


REVIEW

Open Access



Next-generation sequencing for BCR-ABL1 kinase domain mutation testing in patients with chronic myeloid leukemia: a position paper

Simona Soverini^{1*} , Elisabetta Abruzzese², Monica Bocchia³, Massimiliano Bonifacio⁴, Sara Galimberti⁵, Antonella Gozzini⁶, Alessandra Iurlo⁷, Luigiana Luciano⁸, Patrizia Pregno⁹, Gianantonio Rosti¹, Giuseppe Saglio¹⁰, Fabio Stagno¹¹, Mario Tiribelli¹², Paolo Vigneri¹³, Giovanni Barosi¹⁴ and Massimo Breccia¹⁵

Abstract

BCR-ABL1 kinase domain (KD) mutation status is considered to be an important element of clinical decision algorithms for chronic myeloid leukemia (CML) patients who do not achieve an optimal response to tyrosine kinase inhibitors (TKIs). Conventional Sanger sequencing is the method currently recommended to test *BCR-ABL1* KD mutations. However, Sanger sequencing has limited sensitivity and cannot always discriminate between polyclonal and compound mutations. The use of next-generation sequencing (NGS) is increasingly widespread in diagnostic laboratories and represents an attractive alternative. Currently available data on the clinical impact of NGS-based mutational testing in CML patients do not allow recommendations with a high grade of evidence to be prepared. This article reports the results of a group discussion among an ad hoc expert panel with the objective of producing recommendations on the appropriateness of clinical decisions about the indication for NGS, the performance characteristics of NGS platforms, and the therapeutic changes that could be applied based on the use of NGS in CML. Overall, these recommendations might be employed to inform clinicians about the practical use of NGS in CML.

Keywords: Next-generation sequencing, Chronic myeloid leukemia, Sanger sequencing, *BCR-ABL1* mutation

Background

The introduction of three generations of tyrosine kinase inhibitors (first: imatinib; second: dasatinib, nilotinib, and bosutinib; third: ponatinib) has dramatically changed the management and long-term outcome of patients affected by chronic myeloid leukemia (CML). Nevertheless, resistance has been observed [1–4]. In about one-third of patients who experience resistance to first-line therapy, and in up to 50% of patients who experience resistance to second- or subsequent-line therapy, point mutations in the ABL1 kinase domain (KD) that impair

TKI binding can be detected [5]. Mutations may arise at critical contact points between the inhibitor and its target or in key regions of the KD, namely the phosphate-binding loop (P-loop), the catalytic cleft, or the activation loop (A-loop) [5]. One of the most frequent and most challenging mutations is a substitution of threonine with isoleucine at residue 315 (T315I), the so-called “gatekeeper residue” that impairs the binding of imatinib and all second-generation TKIs, and may be overcome only by ponatinib. Detection of a mutation identifies patients at greater risk of subsequent relapse [6]. In the past, mutations (especially the T315I and the P-loop mutations G250E, Q252H, Y253H/F, E255K/V) had also been associated with significantly shorter progression-free and overall survival [7–13]. In more recent times, a negative prognostic impact has not been observed in all studies [14], suggesting that the expanded

* Correspondence: simona.soverini@unibo.it

¹Hematology/Oncology “L. e A. Seràgnoli”, Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy
Full list of author information is available at the end of the article



drug armamentarium and the wider and wider use of mutation testing in routine practice have been beneficial. Indeed, each TKI has its own spectrum of sensitive and insensitive mutations, and the type of mutation detected may, nowadays, drive the choice of TKI to use after failure of first or subsequent lines of treatment (Table 1) [5]. Early identification and quantitative monitoring of mutant *BCR-ABL1* subclones displaying resistance to TKIs are thus important tasks for optimal management of patients with CML.

Conventional Sanger sequencing is the currently recommended method for diagnostic *BCR-ABL1* KD mutation screening [16]. However, Sanger sequencing has limited sensitivity (it cannot robustly identify mutations present in less than 15–20% of transcripts) and, in many cases, cannot provide clear discrimination between polyclonal and compound mutations [17].

Various next-generation sequencing (NGS)-based approaches facilitating sensitive detection and quantitative monitoring of *BCR-ABL1* KD mutations have recently been described. They are able to detect and quantify any sequence variation in *BCR-ABL1* transcripts down to 1% abundance and resolve the clonal architecture in the majority of cases harboring multiple mutations [18–20]. However, the broad clinical implementation of NGS for *BCR-ABL1* KD mutation testing has so far been hampered by a series of issues, including limited availability due to the high capital investment required to acquire an instrument, and the current costs of the tests, which may not be regarded as readily affordable for routine diagnostic monitoring. Additionally, the currently available data on NGS-based *BCR-ABL1* mutational testing in CML patients who experienced resistance to treatment have not yet been used to formulate recommendations on its utilization in clinical practice.

The objective of this project was to produce consensus-based recommendations on the appropriateness of the clinical decisions concerning the indications of NGS in CML, the performance characteristics of NGS platforms, and the therapeutic changes that could be applied based on the results of NGS analysis.

Brief overview of NGS methodologies

The term “NGS” refers to different chemistries and platforms that use different strategies to perform the same series of tasks. Briefly, the pool of DNA molecules to be sequenced (usually termed “library”: may be either fragmented genomic DNA or cDNA, or amplicons) need to be physically isolated in space and clonally amplified by polymerase chain reaction. Next, the instrument performs massively parallel sequencing of each individual molecule, yielding millions of sequence reads in a few hours. Key features of NGS are thus the high throughput and the clonal nature of sequence analysis. The high throughput can be exploited to cover whole genomes, or exomes, or transcriptomes in a single sequencing run, or to focus on a panel of genes or genetic regions of interest, that will be sequenced hundreds or thousands of times, thus achieving high sensitivity. The latter currently represents the main diagnostic application of NGS and is frequently referred to as “deep” or ultra-deep sequencing”. For a more detailed technical overview of NGS methodologies, see Yohe S and Thyagarajan B, 2017 and Muzzey D et al., 2015 [21, 22].

