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Best Practices in Chronic Myeloid Leukemia Monitoring and Management

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Leukemia, chronic myeloid • *BCR-ABL1* • Tyrosine kinase inhibitor • Minimal residual disease • Molecular testing

Abstract

Optimal use of current therapeutic opportunities for chronic myeloid leukemia patients requires integration of clinical and laboratory monitoring. Assessment of molecular response (MR) by real-time quantitative polymerase chain reaction is the most sensitive way to monitor tyrosine kinase inhibitor (TKI) treatment efficacy. Besides major molecular response, which has emerged as a safe haven for survival since the initial studies of first-line imatinib treatment, two additional MR milestones have recently been defined: early molecular response and deep molecular response. The achievement of such MR milestones within defined time points during therapy is thought to draw the ideal trajectory toward optimal long-term outcome and, possibly, successful treatment discontinuation. Sensitive and reproducible MR measurement and proper interpretation of MR results are therefore critical to correctly inform therapeutic decisions. In patients who do not achieve an optimal response to TKI therapy, *BCR-ABL1* mutation screening should also be performed, because it may deliver useful information for TKI choice. This review aims to help clinicians apply and translate the latest response definitions and clinical recommendations into practice. We provide a critical update on how these recommendations have incorporated MR levels in the clinical decision algorithms and how detection of *BCR-ABL1* mutations should be interpreted. We also include a practical guide for pathologists and molecular biologists to best perform molecular testing and for hematologists and oncologists to best integrate it into routine practice. ***The Oncologist*** 2016;21:626–633

Implications for Practice: Ever-more-potent therapeutic strategies have been developed for chronic myeloid leukemia (CML) in parallel with the evolution of therapeutic goals and the refinement of response definitions and monitoring schemes and procedures. Terminology and methodology continue to evolve rapidly, making it difficult for busy hematology/oncology professionals to keep abreast of the newest developments. Optimal CML patient management results from the timely and rational use of molecular testing, the critical assessment of the power and pitfalls of current technology, and the appropriate interpretation and contextualization of results.

Current Options for the Treatment of Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that accounts for 15%–20% of all cases of leukemia in adults [1]. The landmark discovery of the Philadelphia chromosome (Ph) in 1960 [2] and the t(9;22)(q34;q11) chromosomal translocation from which it originates in 1973 [3] provided the first demonstration of a chromosomal abnormality being consistently associated with the development of cancer. The t(9;22) translocation and the resulting *BCR-ABL1* gene rearrangement are detectable in virtually all CML patients. CML is also one of the first malignancies for which the principle of targeted therapy has found successful application. In the late 1980s, several seminal studies contributed to unravel the molecular pathogenesis of CML, showing the central role of the deregulated tyrosine kinase activity of *BCR-ABL1* in the initiation and maintenance of the disease [4–8]. It soon became evident that turning *BCR-ABL1* off would selectively eliminate leukemic cells while sparing the normal ones. Imatinib mesylate [9], the first tyrosine kinase inhibitor (TKI) to enter clinical evaluation as an anticancer drug in 1998, emerged serendipitously from a time-consuming process of random screening of a large number of compounds created using the structure of the adenosine triphosphate binding site [10]. After clinical trials whose success surpassed almost everyone's expectations, in 2002 imatinib became the gold standard for front-line treatment of all newly diagnosed CML patients [11].

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Table 1. Overview of the available TKI options in CML

First line	Second line	Third line	Fourth line
Imatinib	Dasatinib	Dasatinib	Bosutinib (when imatinib, dasatinib, and nilotinib are not considered appropriate)
Dasatinib	Nilotinib (CP and AP)	Nilotinib (CP and AP)	Ponatinib
Nilotinib	Ponatinib (T315I^{+} or when no other TKI is appropriate)	Bosutinib (when imatinib, dasatinib and nilotinib are not considered appropriate)	Omacetaxine (*U.S.* only; CP and AP)
	Bosutinib (when imatinib, dasatinib, and nilotinib are not considered appropriate)	Ponatinib (T315I^{+} or when no other TKI is indicated)	
		Omacetaxine (*U.S.* only; CP and AP)	

For some TKIs, indications may slightly change from country to country.
Abbreviations: AP, accelerated phase; CML, chronic myeloid leukemia; CP, chronic phase; TKI, tyrosone kinase inhibitor.

