Measurable residual disease in chronic myeloid leukemia

Susan Branford[1,2,3] and Jane F. Apperley[4,5]

[1]Department of Genetics and Molecular Pathology, Centre for Cancer Biology, SA Pathology, Adelaide, Australia; [2]School of Medicine, University of Adelaide, Adelaide, Australia; [3]Clinical and Health Sciences, University of South Australia, Adelaide, Australia; [4]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK and [5]Centre for Haematology, Department of Immunology and Inflammation, Faculty of Medicine, Imperial College London, London, UK

Correspondence: S. Branford
susan.branford@sa.gov.au

Received: May 30, 2022.
Accepted: September 9, 2022.

https://doi.org/10.3324/haematol.2022.281493

Abstract

Chronic myeloid leukemia is characterized by a single genetic abnormality resulting in a fusion gene whose mRNA product is easily detected and quantified by reverse-transcriptase polymerase chain reaction analysis. Measuring residual disease was originally introduced to identify patients relapsing after allogeneic stem cell transplantation but rapidly adopted to quantify responses to tyrosine kinase inhibitors. Real-time quantitative polymerase chain reaction is now an essential tool for the management of patients and is used to influence treatment decisions. In this review we track this development including the international collaboration to standardize results, discuss the integration of molecular monitoring with other factors that affect patients' management, and describe emerging technology. Four case histories describe varying scenarios in which the accurate measurement of residual disease identified patients at risk of disease progression and allowed appropriate investigations and timely clinical intervention.

Introduction

Monitoring residual disease has been integral to the management of chronic myeloid leukemia (CML) for more than 30 years, and has paved the way for the introduction of similar methodology for assessing measurable residual disease (MRD) in other malignancies.

Patients with CML have an ideal marker to directly measure therapy response: the *BCR::ABL1* fusion oncogene. *BCR::ABL1* is the product of the t(9:22) chromosomal translocation, which in >95% of patients can be visualized in karyotyping as a shortened chromosome 22, termed the Philadelphia chromosome. The genetic breakpoints occur in well-defined regions leading to common RNA transcript types, termed e13a2 and e14a2, in approximately 98% of patients (Figure 1).[1] These transcripts only differ by 75 base pairs and can be measured in a single assay.

Identifying residual leukemic cells, by cytogenetic and later, molecular technology, was originally employed to recognize disease recurrence after allogeneic stem cell transplantation (SCT).[2-5] The ability to identify early relapse became particularly important after the observation that the infusion of additional donor lymphocytes was capable of restoring durable remissions.[6] Further work confirmed that donor lymphocyte infusions were more likely to be effective if delivered at the point of low disease burden, and necessitated the development of more sensitive methodology to identify and later quantify residual or emerging leukemia.[7]

As a consequence, reverse transcriptase polymerase chain reaction (RT-PCR) became the molecular monitoring workhorse and over time, the methods advanced from qualitative to quantitative *BCR::ABL1* detection when it became apparent that a positive signal after allogeneic SCT had limited predictive value for relapse.[8] Serial analysis of quantitative *BCR::ABL1* mRNA levels provided more information and identified patients at risk of relapse to allow timely therapeutic intervention.[9] This early work in the 1990s heralded the era of quantitative measurement of *BCR::ABL1* transcripts, which became highly relevant after the introduction of tyrosine kinase inhibitor (TKI) therapy when most patients rapidly achieved *BCR::ABL1* levels that could only be measured using sensitive molecular analysis. Techniques advanced from competitive PCR to real-time quantitative PCR (RT-qPCR) and, more recently, to digital PCR.[10-16] Recent reviews have comprehensively discussed the standardization of PCR methods,[17,18] and future molecular technology for monitoring patients with CML.[19]

Molecular monitoring of CML is now well established, widely used, and is the recommended monitoring strategy in international guidelines.[20,21] Evidence-based, milestone-driven molecular results define levels of response, guide therapeutic decisions and direct *BCR::ABL1* kinase domain

mutation analysis to assess for drug resistance. The current recommendations from the European LeukemiaNet (ELN)[20] and the National Comprehensive Cancer Network (NCCN)[21] are summarized in Tables 1 and 2. These have evolved over time but maintain a focus on early molecular response in the first 3-12 months of therapy. The initial degree of *BCR::ABL1* reduction is a powerful predictor of response.[22-29] Patients who achieve a major molecular response (MMR, *BCR::ABL1* ratio ≤0.1% on an international scale [IS]) are highly unlikely to experience disease progression. Deep molecular responses (DMR, *BCR::ABL1* ratio ≤0.01% IS), sustained for 1-2 years, are a prerequisite to trial treatment discontinuation and possible treatment-free remission (TFR).