Methods

An Expert Panel (hereafter referred to as the Panel) composed of Italian physicians and biologists was selected for their individual expertise in research and clinical practice in the management of CML and assembled

Table 1 List of *BCR-ABL1* KD mutations poorly sensitive to imatinib, dasatinib, nilotinib, bosutinib, and ponatinib based on the integration of published studies (2001–2018) reporting the mutation status of TKI-resistant patients and experimental data

Mutations poorly sensitive to imatinib	M237V, I242T, M244V , K247R, L248V, G250E , G250R, Q252R, Q252H , Y253F , Y253H , E255K , E255V , E258D, W261L, L273M, E275K, E275Q, D276G , T277A, E279K, V280A, V289I, V289L, V289A, E292Q, E292V, I293V, L298V, F311L , F311I, T315I , F317L , F317V, F317I, F317C, Y320C, L324Q, Y342H, M343T, A344V, A350V, M351T , E355D, E355G , E355A, F359V , F359I, F359C, F359L, D363Y, L364I, A365V, A366G, L370P, V371A, E373K, V379I, A380T, F382L, L384M , L387F , L387V, M388L, H396R , H396P , H396A, A397P, S417F, S417Y, I418S, I418V, A433T, S438C, E450K, E450G, E450A, E450V, E453G, E453A, E453K, E453V, E453Q, E459K , E459V, E459G, E459Q, M472I, P480L, F486S
Mutations poorly sensitive to dasatinib	V299L, T315I, T315A, F317L, F317V, F317I, F317C
Mutations poorly sensitive to nilotinib	Y253H, E255K, E255V, T315I, F359V, F359I, F359C
Mutations poorly sensitive to bosutinib ^a	E255V, E255K, V299L, T315I
Mutations poorly sensitive to ponatinib	T315M, T315L

^aIn contrast to the other second-generation TKIs, there is still limited data available on mutations associated with clinical resistance to bosutinib in vivo. In vitro data suggest that the E255K and, to a lesser extent, the E255V might be poorly sensitive to bosutinib [15]

TKI tyrosine kinase inhibitor

The most frequent imatinib-resistant mutations are highlighted in boldface

in May 2018. During an initial meeting held the following month, the outline of the project was discussed, and the topics that form the structure of the present document were decided. Key questions were selected through a series of questionnaires, and each panelist drafted statements that addressed one or more questions, while the remaining panelists scored their agreement with those statements and provided suggestions for modifications. Finally, the Panel convened for a consensus conference that was held in Milan, Italy, in November 2018. At this conference, final proposals were given using the nominal group technique [23], by which participants were first asked to comment in a round-robin fashion on their disagreements with the proposed issues and then to vote for a final statement.

Results

A summary of the recommended indications for the use of NGS testing in CML proposed by the Panel is presented in Table 2.

Indications for the use of NGS testing in chronic-phase (CP) CML

Approximately one-third of CP CML patients with primary or acquired resistance to first or second-generation TKIs harbor mutations in the *BCR-ABL1* KD [6]. Such mutations are not induced by TKIs. The selective pressure exerted by TKIs selects for mutations that occur by chance at resistance-causing residues at any time during

Table 2 Summary of the indications for the use of next-generation sequencing (NGS) for *BCR-ABL1* KD mutation testing in chronic myeloid leukemia (CML)

Indications for the use of NGS testing in chronic phase CML

- in patients with failure^a response to TKI therapy, irrespective of the TKI
- in patients with warning^a response to TKI therapy, irrespective of the TKI

Indications for the use of NGS testing before allogeneic stem cell transplant (allo-SCT)

- *BCR-ABL1* KD mutation status by NGS testing before allo-SCT may provide useful information regarding when post-transplant TKI therapy should be reinstated. Patients who do not have *BCR-ABL1* KD mutation results by NGS available at the time of transplant should be tested^b

Indications for the use of NGS testing in advanced CML phases

- all patients with advanced phase (AP or BC) either at diagnosis or during therapy

Indications for the use of NGS testing after TKI therapy discontinuation

- in patients relapsing after a TFR attempt if they fail to re-achieve MMR within 3–6 months after TKI re-treatment

^aIt has to be noted that, at present, failure and warning definitions for third-line and beyond are lacking

^bProvided that *BCR-ABL1* transcript levels are sufficient, i.e., > 0.1%^{IS}

AP accelerated phase, BC blast crisis, MMR major molecular response, TFR treatment-free remission, TKI tyrosine kinase inhibitor, IS International Scale

therapy. Mutations represent a hallmark of patients at higher risk of acquiring further mutations and of relapse on second- or subsequent-line of therapy [24–26]. No robust evidence has conclusively shown that mutations may already be detectable at diagnosis in CP patients, even when using highly sensitive methods. A recent study combining high-resolution Duplex Sequencing and computational simulations has indeed suggested that, because of the low number of leukemia-initiating cells, CP CML is very unlikely to harbor resistant mutations at the time of diagnosis and, regardless, these would be well below the detection limit of NGS [27].

The detection of *BCR-ABL1* mutations in TKI-treated patients may represent a biological hallmark of disease progression: cells from CP patients harboring mutations have gene expression profiles superimposable to those from blast crisis (BC) patients [28]. In a study in which 319 CP CML patients were routinely monitored for mutations by Sanger sequencing regardless of response status, the identification of mutations even without evidence of imatinib resistance was found to be highly predictive for loss of complete cytogenetic response (CCyR) and progression to advanced phase [8]. NGS was found to anticipate the detection of emerging resistant mutants from 2 to 11 months earlier than Sanger sequencing and, in some instances, could reveal TKI-resistant mutations at the time of major or deeper molecular responses [29]. In another backtracking study, the highly resistant T315I substitution was found to be detectable on average 3 months earlier than with Sanger sequencing [30]. A more recent study performed in an unselected series of 121 CML patients who were systematically screened using NGS, irrespective of their response to first-line TKI therapy, showed that detection of a mutation by NGS was associated with significantly worse outcomes [31]. Patients who developed a KD mutation had a lower cumulative incidence of CCyR and major molecular response (MMR) compared to patients without mutations. Patients with mutations also had worse 5-year progression-free survival and a higher 5-year cumulative incidence of progression compared to patients without mutations [31].

At present, however, both the European LeukemiaNet (ELN) recommendations and the National Comprehensive Cancer Network (NCCN) guidelines anchor KD mutation testing to the lack of optimal response [16, 32, 33]. A more accurate picture of *BCR-ABL1* mutation status at the time of failure, when therapy has to be changed, may help in selecting the most active TKI. In imatinib-resistant patients who failed second-line dasatinib or nilotinib therapy because of the selection of a *BCR-ABL1* KD mutation, the same mutation could retrospectively be tracked by NGS back to the time of imatinib failure [29, 34, 35]. The advantage of a more sensitive approach at the time of switching to second-line TKI therapy in imatinib-resistant patients had already been shown by Parker et al. using a

mass spectrometry-based approach of mutation analysis [36, 37]. In the case of a clinical response classified as “warning,” more accurate and frequent monitoring is recommended in order to allow a prompt change in therapy [33]. In this clinical setting, emerging resistant clones could be present below the detection limit of Sanger sequencing and might thus not be identified in a timely manner, ultimately leading to treatment failure. Indeed, in another “backtracking” study performed in 51 CP CML patients who had acquired TKI-resistant mutations on second-line therapy, the first detection by NGS occurred at the time of a “warning” response in many cases [38]. Unfortunately, however, there are currently no formal definitions of failure and warning beyond second-line, although an update of the 2013 ELN recommendations is planned. Patients on second-line therapy may harbor multiple mutations. In these cases, NGS can define whether such mutations are compound or polyclonal, guiding proper clinical management - because compound mutants and, in particular, those including the T315I have been predicted to be highly resistant to all second-generation TKIs [39, 40].