After more than 15 years of clinical use, the safety and efficacy of imatinib are well established. However, imatinib has an Achilles heel that Ph^{+} cells may exploit. Several point mutations in the kinase domain, where imatinib binds, have been observed in patients with recurrent disease [12]. This prompted pharmaceutical companies to rationally develop and test second-generation TKIs with greater potency and/or improved binding modalities. Such second-generation TKIs include dasatinib [13] and nilotinib [14], available in many countries for first-line, second-line, or subsequent use, and bosutinib [15], currently approved only for patients with resistance or intolerance to prior therapy. First-line use of dasatinib and nilotinib has been shown to induce faster and deeper responses, with a lower percentage of cases developing drug resistance and progressing to advanced phase [16–19]. However, the shorter follow-up of patients on nilotinib and dasatinib, together with the occurrence of some severe adverse events (especially cardiovascular and pulmonary) that were never reported in patients treated with imatinib, currently poses a question about the long-term safety of second-generation TKIs [20].

The shorter follow-up of patients on nilotinib and dasatinib, together with the occurrence of some severe adverse events (especially cardiovascular and pulmonary) that were never reported in patients treated with imatinib, currently poses a question about the long-term safety of second-generation TKIs.

The unmet need represented by patients harboring the T315I *BCR-ABL1* mutation (which is cross-resistant to imatinib, dasatinib, nilotinib, and bosutinib) has recently been addressed, to some extent, by omacetaxine [21–25] as well as by the development of the third-generation TKI ponatinib [26–29]. Omacetaxine (formerly homoharringtonine; approved in the U.S. only) is a plant alkaloid long known to have some degree of activity in CML, with a mechanism of action that is unclear but is independent of *BCR-ABL1* binding and inhibition [30–32]. Ponatinib is a TKI active against several kinases, with a binding mode that is less susceptible to disruption by single point mutations [26, 33]. Although safety concerns deriving from serious vascular occlusive events reported in the phase 2 study have halted its clinical development in the first-line setting, ponatinib is regarded as a precious option for patients who have developed the T315I mutation or have failed two lines of TKI therapy. The very recent report that a vascular endothelial growth factor receptor inhibitor approved for renal cancer, axitinib, is also effective, in vitro and in vivo, against T315I-positive CML [34, 35] suggests that among existing or emerging compounds developed for other indications, some might turn out to be useful for TKI-resistant CML as well. Thus, the pharmacologic scenario is likely to evolve further.

Allogeneic stem cell transplantation (aSCT) had an important role in the pre-TKI era [36] and remains the only proven curative option for CML. However, because of the high morbidity and mortality still associated with aSCT, it is now confined to patients who are diagnosed in advanced phase and is kept as a salvage option after multiple TKI failure in all the others [37]. An exception exists in those countries where, because of financial limitations or other bureaucratic/infrastructure restrictions, TKI therapy is difficult to obtain or sustain, and aSCT, if feasible, is still the preferred first-line option [37].

Hematologists and oncologists treating CML patients these days can thus rely on a wide spectrum of treatment opportunities (Table 1). The best clinical outcomes will result from the best use of these opportunities, which in turn requires optimal integration of expert clinical and laboratory monitoring.

Monitoring Response in CML

Three levels of response to therapy can be defined in CML patients: hematologic, cytogenetic, and molecular (Fig. 1) [38–40].

Hematologic response refers to the normalization of blood cell count. The utility of hematologic monitoring is limited to the first few months of therapy, when dose adjustments for hematologic toxicity may be needed.