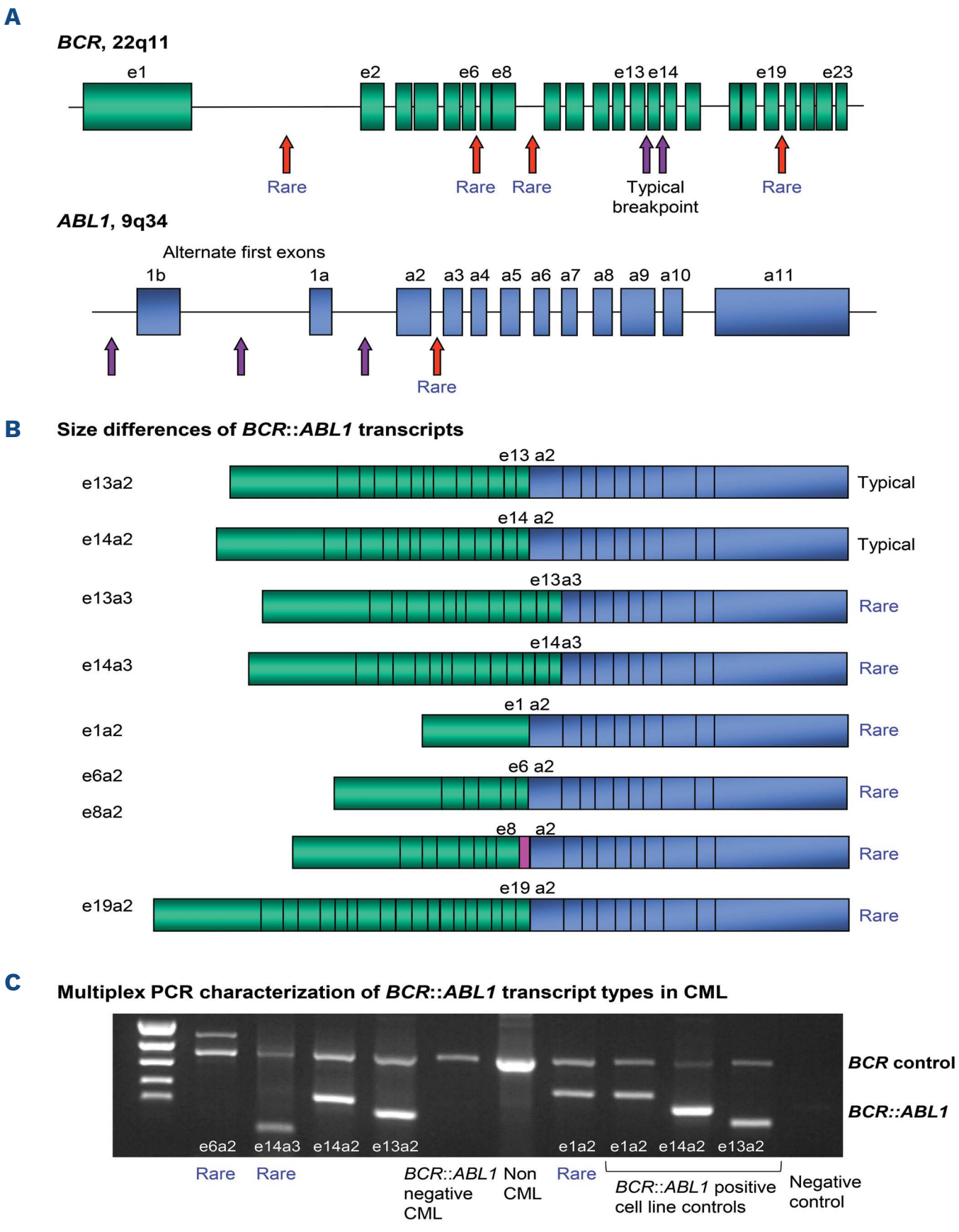


Figure 1. Schematic of *BCR::ABL1* transcripts. (A) *BCR* and *ABL1* genes showing the general location of breakpoints. The red arrows are breakpoint regions that generate rare *BCR::ABL1* transcripts. (B) Size differences of the typical transcripts and some of the rare transcripts. Note that the direct fusion of *BCR* exon 8 and *ABL1* exon 2 does not generate an in-frame protein. The e8a2 *BCR::ABL1* transcript requires an inserted sequence or a genomic break within an exon to generate a constitutively activated protein. (C) Characterization of the *BCR::ABL1* transcript type is essential at the time of diagnosis. Multiplex reverse transcriptase polymerase chain reaction techniques can simultaneously detect various transcript types. Gel image courtesy of Professor Andreas Hochhaus, Universitätsklinikum Jena, Germany. PCR: polymerase chain reaction; CML: chronic myeloid leukemia.

Table 1. European LeukemiaNet[20] and National Comprehensive Cancer Network[21] treatment response milestones for chronic myeloid leukemia expressed as *BCR::ABL1* on the International Scale. Bold text indicates where the recommendations differ.

Milestones	ELN 2020 Optimal	NCCN V3.2022 TKI-sensitive disease	ELN 2020 Warning	NCCN V3.2022 Possible TKI resistance	ELN 2020 Failure	NCCN V3.2022 TKI-resistant disease
Baseline	NA	NA	**High-risk ACA, High-risk ELTS score**	NA	NA	NA
3 months	≤10%	≤10%	>10%	>10%	**>10% if confirmed within 1-3 months**	NA
6 months	**≤1%**	≤10%	**>1-10%**	NA	>10%	>10%
12 months	**≤0.1%**	*≤0.1% or >0.1-1%	**>0.1-1%**	>1-10%	>1%	>10%
Any time	≤0.1% or ≤0.01% for patients with the aim to achieve TFR		>0.1-1%, loss of MMR indicates failure after TFR		>1%, resistance mutations and high-risk ACA	