Consensus statements

Neither conventional Sanger sequencing nor NGS for *BCR-ABL1* KD mutation testing are indicated in patients with CP CML at diagnosis, before the start of first-line TKI therapy.

BCR-ABL1 KD mutation testing by NGS is indicated in CP CML after first-line TKI therapy in patients with a “warning” response, regardless of the generation of TKI used for first-line therapy. In these patients, NGS may detect emerging resistant mutants earlier than Sanger sequencing and could allow a timely therapeutic switch, when appropriate.

BCR-ABL1 KD mutation testing by NGS is indicated in CP CML after first-line TKI therapy in patients with a “failure” response, independently from the generation of TKI used for first-line therapy. In these patients, NGS could detect a complex pattern of mutations, including low-level and/or compound mutations, and could allow more individualized therapeutic decision-making.

BCR-ABL1 KD mutation testing by NGS is also indicated in case of a “warning” or “failure” response after second-line TKI therapy. In this situation, the identification of mutations not detectable by Sanger sequencing could allow a more appropriate change of therapy.

The panel agreed that, at present, it is not possible to provide recommendations on NGS testing in patients who are receiving third- or later-line TKI therapy due to the lack of definitions of “optimal,” “warning” and “failure” responses in that setting.

Indications for the use of NGS testing before allogeneic stem cell transplant

Allogeneic stem cell transplant (allo-SCT) is still the only available treatment that is considered as a curative option, although the high procedural morbidity and mortality remain a major deterrent. The current indications for allo-SCT in CP CML are as follows: failure of nilotinib or dasatinib in the first line or failure of two TKIs or evidence of T315I mutation [33]. The majority of patients who underwent an allo-SCT in the European Group for Blood and Marrow Transplantation (EBMT) database were treated in the pre-ponatinib era, limiting the possibility of establishing whether specific settings, such as CP patients with T315I mutation or patients resistant to a frontline second-generation TKI, are in fact real candidates for this procedure. Indeed, allo-SCT is recommended for all BC patients and for accelerated phase (AP) patients who do not achieve an optimal response [33, 41, 42].

The detection of low-level mutations after TKI failure may become important in opting for stem cell transplant procedure in the presence of a T315I mutation or when multiple mutations are present. In the latter case, it will be important to distinguish between compound and polyclonal mutations with or without T315I. Relapse after allo-SCT in CML is observed in 20 to 40% of patients. In this setting, a continued regular long-term longitudinal monitoring of quantitative *BCR-ABL1* transcript levels post-transplant is crucial to anticipate the occasional late-relapsing patients. The detection of *BCR-ABL1* transcripts in the first few months after transplant seems not to be associated with a worse long-term outcome. Furthermore, it has been shown that persistence of very low levels of residual disease (*BCR-ABL1* < 0.1%^{IS}) detectable up to 10 years post-transplant has less implication for relapse [43]. It has been suggested that pre-transplant mutation analysis should be considered when selecting a TKI for post-transplant prophylaxis, based on the observation that the majority of resistant mutations are still detectable after transplantation and that patients often relapse with these mutant clones despite receiving TKI therapy [44, 45].

Consensus statements

Although the decision to direct a patient to SCT is based on his/her past clinical history of treatment failure, the Panel recognized that the knowledge of pre-transplant mutation status might be useful should TKI treatment be restarted afterward. Thus, cases who did not undergo NGS testing at the last treatment failure before transplant should be analyzed, provided that *BCR-ABL1* transcript levels make it feasible (e.g., are > 0.1%^{IS}).

In case of disease relapse or failure to achieve an optimal response with the re-administration of TKIs after

allo-SCT, the recommended method of assessing *BCR-ABL1* KD mutation status remains Sanger sequencing because no studies have yet investigated the clinical value of low-level mutations in this setting. However, it can reasonably be assumed that low-level mutations may display the same kinetics of selection under TKI pressure. The Panel agreed that the use of NGS testing for *BCR-ABL1* KD mutations in this setting should be investigated.

Indications for the use of NGS testing in advanced CML phases

Fewer than 5% of patients with CML are diagnosed in advanced disease, as AP or BC. Furthermore, a small proportion of patients (5–6%) diagnosed in CP become resistant and progress to advanced disease during treatment [46, 47]. One of the hallmarks of AP and BC is genetic instability, which fosters the development of additional cytogenetic abnormalities and point mutations, including mutations in the *BCR-ABL1* KD [48–50]. Accordingly, the frequency of *BCR-ABL1* KD mutations has been reported to be much higher in AP/BC patients (70–80%) than in CP patients [51]. Additionally, BC patients frequently harbor multiple mutations whose clonal relationship cannot always be easily established by Sanger sequencing, unless a cumbersome process of cloning and sequencing is undertaken [39]. In BC patients, NGS has shown that different Ph + subpopulations may follow different routes to escape TKI inhibition, so that mutants detectable by Sanger sequencing may coexist with mutants detectable only by NGS [34]. A series of early case reports has also suggested that mutations may be detectable already at the time of diagnosis in patients who present in AP/BC, especially when highly sensitive assays are used [52–54].

Consensus statements

In patients with advanced-stage CML at diagnosis or in patients who progress to AP/BC during therapy, the use of NGS testing for *BCR-ABL1* KD mutations could reveal both single mutations, especially T315I, and low-level compound mutations more frequently than Sanger sequencing. The Panel agreed that searching for *BCR-ABL1* KD mutations by NGS testing is indicated to allow for personalized therapy planning.

Indications for the use of NGS testing after TKI therapy discontinuation

Treatment discontinuation, commonly referred to as “treatment-free remission” (TFR), is an appealing goal of CML therapy. CML patients with a sustained and stable deep molecular response (MR⁴ or greater) for more than 2 years are possible candidates for discontinuation. Since the first prospective TFR trial (the STIM-1 study in 2007), more than 2000 CML patients worldwide have

pursued TKI discontinuation in a clinical trial [55]. Reports from these studies (recently reviewed in [56]) have demonstrated that not all patients eligible for TFR will maintain a deep molecular response once TKI therapy has been discontinued. Invariably, 40–60% of patients will lose their molecular response (defined as the loss of MMR) and will have to resume therapy. The long-term follow-up is consistent across several studies, including patients treated with second-generation TKIs. In the majority of patients, relapses occur rapidly within 6 months after treatment discontinuation [56].