Cytogenetic response (CyR) denotes the percentage of residual bone marrow metaphases showing evidence of Ph, as assessed by chromosome G banding analysis (CBA). Fluorescence in situ hybridization (FISH) on interphase nuclei from peripheral blood can also be used, as an alternative, to monitor CyR. CBA was the main tool to monitor residual disease burden in the pre-TKI era. CBA or FISH is still used now if molecular monitoring is not available; however, they do not allow stratification of responders below the level of complete CyR (CCyR), which is achieved by more than 80% of patients receiving TKI therapy.

Molecular response (MR) measures the reduction of *BCR-ABL1* fusion transcripts and, as such, has the greatest

CME

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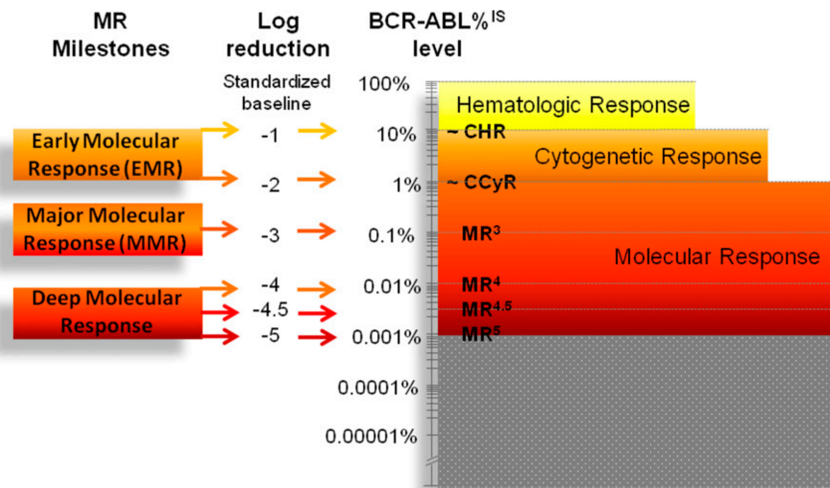

Figure 1. Levels of hematologic, cytogenetic, and molecular response in CML and the novel concepts of early molecular response and deep molecular response. The gray area indicates that RQ-PCR cannot measure MRD below MR^5 (*BCR-ABL1* <0.001%); nevertheless, residual leukemia cells may still be present. Novel technologies (such as digital PCR) and approaches (such as assessing genomic DNA rather than RNA), might, in the future, extend the dynamic range of MR detection below MR^5.

Abbreviations: CCyR, complete cytogenetic response; CHR; complete hematologic response; CML, chronic myeloid leukemia; IS, International Scale; MMR, major molecular response; MR, molecular response; MRD, minimal residual disease; RQ-PCR, real-time quantitative reverse-transcription polymerase chain reaction.

sensitivity. Once CCyR is achieved, only molecular methods make it possible to follow the dynamics of minimal residual disease (MRD) over time. Currently, the gold standard for MR monitoring is real-time quantitative reverse-transcription polymerase chain reaction (RQ-PCR), which was introduced with the very first clinical trials of imatinib [41].

Clinical Value of Molecular Monitoring

Over the years, dozens of clinical studies with imatinib, dasatinib, and nilotinib worldwide have soundly demonstrated the clinical relevance of MR monitoring (recently reviewed by Hanfstein et al. [42]). The results have been used to establish key MR milestones to be achieved at specific time points during therapy.

BCR-ABL1 levels ≤10% at 3 months and ≤1% at 6 months represent the so-called early molecular response. Early molecular response has been shown to predict the rate and depth of any subsequent response (CCyR and major molecular response [MMR]) and to correlate with significantly improved long-term outcomes (progression-free survival [PFS] and overall survival [OS]). *BCR-ABL1* ≤0.1% is MMR, the "safe haven" for survival, to be achieved within 12 months of therapy. Finally, *BCR-ABL1* ≤0.01%, down to 0.001%, defines the so-called deep or deeper MR that has also been shown to predict significantly better long-term outcomes (failure-free survival, transformation-free survival, PFS, and OS).