NA: not applicable; ACA: additional chromosome abnormalities in Philadelphia-positive cells; ELTS: EUTOS long-term survival; MMR: major molecular response (≤0.1%); ELN: European LeukemiaNet; NCCN: National Comprehensive Cancer Network; TKI: tyrosine kinase inhibitor; TFR: treatment-free remission. *NCCN guidelines: ≤0.1% is optimal if the treatment goal is treatment-free remission and ≤1% is optimal if the treatment goal is long-term survival.

Despite the widespread use and clinical applicability of monitoring *BCR::ABL1* ratios, the molecular methodology is not perfect. It is not always easy to maintain consistency in the results and clinicians should be aware of the pitfalls. As a consequence, it is important to consider trends in *BCR::ABL1* ratios, and avoid making management decisions on the basis of a single result. The use of an internal control gene is essential to maintain reliability and reproducibility as it determines the quality of individual RNA samples and compensates for differences in the *BCR::ABL1* transcript level due to sample degradation.[30] Appropriate control genes are *ABL1*, *GUSB* and *BCR* and molecular values are reported as the percentage ratio of *BCR::ABL1* transcripts to the control gene transcripts on the IS. *ABL1* is the most widely used control gene. The effective measurement range on the IS is for *BCR::ABL1* ratios of ≤10% due to potential methodological inaccuracies at higher levels related to the control genes.[31,32] In the laboratory, vigilance is required to monitor and detect any shift in the ratios that might occur with a myriad of factors, such as something as simple as a new lot of reagents. Enrolment in quality assurance programs and regular use of quality control material to identify and mitigate these trends are essential. A change of methodology may require re-calculation of the IS conversion factor. Unfortunately, despite being recommended from the early days of the international effort to harmonize methods, it is often unclear how rigorously these quality controls are used in daily practice.[30,31]