Patients in molecular relapse who promptly restart TKI therapy remain responsive to re-treatment and rapidly regain MMR after 2–3 months, and deeper responses thereafter [57]. So far, only one case has been reported to have developed a mutation at the time of molecular relapse [58].

Consensus statement

While NGS testing for *BCR-ABL1* KD mutations has no additional value in patients losing deep molecular response after TKI discontinuation, it is indicated in patients relapsing after a TFR attempt if they fail to re-achieve MMR within 3–6 months after TKI re-treatment.

Impact of NGS on therapeutic decisions

The recent study by Kizilors et al. in an unselected series of patients systematically analyzed by NGS regardless of their response to TKI therapy showed that patients positive for mutations by NGS have significantly worse outcomes in terms of loss of MMR and CCyR and probability of progression [31]. In that study, a threshold of 3% was established based on a thorough methodological validation, which also led to ISO 15189 accreditation of the assay [31]. In the NGS studies in TKI-resistant patients published so far, no low-level mutation known to be resistant to the TKI the patient was receiving failed to undergo clonal selection [29–31, 34, 35, 38]. Some studies even included a control group of patients who responded to therapy or who relapsed with no evidence of mutation selection by Sanger sequencing [30, 35, 38], and in these groups, no low-level mutation resistant to the TKI in use were detected. In all of these studies, the lower detection limit of NGS was set to 1–3% [29–31, 34, 35, 38, 59–62]. In the prospective multicenter study “NEXT-in-CML,” a lower detection limit of 3% was chosen after a control round aimed at testing inter-laboratory reproducibility, showing that in between 1 and 3% of variant frequency, some false positive and false negative mutation calls may occur [63]. Additional studies will be needed to confirm a threshold showing the relevance and robustness at both the technical and clinical levels. In particular, the relevance of low-level mutations for whom inconclusive or no in vitro or in vivo sensitivity data are available is still unknown, and whether

they can represent at least a marker of greater genetic instability is under investigation.

Consensus statements

In general, the interpretation of the results of *BCR-ABL1* KD mutation testing by NGS and the consequent clinical decisions should involve both biologists and clinicians expert in CML biology and treatment.

The Panel agreed that whether a positive mutation result by NGS testing should lead to an immediate change of treatment depends on the level of non-optimal response to TKIs, the type and level of mutation(s) detected by NGS, and clinical considerations as to the suitability of therapeutic alternatives for each individual patient.

The Panel argued that the greatest utility of *BCR-ABL1* KD mutation testing by NGS is in CP CML patients with a “failure” or “warning” response. In the latter setting, the detection of any mutation known to be associated with resistance to the TKI the patient is receiving at a level > 3% should be strongly considered for a change of therapy; if the mutation level is between 1 and 3%, testing a subsequent sample in 1 month is recommended in order to check for mutation kinetics. An increase in mutation burden should trigger a change of therapy.

Performance characteristics of NGS testing in CML

TKI-resistant mutations have been reported all over the KD [6]. Mutations outside the KD have been investigated and described in only a single study [64], but their clinical relevance, if any, is unknown. There are currently no European Conformity (CE)-marked for in vitro diagnosis (CE-IVD) or Food and Drug Administration (FDA)-approved commercial kits available for NGS-based *BCR-ABL1* KD mutation testing. The so-called “myeloid panels” include the *ABL1* exons coding for the KD, but DNA-based mutation screening would mainly have untranslocated *ABL1* as a substrate, thus dangerously “diluting” mutations down to a level that might be undetectable even by NGS. Some studies have reported the setup and application of in-house-developed protocols for *BCR-ABL1* KD mutations screening implemented on different NGS platforms [29–31, 34, 35, 38, 60–62].

The results of these studies suggest that NGS-based *BCR-ABL1* KD mutation screening is technically feasible in expert laboratories and may provide accurate and reproducible results even down to variant frequencies of 1%. However, inter-lab reproducibility is equally fundamental for diagnostic accuracy and homogeneity of patient management. National or international expert networks exist that have played, and will continue to play, a key role in the standardization and harmonization of diagnostic tools. For example, at the European level, the “EUTOS (European Treatment and Outcome Study) for CML” initiative, that over the past decade has been running regular control

rounds for real-time quantitative polymerase chain reaction (PCR)-based molecular response monitoring, has recently undertaken the first control round of NGS-based *BCR-ABL1* KD mutation screening.

Consensus statements

Any NGS-based *BCR-ABL1* KD screening assay should have three mainstays:

a) RNA from peripheral blood buffy coat as a template. Any in-house or commercial method of RNA extraction may be used provided that it ensures at least one microgram of high-quality RNA;

b) Selective analysis of the *ABL1* KD derived from the fusion *BCR-ABL1* allele. This may be accomplished using two alternative forward primers, either on *BCR* exon 1 (for the e1a2 breakpoint; p190) or on exon 13 (for the b2a2 and b3a2 breakpoints; p210) and a common reverse primer on *ABL1* exon 10. The resulting amplicon may either be used as a template for a nested PCR or may be enzymatically fragmented, provided that the resulting amplicons or fragments are of adequate length, as detailed below;

c) Amplicon/fragment length not shorter than 300 base pairs (bp; excluding adapters and indexes). Accordingly, sequencing chemistry and cycles producing reads shorter than 400 bp are not recommended, since this would be of limited value for the detection of compound mutations. The minimal region to be screened for mutations must include an mRNA sequence (reference: Genbank accession no. NM_005157.5) corresponding to amino acids 235 through 498 of the *ABL1* 1a protein isoform (KD). Mutations outside this region, if detected, should not be reported.

The minimum recommended depth of coverage is 1000×; better if greater. Until commercial kits are available, each individual laboratory will be responsible for the optimization of assay conditions and will have to carefully assess accuracy, precision (repeatability), and analytical sensitivity.

Given the inherent differences between NGS platforms, protocols, and bioinformatics tools, specific recommendations on ranges and thresholds cannot be provided. It is recommended that the performance requirements for each individual assay be established during the validation procedure, and the same procedure is used to monitor the performance of the assay for each run. It is also recommended that each lab engages in regular external quality assurance programs.

Mutations with a variant allele frequency below 1% should not be reported. The exact reportable range, however, should be established and validated by each individual lab. Any variant should be reported, but those for whom experimental or clinical information regarding the sensitivity profile is available should be clearly

distinguished from those with an unknown sensitivity/resistance profile, and the TKIs not likely to be effective against those mutants should be indicated, with references to the existing literature.

Strength of the recommendations on indications of NGS testing

The Panel discussed the issue of how binding clinical centers that do not have access to NGS technology should consider the indications for the use of NGS testing for *BCR-ABL1* KD mutation issued in this document.