Besides predicting optimal outcome, deep or deeper MR is currently considered to be the gateway to treatment-free remission, which is becoming a high-priority goal of CML treatment, from both a patient perspective and a health-economics perspective [43]. Permanent TKI treatment discontinuation (often referred to as functional cure) is particularly desirable in younger patients with CML, since it would allow safe conception and pregnancy and dissipate concerns of potential late, off-target complications. The relevance of discontinuation to the health care system is also not negligible. Interim analysis of the first 200 patients enrolled in the European Stop Tyrosine Kinase Inhibitor (EURO-SKI) trial, presented at the December 2014 ASH meeting in San Francisco, estimated drug-related savings for the eight participating countries to be more than 7 million euros just in the first year of the study [44].

Best Practices in Molecular Monitoring of CML Patients: What Clinicians Need to Know

As mentioned above, MR is assessed by RQ-PCR: after total RNA isolation from peripheral blood leukocytes and reverse transcription (RT) of RNA to cDNA, the absolute copy number of the target transcript together with that of a control gene transcript (necessary to correct for differences in RNA quantity and quality and RT efficacy across samples) are determined using plasmid calibration curves [45–47]. Results are expressed as a percentage ratio between the sum of *BCR-ABL1* copies and the sum of control gene (CG) copies across replicates (typically two replicates, with some laboratories using three replicates and a few using a single measurement) [48]. Recommended CGs are *ABL*, *BCR*, or *GUSB* [48]. *ABL* and *BCR* historically were, and continue to be, the most widely used. However, they both may introduce biases in quantitation at very high and very low levels of residual disease, respectively (with use of *ABL* resulting in an underestimation and use of *BCR* resulting in an overestimation of MRD), because the assay cannot discriminate between sequences of the nontranslocated versus the translocated allele [49]. For this reason, and given the ever-increasing importance of both early and deep MR, *GUSB* (another CG transcript shown, in initial studies [47], to be a suitable candidate for its stability over time irrespective of treatment and for its half-life, comparable to that of *BCR-ABL1*) is currently being considered as an alternative [48].

To be used in clinical decision making, MR has to be measured reliably and reproducibly. Sources of technical and

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experimental variability intervening at each analytical step include (a) sample quantity, quality, and delivery time; (b) RNA extraction method, yield, and purity; (c) starting quantity and quality of the RNA that is reverse-transcribed to cDNA and efficiency of the reverse transcriptase enzyme/kit; and (d) type of RQ-PCR instrument and chemistry, protocol (homebrew assay vs commercial kit), and type of CG used. Indeed, soon after the introduction of RQ-PCR for molecular monitoring of CML patients, it was realized that results were not comparable, even among expert laboratories. International standardization efforts were thus undertaken. It was decided that each laboratory could maintain local instruments and protocols provided that (a) results be comparable and (b) adequate levels of sensitivity be routinely achieved [49–51]. This led, in 2005, to the introduction of the International Scale (IS) [49]. On the IS, MR is traditionally defined in terms of log-reduction from a standardized baseline, set to 100% (Fig. 1). According to the depth of MR, patients can be stratified into those who are in MR^{4}, $MR^{4.5}$, and MR^{5}, with the superscript indicating the log-reduction (Fig. 1). To be used in clinical decision making according to the international (European LeukemiaNet [ELN] or National Comprehensive Cancer Network [NCCN]) treatment recommendations [40, 52], MR has to be reported on the IS. The depth of response that can be measured by the assays (i.e., sensitivity) may vary from sample to sample and from laboratory to laboratory, because it is subordinated to the CG copy number. According to the latest recommendations, *ABL* CG copy number must be $\geq$10,000 to define MR^{4}, $\geq$32,000 to define $MR^{4.5}$, and $\geq$100,000 to define MR^{5}; *GUSB* CG copy number must be $\geq$24,000 to define MR^{4}, $\geq$77,000 to define $MR^{4.5}$, and $\geq$240,000 to define MR^{5} [48]. If, even in a single replicate of two or three, *ABL* copy number is $<$10,000 or *GUSB* copy number is $<$24,000, the sample must be considered inevaluable for MR regardless of what the *BCR-ABL1* copy number is [48].