Molecular monitoring, ongoing for very many years will be essential in the management of most patients. In most patients the *BCR::ABL1* decline is rapid upon initiation of TKI treatment but the trend and dynamics of *BCR::ABL1* ratios over time provide information to guide clinical decisions.[33,34] The dynamics of an initial *BCR::ABL1* decline can be measured as the *BCR::ABL1* halving time. A number of studies using various control genes to measure *BCR::ABL1* (*ABL1*, *GUSB* or *BCR*) have reported an association between the halving time and molecular response.[32,34-37] Similarly, *BCR::ABL1* doubling times provide prognostic information for the disease phase at loss of response, using *BCR* or *ABL1* control genes.[35,38] At this stage, *BCR::ABL1* halving and doubling times are non-standardized metrics and as such, are not included in guidelines for routine monitoring of CML.

In this review we present examples of long-term molecular results, their clinical interpretation and guidance on therapeutic decisions for individual patients diagnosed in chronic phase (CP). Our aim is to provide advice that may ultimately enhance patients' management and outcome.

Transcript types: relevance for molecular monitoring and treatment outcome

By 2013, there was a substantial body of evidence demonstrating the importance of standardized molecular monitoring for the prediction of response for TKI-treated patients.[22-29] At this point the ELN recommended that *BCR::ABL1* ratios at specific timepoints should be used to guide therapy decisions.[39] This was a decade after the association between achievement of MMR and a reduced risk of progression had been reported.[40] All of this work was performed for patients with the most frequent *BCR::ABL1* transcript types, e14a2 and e13a2. Note that e14a2 is the primary transcript for patients who co-ex-

press e13a2 and e14a2. In these patients e13a2 is expressed due to alternative splicing.[41] The transcript type must be characterized at diagnosis to ensure an appropriate method is used to monitor the remaining 2% of patients with atypical *BCR::ABL1* transcripts. Standardized molecular methods for *BCR::ABL1* monitoring are not designed for patients with atypical transcripts and if used will generate false negative results.[20,42]

Different proteins are translated from each of the *BCR::ABL1* transcript types that can theoretically influence the biological properties of the disease and potentially affect response to therapy. In the pre-TKI era some studies reported an inferior outcome for patients with the e14a2 transcript, including shorter duration of CP and shorter time to the onset of blast phase (BP)[43,44] but these findings were not always corroborated.[45-48] Fast forward to the TKI era and several studies have assessed the influence of transcript type on outcome.[49-54] Although the findings were occasionally contradictory[55,56] the body of evidence suggested that patients with the e13a2 transcript reached

Table 2. European LeukemiaNet[20] and National Comprehensive Cancer Network[21] treatment response and testing recommendations.