The Panel agreed that NGS testing should be encouraged in the proper indications for use in all the clinical centers that care for patients with CML. However, the Panel also argued that in no CML setting (CP, AP, BC) has the lack of access to NGS testing been demonstrated to hamper the possibility of properly managing CML patients. Thus, failure to perform an NGS test for *BCR-ABL1* KD mutations currently does not represent inappropriate clinical management of patients.

The Panel also suggested that NGS testing for *BCR-ABL1* KD mutations should be performed in a limited number of highly specialized and qualified laboratories within regional or national networks, undergoing periodic and regular quality control rounds.

Discussion

BCR-ABL1 KD mutation testing is recommended both by the ELN and by the NCCN in CML patients who do not achieve an optimal response to TKI therapy. While NCCN does not address any methodological aspect, the ELN, in 2011, endorsed Sanger sequencing as the gold standard for *BCR-ABL1* KD mutation screening. The ELN panel acknowledged that the limited sensitivity (15–20%) of Sanger sequencing might be a drawback, but admitted that Sanger sequencing was, at that time, the only method enabling the scanning of the entire KD for the multitude of mutations associated with imatinib and second-generation TKI resistance [16]. At present, however, an increasing number of laboratories are in the process of implementing the NGS technology and integrating NGS results into the diagnostic algorithms of patients with various hematological malignancies. Over the past five years, a series of studies exploring the use of NGS for *BCR-ABL1* mutation testing have assessed its accuracy and reproducibility and have demonstrated that it may provide a more accurate picture of KD mutation status, which may better inform therapeutic decisions [29–31, 34, 35, 38, 60, 62].

In this article, experts in CML judged whether the body of evidence was sufficient to provide recommendations regarding the use of NGS for detecting low-level mutations in the *BCR-ABL1* transcript. Randomized

clinical trials assessing the advantage of a change of therapy based on low-level mutations detectable by NGS (as opposed to mutations detectable by Sanger, or as opposed to no mutation testing at all) are lacking. Thus, the advantage of NGS-based treatment decision-making has not yet been formally demonstrated. This forced the Panel to use the method of consensus to shape the recommendations reported in this manuscript, and which are summarized in Table 2.

After careful evaluation of the literature, the Panel recognized that the body of data available supports the use of NGS in case of warning and failure responses to first- or second-line therapy and in case of disease progression from CP to AP or BC. In patients with warning, early detection of an emerging TKI-resistant mutation may play a key role in identifying those patients who might benefit from a proactive TKI switch rather than from a “wait-and-watch” approach. In patients with failure, it has been shown that some cases may harbor mutations below the lower detection limit of Sanger sequencing, and that, even in Sanger sequencing-positive patients, additional low-level mutations relevant for TKI selection may be detectable by NGS. For patients receiving third-line treatment and beyond, there are currently no formal definitions of failure and warning response, hence no published NGS data. Nevertheless, it can be assumed that NGS may equally be useful in patients who do not improve their responses on third-line therapy or beyond, to prevent them from continuing on an inappropriate therapy. In patients who progress to AP or BC, multiple mutations are often detectable [34]; in this setting, NGS may provide a straightforward way to identify compound mutants.

The Panel discussion also highlighted a series of scenarios where very little data are available regarding the frequency and clinical significance of mutations, either detected with Sanger sequencing or NGS. They include the setting of patients who present in AP or BC, the setting of patients who lose MMR after TKI discontinuation or who fail to regain MMR after therapy has been reintroduced, and the post-SCT setting. Further studies are definitely needed in those specific patient populations.

According to the ELN (and the European Society for Medical Oncology [ESMO]) recommendations, detection of a *BCR-ABL1* KD mutation by Sanger sequencing denotes a failure of TKI therapy and mandates a change of the therapeutic strategy [33, 65]. A variety of studies comparing NGS and Sanger sequencing results have shown that NGS and Sanger results are highly concordant for all variants with a frequency of 15% or higher [29–31, 34, 35, 38, 60, 66–72]. The clinical significance of mutation loads between 1 and 15% has been shown by some retrospective studies and, very recently, by a prospective study in which a consecutive series of

patients were monitored by NGS irrespective of their response to the ongoing TKI therapy [29–31, 34]. Moreover, a recently completed prospective multicenter study (“NEXT-in-CML”) has shown that low-level, known TKI-resistant mutants identified by NGS remain consistently detectable and increase in burden whenever the TKI they are insensitive to is not changed [63]. In both of these prospective studies, mutations between 1 and 3% were excluded because, in that range, some false positive and false negative results were found to occur. Thus, there are two critical issues around the relevance of low-level mutations: on one hand, what is the “technical” threshold above which a true low-level mutation (and not an amplification or sequencing artifact) can be detected; on the other hand, what is the “clinical” threshold above which a low-level mutation should be incorporated into therapeutic decision algorithms. Both are equally important, and both deserve further investigation. Pragmatically, it is unlikely that a unique threshold of significance can be defined and applied to all mutations and to all patients. While the resistance profile of some mutations is well established, for others there are inconclusive *in vitro* sensitivity data, or even no data at all. The latter might be passengers rather than drivers and should not trigger an immediate change of therapy in patients not exhibiting a clear “failure” response. Besides this, in each individual case, NGS results should be contextualized and integrated with disease- and patient-specific characteristics and considerations. In this light, and taking into account the uncertainty due to the inherent error rate of the methodology, the Panel agreed that for mutations detected with a frequency between 1 and 3%, validation and monitoring of mutation kinetics in at least one subsequent sample collected in 1 month should be undertaken before taking any decision. In the future, Digital PCR [18] might become the method of choice for rapid and inexpensive orthogonal validation of clinically relevant low-level mutations detected by NGS. However, the existing commercial kits are not suitable for the detection of mutations in *BCR-ABL1* transcripts.

Finally, the Panel compiled a series of methodological recommendations, defining the key features and minimal performance characteristics for an NGS-based *BCR-ABL1* KD mutation testing assay. This was felt to be important since no commercial assays are yet available. Thus, each laboratory will be responsible for the design, set up and validation of its own, “home brew” protocol as well as for the regular monitoring of assay performance. For the same reason, the Panel underlined the importance that NGS be performed only by highly qualified laboratories, belonging to specialized national or international networks, ensuring continuous scientific update and cooperating in the standardization of protocols and periodical quality control rounds. Designation

of a few expert referral laboratories where samples can be centralized will also be mandatory to ensure reasonable costs and turnaround times.