The main challenges facing laboratories performing molecular monitoring of CML patients are, therefore, the following: (a) all the analytical steps from sampling to performing RQ-PCR must be optimized, to maximize the limit of detection (sensitivity) of *BCR-ABL1* transcripts and ensure reliable measurements; (b) established criteria for quality control of each run (intercept, slope, and correlation coefficient of the plasmid standard curves) and definitions of acceptable/unacceptable results and detectable/undetectable disease must be followed strictly [48]; and (c) results must be reported on the IS, to allow comparability of results and adoption of ELN or NCCN molecular checkpoints for the definition of response. This requires the testing laboratory either to obtain (and periodically revalidate) a conversion factor by sample exchange with an established reference laboratory (the *BCR-ABL1*/CG ratio percentage will have to be multiplied by this factor) or to use kits and reagents that have been calibrated to the World Health Organization International Genetic Reference Panel, recently made available [53, 54].

Hematologists and oncologists sending samples for MR assessment should verify that the testing laboratory is producing and scoring results according to the latest international recommendations [48].

What is the optimal timing of molecular monitoring? In all patients at diagnosis, a qualitative multiplex RT-PCR should be performed, to assess the transcript type [40]. The great majority of newly diagnosed CML patients test positive for the e13a2 (also known as b2a2) or e14a2 (also known as b3a2) *BCR-ABL1* rearrangement, but some may rather harbor atypical transcripts such as e13a3, e14a3, e1a2, e1a3, e19a2, e19a3, e6a2, e8a2, or e18a2 [55]. If multiplex RT-PCR is not performed at diagnosis, detecting atypical transcripts later on may not be possible. Qualitative RT-PCR is also useful for diagnostic confirmation of CML in all cases (up to 5%) with a cryptic translocation [55]. Afterward, during TKI therapy, all patients should be monitored by RQ-PCR on a regular basis: every 3 months until MMR has been achieved, then every 3–6 months [40].

ELN treatment recommendations have established critical checkpoints at 3, 6, and 12 months, so that, depending on MR levels, more careful monitoring or treatment change can be undertaken as appropriate [40]. Namely, the ELN recommendations categorize response into optimal (no change of therapy is indicated); warning (formerly, "suboptimal") (more frequent monitoring is recommended to permit timely change in case of subsequent treatment failure); and failure (the patient should receive a different treatment) (Table 2). NCCN guidelines may also be followed, as an alternative (Table 2) [52]. Updated annually, the NCCN guidelines provide algorithms for decision making at 3, 6, and 12 months, based on the response level. The main difference between the ELN recommendations and the NCCN guidelines lies in the clinical implications of the 3-month monitoring results. At 3 months, the ELN considers a *BCR-ABL1* transcript level greater than 10% a warning, irrespective of the TKI. In contrast, the NCCN mandates a dose increase or a change to an alternate TKI if the primary treatment is imatinib, whereas treatment continuation at the same dose or change to an alternate TKI is possible if the primary treatment is dasatinib or nilotinib. At 6 months, both ELN and NCCN are concordant in mandating a change of therapy in all patients with a *BCR-ABL1* level persistently above 10%. At 12 months, *BCR-ABL1* levels lower than 0.1% (MMR or better) are an optimal response, and *BCR-ABL1* levels greater than 1% are a failure according to both ELN and NCCN. The 2013 ELN recommendations also established definitions of optimal response, warning, and failure to be used in patients receiving second-line TKI therapy after imatinib failure (Table 3). Again, key MR levels appear at every time point [40].

