventional cytogenetics showed monosomy 7 in 18 of 20 metaphases and no t(9;22). Unlike most patients developing new non-CML cytogenetic abnormalities on TKI therapy, this patient developed a bona fide myelodysplastic syndrome and required a bone marrow transplant

The progression to advanced phases of disease is caused by the development of genetic lesions in addition to *BCR-ABL*; secondary cytogenetic changes are identified in 85% of patients by the time of progression to CML-BC [72]. Even in the absence of other features of CML-AP or CML-BC, the acquisition of new cytogenetic abnormalities in patients treated with imatinib is associated with loss of response and poor prognosis [30, 73]. FISH appears to increase the yield of identifying the most common additional chromosomal abnormalities associated with disease progression, but it is unclear if these small clones with secondary abnormalities have clinical relevance [74]. Thus, the use of FISH to examine for additional secondary changes that are not evident on routine karyotype is not currently indicated.

The most frequent secondary cytogenetic changes in advanced phase CML are trisomy 8, one or more additional Philadelphia chromosomes (derivative 22), and an isochromosome 17 [75]. The molecular events associated with CML progression are complex and are likely related to genetic instability and impaired DNA repair mechanisms that are consequences of *BCR-ABL* activity [76–78]. In addition to cytogenetic aberrations, *BCR-ABL* amplification, gene mutations, and epigenetic changes such as gene methylation also appear to play a role in disease progression [79]. Interestingly, different blast crisis phenotypes have been associated with unique patterns of molecular evolution: *ABL* kinase domain mutations that confer resistance to TKIs occur frequently in lymphoid blast crisis, while myeloid blast crisis is more commonly associated with cytogenetic evolution. *BCR-ABL* amplification is seen in about one third of both myeloid and lymphoid blast crisis cases [4]. The criteria for CML-AP in patients progressing on TKI therapy are still poorly defined and disease progression in CML is currently defined more practically in terms of responsiveness to TKI therapy [50]. Evidence of therapeutic refractoriness (such as rising RQ-PCR *BCR-ABL* levels or loss of CCR) should elicit a bone marrow examination [80]. However, even if standard features of CML-AP or CML-BC are not pathologically evident, a switch in therapy may be indicated in such cases. Of note, increased reticulin fibrosis on imatinib therapy is independently associated with treatment failure and appears more significant than increased reticulin present at the initial diagnosis. For these reasons, it is important to perform a reticulin stain on CML bone marrow samples taken on TKI therapy [27].

Other pathologic processes occurring in CML patients treated with tyrosine kinase inhibitors

While therapeutic refractoriness to imatinib is often associated with cytogenetic evolution, between 2% and

8% of CML patients treated with imatinib develop clonal cytogenetic abnormalities in non-CML cells lacking a t(9;22) [81, 82]. The abnormalities include changes often associated with myelodysplastic syndromes, such as trisomy 8 (the most frequently observed), deletion 7q, deletion 20q, and loss of the Y chromosome. Although often associated with cytopenias, these changes are usually transient and only rare patients develop myelodysplastic syndromes [83] (Fig. 5). The etiology of these cytogenetic abnormalities is uncertain: normal hematopoiesis following successful TKI therapy has been shown to be polyclonal in most patients and imatinib does not appear to be mutagenic in vitro [84, 85]. Further follow-up is required to determine the incidence and significance of these cytogenetic changes. Until their significance and follow-up are clarified, bone marrow examination is recommended in CML patients on TKI therapies who develop new onset unexplained cytopenias [49].

Conflicts of interest The author declares that he has no conflict of interest.

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