Conclusions

Different situations in which NGS may be, to a greater degree than Sanger sequencing, useful in the optimization of the therapeutic strategies have been discussed by a Panel of experts, and a consensus opinion has been reached. With this, we did not mean to compete with or substitute for, but rather to lay the foundations for, the international efforts under the auspices of ELN or other expert networks, that can be predicted to take the lead in the worldwide dissemination of clinical and technical recommendations regarding mutation testing in the near future. Of note, the Panel pointed out that even though encouraged, NGS testing cannot yet be considered mandatory in clinical practice, so that lack of access to NGS cannot be claimed to result in inappropriate patient management. However, the indications for the use of NGS described in this position paper can be expected to improve CML diagnostics by encouraging more widespread use of NGS for detecting *BCR-ABL1* KD mutations while preventing unnecessary and/or poor-quality testing. This will also foster the interaction between molecular pathology centers and provide a stepping-stone towards a common NGS language between different platforms and centers.

Abbreviations

allo-SCT: Allogeneic stem cell transplant; AP: Accelerated phase; BC: Blast crisis; CCyR: Complete cytogenetic response; CML: Chronic myeloid leukemia; CP: Chronic phase; EBMT: European Group for Blood and Marrow Transplantation; ELN: European LeukemiaNET; ESMO: European Society for Medical Oncology; EUTOS: European Treatment and Outcome Study; KD: Kinase domain; MMR: Major molecular response; NCCN: National Comprehensive Cancer Network; NGS: Next-generation sequencing; PCR: Polymerase chain reaction; Ph: Philadelphia; TFR: Treatment-free remission; TKI: Tyrosine kinase inhibitor

Acknowledgements

We thank Ray Hill and Nicola Ryan, two independent medical writers, who provided English-language editing and styling support prior to submission, on behalf of Springer Healthcare Communications.

Authors' contributions

SS, EA, MB, MB, SG, AG, AI, LL, PP, GR, GS, FS, MT, PV, GB, and MB contributed to the conception and design of the work, analysis and interpretation of the literature, and manuscript drafting. All authors read and approved the final manuscript.

Funding

Unrestricted funding for this project was provided by Incyte Biosciences. The funding source had no role in identifying statements, abstracting data, synthesizing results, or preparing the manuscript or in the decision to submit the manuscript for publication. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Incyte Biosciences.

Availability of data and materials

The material supporting the conclusion of this review has been included within the article.

Ethics approval and consent to participate

This is not applicable for this review.

Consent for publication

This is not applicable for this review.

Competing interests

SS: honoraria from Incyte Biosciences, Novartis, Bristol-Myers Squibb; MB: honoraria from Novartis, Incyte Biosciences, Pfizer, Celgene; SG: speaker fees from Pfizer, Novartis, Incyte Biosciences; Al: honoraria from Novartis, Pfizer and Incyte Biosciences; GR: honoraria from Novartis, Bristol-Myers Squibb, Pfizer, Incyte Biosciences; FS, honoraria from BMS, Incyte Biosciences, Novartis, Pfizer; MT: honoraria from and speakers' bureau for Novartis, BMS, Pfizer, and Incyte Biosciences; PV: honoraria from Astra Zeneca, Celgene, Incyte Biosciences, Italfarmaco, Novartis, Pfizer, Tesaro, Teva and research funding from Novartis and Pfizer; PP: honoraria from Novartis, Incyte Biosciences, Bristol-Myers Squibb, Pfizer. The remaining authors have no conflicts of interest to disclose.

Author details

¹Hematology/Oncology "L. e A. Seràgnoli", Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy. ²Hematology, S. Eugenio Hospital, ASL Roma2, Rome, Italy. ³Hematology Unit, Azienda Ospedaliera Universitaria Senese, University of Siena, Siena, Italy. ⁴Department of Medicine, Section of Hematology, University of Verona, Verona, Italy. ⁵Department of Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, Italy. ⁶Department of Cellular Therapies and Transfusion Medicine, AOU Careggi, Florence, Italy. ⁷Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy. ⁸Hematology Unit, AUOP Federico II, Naples, Italy. ⁹Hematology Unit, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Turin, Italy. ¹⁰Department of Clinical and Biological Sciences of the University of Turin, Mauriziano Hospital, Turin, Italy. ¹¹Hematology Section and BMT Unit, Rodolico Hospital, AOU Policlinico-V. Emanuele, Catania, Italy. ¹²Division of Hematology and Bone Marrow Transplantation, Department of Medical Area, University of Udine, Udine, Italy. ¹³Department of Clinical and Experimental Medicine and Center of Experimental Oncology and Hematology, A.O.U. Policlinico-Vittorio Emanuele, Catania, Italy. ¹⁴Center for the Study of Myelofibrosis, IRCCS Policlinico S. Matteo Foundation, Pavia, Italy. ¹⁵Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy.