In addition to the correct timing of MR evaluations, the importance of correct sampling and delivery modalities should not be overlooked. It is now well established that a peripheral blood sample is sufficient for MR response assessment. Bone marrow is not necessary, and alternating bone marrow and peripheral blood samples should be avoided. To facilitate achieving sensitivity levels necessary to score $MR^{4.5}$ and MR^{5}, sample quantity is important. Ten milliliters of blood may be sufficient, but more (up to 20 mL) would be preferable. Because progressive degradation of RNA starting soon after blood collection is an inevitable physiological phenomenon [56], samples should be delivered to the laboratory and processed within 24 hours, which mandates avoiding sampling on Fridays and holiday eves unless agreed on with the laboratory. Whole blood can be shipped at room temperature or refrigerated, but it must never be frozen unless specific vials containing RNA stabilizing solutions [57–59] are used.

Drug Resistance and *BCR-ABL1* Mutation Analysis

Resistance can be defined using the ELN or NCCN criteria for failure. Patients with failure on first-line imatinib treatment have been shown to have decreased PFS and OS compared

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Table 2. Definitions of response to first-line TKI therapy according to ELN recommendations and comparison with the NCCN guidelines

	Optimal response		Failure		Warning
Time	**ELN (2013)**	**NCCN (2016)**	**ELN (2013)**	**NCCN (2016)**	**ELN (2013)**
Baseline	NA	NA	NA	NA	High risk; CCA/Ph$^+$, major route
3 mo	*BCR-ABL1* ≤10% and/or Ph$^+$ ≤35%	*BCR-ABL1* ≤10% or Ph$^+$ ≤35%	No CHR, Ph$^+$ >95%	*BCR-ABL1* >10% and/or Ph$^+$ >35%	*BCR-ABL1* >10% and/or Ph$^+$ 36%–95%
6 mo	*BCR-ABL1* ≤1% and/or Ph$^+$ 0%	*BCR-ABL1* ≤1% or Ph$^+$ 0%	*BCR-ABL1* >10% and/or Ph$^+$ >35%	*BCR-ABL1* >10% and/or Ph$^+$ >35%	*BCR-ABL1* 1%–10% and/or Ph$^+$ 1%–35%
12 mo	*BCR-ABL1* ≤0.1%	*BCR-ABL1* ≤1% or Ph$^+$ 0%	*BCR-ABL1* >1% and/or Ph$^+$ >0%	*BCR-ABL1* >1% and/or Ph$^+$ >0%	*BCR-ABL1* 0.1%–1%
Anytime thereafter	*BCR-ABL1* ≤0.1%	*BCR-ABL1* ≤0.1% or Ph$^+$ 0%	Loss of CHR, or loss of CCyR, or confirmed loss of MMR with *BCR-ABL1* ≥1%, or *BCR-ABL1* mutations, or CCA/Ph$^+$	*BCR-ABL1* >0.1% and/or Ph$^+$ >0%	CCA/Ph$^-$

Abbreviations: CCA, clonal chromosome abnormality [major route CCAs: trisomy 8, trisomy Ph (1der(22)t(9;22)(q34;q11)), isochromosome 17(i(17)(q10)), trisomy 19, and ider(22)(q10)t(9;22)(q34;q11)]; CCyR complete cytogenetic response; CHR, complete hematologic response; ELN, European LeukemiaNet; MMR, major molecular response; NA, not applicable; NCCN, National Comprehensive Cancer Network; Ph, Philadelphia chromosome; TKI, tyrosone kinase inhibitor.