Received: 13 August 2019 Accepted: 27 October 2019

Published online: 05 December 2019

References

- Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med*. 2017;376(10):917–27.
- Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. *Leukemia*. 2016;30(5):1044–54.
- Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boque C, et al. Final 5-year study results of DASISION: the Dasatinib versus Imatinib study in treatment-naïve chronic myeloid leukemia patients trial. *J Clin Oncol*. 2016;34(20):2333–40.
- Talati C, Pinilla-Ibarz J. Resistance in chronic myeloid leukemia: definitions and novel therapeutic agents. *Curr Opin Hematol*. 2018;25(2):154–61.
- Soverini S, Mancini M, Bavaro L, Cavo M, Martinelli G. Chronic myeloid leukemia: the paradigm of targeting oncogenic tyrosine kinase signaling and counteracting resistance for successful cancer therapy. *Mol Cancer*. 2018;17(1):49.
- Soverini S, Branford S, Nicolini FE, Talpaz M, Deininger MW, Martinelli G, et al. Implications of BCR-ABL1 kinase domain-mediated resistance in chronic myeloid leukemia. *Leuk Res*. 2014;38(1):10–20.
- Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood*. 2003;102(1):276–83.
- Khorashad JS, de Lavallade H, Apperley JF, Milojkovic D, Reid AG, Bua M, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. *J Clin Oncol*. 2008;26(29):4806–13.
- Nicolini FE, Corm S, Le QH, Sorel N, Hayette S, Bories D, et al. Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukemia patients: a retrospective analysis from the French intergroup of CML (Fi(phi)-LMC GROUP). *Leukemia*. 2006;20(6):1061–6.
- Nicolini FE, Ibrahim AR, Soverini S, Martinelli G, Muller MC, Hochhaus A, et al. The BCR-ABL1 T315I mutation compromises survival in chronic phase chronic myelogenous leukemia patients resistant to tyrosine kinase inhibitors, in a matched pair analysis. *Haematologica*. 2013;98(10):1510–6.
- Sharma P, Mohanty S, Kochupillai V, Kumar L. Mutations in ABL kinase domain are associated with inferior progression-free survival. *Leuk Lymphoma*. 2010;51(6):1072–8.
- Soverini S, Martinelli G, Rosti G, Bassi S, Amabile M, Poerio A, et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. *J Clin Oncol*. 2005; 23(18):4100–9.
- Xue M, Cheng J, Zhao J, Zhang S, Jian J, Qiao Y, et al. Outcomes of 219 chronic myeloid leukaemia patients with additional chromosomal abnormalities and/or tyrosine kinase domain mutations. *Int J Lab Hematol*. 2019;41(1):94–101.
- Etienne G, Dulucq S, Hugué F, Schmitt A, Lascaux A, Hayette S, et al. Incidence and outcome of BCR-ABL mutated chronic myeloid leukemia patients who failed to tyrosine kinase inhibitors. *Cancer Med*. 2019;8(11): 5173–82.
- Redaelli S, Piazza R, Rostagno R, Magistroni V, Perini P, Marega M, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol*. 2009;27(3):469–71.
- Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood*. 2011;118(5):1208–15.
- Alikian M, Gerrard G, Subramanian PG, Mudge K, Foskett P, Khorashad JS, et al. BCR-ABL1 kinase domain mutations: methodology and clinical evaluation. *Am J Hematol*. 2012;87(3):298–304.
- Alikian M, Gale RP, Apperley JF, Foroni L. Molecular techniques for the personalised management of patients with chronic myeloid leukaemia. *Biomol Detect Quantif*. 2017;1:4–20.
- Bacher U, Shumilov E, Flach J, Porret N, Joncourt R, Wiedemann G, et al. Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use. *Blood Cancer J*. 2018;8(11):113.
- Kohlmann A, Grossmann V, Nadarajah N, Haferlach T. Next-generation sequencing - feasibility and practicality in haematology. *Br J Haematol*. 2013;160(6):736–53.
- Yohe S, Thyagarajan B. Review of clinical next-generation sequencing. *Arch Pathol Lab Med*. 2017;141(11):1544–57.
- Muzzey D, Evans EA, Lieber C. Understanding the basics of NGS: from mechanism to variant calling. *Curr Genet Med Rep*. 2015;3(4):158–65.
- Williams PL, Webb C. The Delphi technique: a methodological discussion. *J Adv Nurs*. 1994;19(1):180–6.
- Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Muller MC, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. *J Clin Oncol*. 2009;27(25):4204–10.
- Muller MC, Cortes JE, Kim DW, Druker BJ, Erben P, Pasquini R, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to preexisting BCR-ABL mutations. *Blood*. 2009; 114(24):4944–53.
- Soverini S, Gnani A, Colarossi S, Castagnetti F, Abruzzese E, Paolini S, et al. Philadelphia-positive patients who already harbor imatinib-resistant Bcr-Abl kinase domain mutations have a higher likelihood of developing additional mutations associated with resistance to second- or third-line tyrosine kinase inhibitors. *Blood*. 2009;114(10):2168–71.
- Schmitt MW, Pritchard JR, Leighow SM, Aminov BI, Beppu L, Kim DS, et al. Single-molecule sequencing reveals patterns of preexisting drug resistance