Table 3. Definitions of response to second-line TKI therapy after imatinib failure according to ELN recommendations

Time	Optimal	Failure	Warning
Baseline	NA	NA	No CHR, or loss of CHR on imatinib, or lack of CyR to first-line TKI or high risk
3 mo	*BCR-ABL1* ≤10% and/or Ph$^+$ <65%	No CHR or Ph$^+$ >95% or new mutations	*BCR-ABL1* >10% and/or Ph$^+$ 65%–95%
6 mo	*BCR-ABL1* ≤10% and/or Ph$^+$ <35%	*BCR-ABL1* >10% and/or Ph$^+$ >65% and/or new mutations	Ph$^+$ 35%–65%
12 mo	*BCR-ABL1* ≤1% and/or Ph$^+$ 0%	*BCR-ABL1* >10% and/or Ph$^+$ >35% and/or new mutations	*BCR-ABL1* 0.1%–1% and/or Ph$^+$ 1%–35%
Anytime thereafter	*BCR-ABL1* ≤0.1%	Loss of CHR or loss of CCyR or PCyR; confirmed loss of MMR with *BCR-ABL1* ≥1%, or new mutations, or CCA/Ph$^+$	CCA/Ph$^-$ or *BCR-ABL1* >0.1%

Abbreviations: CCA, clonal chromosome abnormality; CCyR complete cytogenetic response; CHR, complete hematologic response; ELN, European LeukemiaNet; MMR, major molecular response; PCyR, partial cytogenetic response; Ph, Philadelphia chromosome; TKI, tyrosone kinase inhibitor.

with patients with optimal responses [60]. Resistance is thus associated with a greater risk of disease progression. Biologically, it is believed that the increased *BCR-ABL1* expression and its functional reactivation, associated with resistance [61–63], are responsible for the enhanced genomic instability and perturbed differentiation [64–66] that are intrinsic features of blast crisis (BC). Even in the TKI era, treatment of BC remains a challenge, and patients who progress have a dismal outcome [67, 68]; hence, preventing disease progression from CP to BC must be the main aim of every clinician treating CML.

Although drug resistance mechanisms are likely to be many and not necessarily mutually exclusive, point mutations in the *BCR-ABL1* kinase domain (detectable at frequencies ranging from 25% to 70%, depending on whether the patient is in chronic or advanced phase) [12, 69] are the only ones whose assessment is currently recommended by both ELN and NCCN. More than 60 different mutations are known to be associated with resistance to imatinib, and not all of them are equally sensitive to other TKIs [70]. In case of failure (once low compliance and inadequate dosing are excluded), mutational analysis should be performed [52, 71]. ELN also recommends mutational analysis in case of suboptimal response (warning) [40, 71]: although mutation frequency seems to be low in patients with warning, detection of a mutation would definitively establish the need of a treatment change and, in some cases, provide indications about which alternative TKI is most likely to be effective. *BCR-ABL1* mutation analysis should not be performed at diagnosis (except in those rare patients who are diagnosed in accelerated or blastic phase) or in patients who have an optimal response to therapy.

Biologically, it is believed that the increased *BCR-ABL1* expression and its functional reactivation, associated with resistance, are responsible for the enhanced genomic instability and perturbed differentiation that are intrinsic features of blast crisis.

Generally, an aliquot of the same RNA (or even cDNA) used for RQ-PCR is sufficient to perform *BCR-ABL1* mutation analysis. If neither RNA nor cDNA are available, sampling has

Table 4. Updated indications on how to choose the TKI according to *BCR-ABL1* mutation status

Mutation	Action
T315I	Consider ponatinib
T315A, F317L/V/I/C	Consider nilotinib or bosutinib rather than dasatinib
Y253H, F359V/C/I	Consider dasatinib or bosutinib rather than nilotinib
V299L	Consider nilotinib rather than bosutinib or dasatinib
E255K/V	Consider dasatinib rather than bosutinib or nilotinib
Any other mutation	Consider dasatinib or nilotinib or bosutinib

Modified from Soverini et al. [71] to include the therapeutic options most recently made available. Ponatinib is also an option whenever no other TKI is indicated. In patients with the T315I mutation with failure on or not eligible for ponatinib, and in patients with multiple TKI failure, hematopoietic stem cell transplant should also be considered.
Abbreviation: TKI, tyrosone kinase inhibitor.

to be done following exactly the same recommendations in terms of source of material, quantity, and delivery requirements as for RQ-PCR samples [72].