- that suggest treatment strategies in Philadelphia-positive leukemias. *Clin Cancer Res.* 2018;24(21):5321–34.
28. Radich JP, Dai H, Mao M, Oehler V, Schelter J, Druker B, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. *Proc Natl Acad Sci U S A.* 2006;103(8):2794–9.
 29. Machova Polakova K, Kulvait V, Benesova A, Linhartova J, Klamova H, Jaruskova M, et al. Next-generation deep sequencing improves detection of BCR-ABL1 kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. *J Cancer Res Clin Oncol.* 2015;141(5):887–99.
 30. Baer C, Kern W, Koch S, Nadarajah N, Schindela S, Meggendorfer M, et al. Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia. *Haematologica.* 2016;101(7):830–8.
 31. Kizilors A, Crisa E, Lea N, Passera R, Mian S, Anwar J, et al. Effect of low-level BCR-ABL1 kinase domain mutations identified by next-generation sequencing in patients with chronic myeloid leukaemia: a population-based study. *Lancet Haematol.* 2019;6(5):e276–e84.
 32. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: chronic myeloid leukemia version 1.2019. Accessed 17 October 2018. Available from: www.nccn.org/professionals/physician_gls/pdf/cml.pdf. 2018.
 33. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukaemia: 2013. *Blood.* 2013;122(6):872–84.
 34. Soverini S, De Benedittis C, Machova Polakova K, Brouckova A, Horner D, Iacono M, et al. Unraveling the complexity of tyrosine kinase inhibitor-resistant populations by ultra-deep sequencing of the BCR-ABL kinase domain. *Blood.* 2013;122(9):1634–48.
 35. Soverini S, De Benedittis C, Polakova KM, Linhartova J, Castagnetti F, Gugliotta G, et al. Next-generation sequencing for sensitive detection of BCR-ABL1 mutations relevant to tyrosine kinase inhibitor choice in Imatinib-resistant patients. *Oncotarget.* 2016;7(16):21982–90.
 36. Parker WT, Ho M, Scott HS, Hughes TP, Branford S. Poor response to second-line kinase inhibitors in chronic myeloid leukemia patients with multiple low-level mutations, irrespective of their resistance profile. *Blood.* 2012;119(10):2234–8.
 37. Parker WT, Lawrence RM, Ho M, Irwin DL, Scott HS, Hughes TP, et al. Sensitive detection of BCR-ABL1 mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. *J Clin Oncol.* 2011;29(32):4250–9.
 38. Soverini S, De Benedittis C, Castagnetti F, Gugliotta G, Mancini M, Bavaro L, et al. In chronic myeloid leukemia patients on second-line tyrosine kinase inhibitor therapy, deep sequencing of BCR-ABL1 at the time of warning may allow sensitive detection of emerging drug-resistant mutants. *BMC Cancer.* 2016;16:572.
 39. Khorashad JS, Kelley TW, Szankasi P, Mason CC, Soverini S, Adrian LT, et al. BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. *Blood.* 2013;121(3):489–98.
 40. Zabriskie MS, Eide CA, Tantravahi SK, Vellore NA, Estrada J, Nicolini FE, et al. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia. *Cancer Cell.* 2014;26(3):428–42.
 41. Barrett J. Allogeneic stem cell transplantation for chronic myeloid leukemia. *Semin Hematol.* 2003;40(1):59–71.
 42. Nicolini FE, Basak GW, Kim DW, Olavarria E, Pinilla-Ibarz J, Apperley JF, et al. Overall survival with Ponatinib versus allogeneic stem cell transplantation in Philadelphia chromosome-positive leukemias with the T315I mutation. *Cancer.* 2017;123(15):2875–80.
 43. Craddock CF. We do still transplant CML, don't we? *Hematology Am Soc Hematol Educ Program.* 2018;2018(1):177–84.
 44. Jabbour E, Cortes J, Santos FP, Jones D, O'Brien S, Rondon G, et al. Results of allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia patients who failed tyrosine kinase inhibitors after developing BCR-ABL1 kinase domain mutations. *Blood.* 2011;117(13):3641–7.
 45. Egan DN, Beppu L, Radich JP. Patients with Philadelphia-positive leukemia with BCR-ABL kinase mutations before allogeneic transplantation predominantly relapse with the same mutation. *Biol Blood Marrow Transplant.* 2015;21(1):184–9.
 46. Hehlmann R, Saussele S, Voskanyan A, Silver RT. Management of CML-blast crisis. *Best Pract Res Clin Haematol.* 2016;29(3):295–307.
 47. Saussele S, Silver RT. Management of chronic myeloid leukemia in blast crisis. *Ann Hematol.* 2015;94(Suppl 2):S159–65.
 48. Branford S, Wang P, Yeung DT, Thomson D, Purins A, Wadham C, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. *Blood.* 2018;132(9):948–61.
 49. Chen Z, Shao C, Wang W, Zuo Z, Mou X, Hu SJ, et al. Cytogenetic landscape and impact in blast phase of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. *Leukemia.* 2017;31(3):585–92.
 50. Soverini S, de Benedittis C, Mancini M, Martinelli G. Mutations in the BCR-ABL1 kinase domain and elsewhere in chronic myeloid leukemia. *Clin Lymphoma Myeloma Leuk.* 2015;15(Suppl):S120–8.
 51. Soverini S, Colarossi S, Gnani A, Rosti G, Castagnetti F, Paoletti A, et al. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin Cancer Res.* 2006;12(24):7374–9.
 52. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell.* 2002;2(2):117–25.
 53. Roche-Lestienne C, Soenen-Cornu V, Gardel-Duflos N, Lai JL, Philippe N, Facon T, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood.* 2002;100(3):1014–8.
 54. Willis SG, Lange T, Demehri S, Otto S, Crossman L, Niederwieser D, et al. High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naïve patients: correlation with clonal cytogenetic evolution but not response to therapy. *Blood.* 2005;106(6):2128–37.
 55. Saussele S, Richter J, Hochhaus A, Mahon FX. The concept of treatment-free remission in chronic myeloid leukemia. *Leukemia.* 2016;30(8):1638–47.
 56. Breccia M, Foa R. Current information and recommendations on the discontinuation of TKI inhibitors in chronic myeloid leukemia. *Curr Oncol Rep.* 2018;20(3):23.
 57. Rea D, Mahon FX. How I manage relapse of chronic myeloid leukaemia after stopping tyrosine kinase inhibitor therapy. *Br J Haematol.* 2018;180(1):24–32.
 58. Hochhaus A, Masszi T, Giles FJ, Radich JP, Ross DM, Gomez Casares MT, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. *Leukemia.* 2017;31(7):1525–31.
 59. Deininger MW, Hodgson JG, Shah NP, Cortes JE, Kim DW, Nicolini FE, et al. Compound mutations in BCR-ABL1 are not major drivers of primary or secondary resistance to ponatinib in CP-CML patients. *Blood.* 2016;127(6):703–12.
 60. Erbilgin Y, Eskazan AE, Hatirnaz Ng O, Salihoglu A, Elverdi T, Firtina S, et al. Deep sequencing of BCR-ABL1 kinase domain mutations in chronic myeloid leukemia patients with resistance to tyrosine kinase inhibitors. *Leuk Lymphoma.* 2019;60(1):200–7.
 61. Preuner S, Barna A, Frommlet F, Czurda S, Konstantin B, Alikian M, et al. Quantitative analysis of mutant subclones in chronic myeloid leukemia: comparison of different methodological approaches. *Int J Mol Sci.* 2016;17(5).
 62. Szankasi P, Schumacher JA, Kelley TW. Detection of BCR-ABL1 mutations that confer tyrosine kinase inhibitor resistance using massively parallel, next generation sequencing. *Ann Hematol.* 2016;95(2):201–10.
 63. Soverini S, Bavaro L, De Benedittis C, Martelli M, Iurlo A, Orofino N, et al. Prospective assessment of NGS-detectable mutations in CML patients with non-optimal response: the NEXT-in-CML study. *Blood.* In press.
 64. Sherbenou DW, Hantschel O, Kaube I, Willis S, Bumm T, Turaga LP, et al. BCR-ABL SH3-SH2 domain mutations in chronic myeloid leukemia patients on imatinib. *Blood.* 2010;116(17):3278–85.
 65. Hochhaus A, Saussele S, Rosti G, Mahon FX, Janssen J, Hjorth-Hansen H, et al. Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29(Supplement_4):iv261.
 66. Arsenic R, Treue D, Lehmann A, Hummel M, Dietel M, Denkert C, et al. Comparison of targeted next-generation sequencing and Sanger sequencing for the detection of PIK3CA mutations in breast cancer. *BMC Clin Pathol.* 2015;15:20.
 67. Baudhuin LM, Lagerstedt SA, Klee EW, Fadra N, Oglesbee D, Ferber MJ. Confirming variants in next-generation sequencing panel testing by Sanger sequencing. *J Mol Diagn.* 2015;17(4):456–61.

68. Hung SS, Meissner B, Chavez EA, Ben-Neriah S, Ennishi D, Jones MR, et al. Assessment of capture and amplicon-based approaches for the development of a targeted next-generation sequencing pipeline to personalize lymphoma management. *J Mol Diagn*. 2018;20(2):203–14.
69. Kohlmann A, Klein HU, Weissmann S, Bresolin S, Chaplin T, Cuppens H, et al. The Interlaboratory ROBustness of Next-generation sequencing (IRON) study: a deep sequencing investigation of TET2, CBL and KRAS mutations by an international consortium involving 10 laboratories. *Leukemia*. 2011; 25(12):1840–8.
70. Misyura M, Zhang T, Sukhai MA, Thomas M, Garg S, Kamel-Reid S, et al. Comparison of next-generation sequencing panels and platforms for detection and verification of somatic tumor variants for clinical diagnostics. *J Mol Diagn*. 2016;18(6):842–50.
71. Simen BB, Yin L, Goswami CP, Davis KO, Bajaj R, Gong JZ, et al. Validation of a next-generation-sequencing cancer panel for use in the clinical laboratory. *Arch Pathol Lab Med*. 2015;139(4):508–17.
72. Sutton LA, Ljungstrom V, Mansouri L, Young E, Cortese D, Navrkalova V, et al. Targeted next-generation sequencing in chronic lymphocytic leukemia: a high-throughput yet tailored approach will facilitate implementation in a clinical setting. *Haematologica*. 2015;100(3):370–6.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