The recommended method for *BCR-ABL1* mutation screening is conventional Sanger sequencing [71]. Hematologists and oncologists should base their clinical decisions on conventional sequencing results and treat results coming from other techniques (e.g., allele-specific PCR assays such as allele-specific oligonucleotide or amplification-refractory mutation system PCR) for research use only [71]. Although next-generation sequencing (NGS) has recently been shown to be a promising candidate alternative to conventional sequencing [73–75], international standardization efforts are still at the very beginning, and their progress is hampered by the continuous evolution of chemistries and platforms. In addition, software programs for alignment to mRNA sequences and reliable mutation calling still need to be optimized, and common definitions of quality control metrics and acceptable performances are still in progress.

Table 4 reports updated recommendations on how to interpret *BCR-ABL1* mutation results in light of the recent availability of bosutinib and ponatinib in many countries. For nilotinib and dasatinib, these recommendations come from the integration of in vitro observations (IC_{50}, the intracellular drug concentration needed to inhibit by 50% the growth of a cell line transfected to express a specific mutated *BCR-ABL1* isoform) [71, 76] and in vivo evidence (the *BCR-ABL1* mutations that have been found to be selected in patients who relapsed on dasatinib or nilotinib) [77–83]. For bosutinib, in vitro data are available [84, 85], but clinical data are still scarce and mainly come from company-sponsored phase 2 and 3 trials [86–90].

Optimal Management of CML Patients: Teamwork

Optimal management of CML patients requires peer dialog and synergy between pathologists/molecular biologists and hematologists/oncologists. The former bring a high degree of expertise and have an essential role in communicating results and their implications to the latter.

In follow-up MR reports, the pathologist or molecular biologist should explicitly indicate whether the sample is evaluable for MR and whether the MR level indicates an optimal response, a warning, or a failure according to ELN recommendations or NCCN guidelines. If the sample is inevaluable, the pathologist should comment on whether sample quality or quantity were adequate and recommend timely resampling. If the sample is evaluable, the pathologist or molecular biologist should indicate what level of MR can be defined. In case of inconsistencies with MR results at previous time points, *BCR-ABL1* transcript level fluctuations in the absence of MMR loss, and borderline results, the pathologist or molecular biologist should also recommended resampling and reconfirmation of results before any clinical decision is taken.

The pathologist and molecular biologist should also suggest (or recommend) a mutation analysis in case *BCR-ABL1* transcript levels indicate warning (or failure). If the patient tests positive for a mutation, the pathologist should suggest more thorough monitoring of the patient, who will have a significantly higher probability of developing additional mutations under second- or subsequent-line TKI therapy. If specific mutations (Y253H, E255K/V, V299L, T315I/A, F317L/V/I/C, F359V/I/C) are detected, the pathologist should also indicate which TKI or TKIs are more likely to be effective against that specific mutant (Table 4). The clinician will thus integrate *BCR-ABL1* mutation status with comorbidities and risk factors, bearing in mind that detection of any of the mutations mentioned above is a stronger predictor of TKI inefficacy than comorbidities and risk factors are for the occurrence of adverse events.

Conclusion

Although the advent of TKIs has dramatically improved patient outcomes, CML is not easy to manage. Both the therapeutic arsenal and the technologic solutions for molecular testing are still evolving. The NCCN guidelines for patient treatment are updated on an yearly basis, and the next ELN recommendations are expected by the end of 2016. In such a rapidly changing scenario, education and constant update of clinicians involved in patient management about MR definitions, monitoring schemes, decision algorithms, and technological advances—as well as perfect synergy between hematology/oncology and laboratory professionals—are of utmost importance.

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Disclosures

Simona Soverini: Ariad Pharma, Novartis, Bristol-Myers Squibb (C/A); **Giovanni Martinelli:** Ariad, Amgen, Pfizer (C/A), Novartis, Bristol-Myers Squibb (H). The other authors indicated no financial relationships.
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