ELN	NCCN
Optimal Current treatment should be continued For patients considering a TFR trial, the optimal response is *BCR::ABL1* ≤0.01% (MR4)	**TKI-sensitive disease** Continue same TKI Monitor response and side effects If *BCR::ABL1* is not ≤0.1% in patients for whom the treatment goal is TFR, shared decision-making with the patient is recommended
Warning Current treatment should be carefully considered for continuation or change, depending on patients´ characteristics, comorbidities and tolerance	**Possible TKI resistance** Evaluate the patient's compliance and drug interactions Consider *BCR::ABL1* kinase domain mutation analysis Consider bone marrow cytogenetic analysis to assess for a major cytogenetic response (<35% Ph-positive) at 3 months or a complete cytogenetic response (Ph-negative) at 12 months Switch to alternate TKI, or Continue same TKI (other than imatinib), or Increase imatinib dose to a maximum of 800 mg, and Consider evaluation for allogeneic hematopoietic cell transplant
Failure Change of therapy is mandatory *BCR::ABL1* kinase domain mutation analysis is mandatory Cytogenetic analysis to assess for clonal evolution	**TKI-resistant disease** Evaluate the patient's compliance and drug interactions Consider *BCR::ABL1* kinase domain mutation analysis Switch to alternative TKI and evaluate for allogeneic hematopoietic cell transplant
Other recommendations Additional molecular testing may be indicated if the kinetics of the response are not clear, or if toxicity or intolerance causes dose interruptions or reductions A change of treatment may be considered if MMR is not reached by 36-48 months Failure to respond may be related to poor or intermittent compliance with treatment, and patients should be questioned closely about their adherence In the future, analyzing the genome and expression profiles of resistant CML cells may lead to identifying somatic mutations as early signs of progression and to a genomically based risk classification with the potential for non-*BCR::ABL1* targeted therapy for resistant patients	**Other recommendations** Patients with levels only slightly >10% at 3 months and/or a steep decline from baseline may achieve <10% at 6 months and have generally favorable outcomes. Therefore, it is imperative to interpret the value at 3 months in this context before making drastic changes to the treatment strategy Consider *BCR::ABL1* mutation analysis for: Loss of hematologic response Loss of a complete cytogenetic response or its molecular equivalent (*BCR::ABL1* >1% IS) 1-log increase in *BCR::ABL1* transcript levels and loss of a major molecular response (≤0.1%) Disease progression to accelerated or blast phase Consider myeloid mutation panel for patients with accelerated phase or blast phase to identify *BCR::ABL1*-independent resistance in patients with no *BCR::ABL1* kinase domain mutations

ELN: European LeukemiaNet; NCCN: National Comprehensive Cancer Network; TFR: treatment-free remission; TKI: tyrosine kinase inhibitor; Ph: Philadelphia chromosome; IS: International Scale.

milestones, such as complete cytogenetic response (CCyR), MMR and DMR, more slowly than those expressing e14a2. Two studies found a difference in overall survival, but one favored the e14a2 transcript[50] and the other the e13a2 transcript.[54] Overall, the data do not currently support a specific upfront therapy recommendation based on transcript type.[20,21]

It is also possible that methodological differences could be responsible for some of the associations observed between the *BCR::ABL1* transcript type and molecular responses.[57-60] Most real-time PCR methods amplify the e14a2 and the shorter e13a2 transcript in a single reaction using a calibration standard that contains the e14a2 fusion junction. Theoretically, the efficiency of PCR amplification could be enhanced for the shorter e13a2 transcript and generate artificially higher *BCR::ABL1* values. A bias in the reported *BCR::ABL1* values has indeed been demonstrated between the transcript types for a number of methods.[57,59,60] The degree of bias varied and may or may not alter the interpretation of the reported value and influence treatment decisions.[57,60]

Technical differences in the efficiency of PCR amplification do not easily explain recent observations that the *BCR::ABL1* transcript type might influence the achievement of TFR. The molecular relapse rate after stopping therapy was twice as high for patients with the e13a2 transcript compared with e14a2 in the Destiny study of TKI de-escalation prior to treatment cessation.[61] Similar results from smaller studies were reported in patients who stopped TKI therapy after achieving sustained DMR.[62-64]

Role of digital polymerase chain reaction in the assessment of rare transcripts and prediction of successful treatment-free remission

Digital PCR provides absolute quantification and should not be subject to bias associated with differences in amplification efficiency (Figure 2). These methods allow replicate analysis to improve the detection of rare transcripts. The sample is divided into thousands of individual replicate PCR using nanofluidic technology and the reagents and workflows are similar to those of real-time PCR using hydrolysis probes. Digital droplet PCR (ddPCR) uses a water-oil emulsion droplet system and the sample is partitioned into droplets. PCR amplification occurs in each of the droplets and at the end of the reaction each droplet is assessed to establish the fraction of positive droplets. The high number of replicates enhances the precision of target detection.

Digital PCR has demonstrated higher sensitivity for the detection of *BCR::ABL1* transcripts[10] and has been used to detect residual disease at the time of stopping TKI in patients who attempted a trial of TFR.[11,12,14] More sensitive detection of *BCR::ABL1* was associated with a higher rate of molecular relapse after TKI discontinuation. In two studies a cut-off *BCR::ABL1* positivity value was established in order to predict relapse.[12,14] Nicolini and colleagues in their

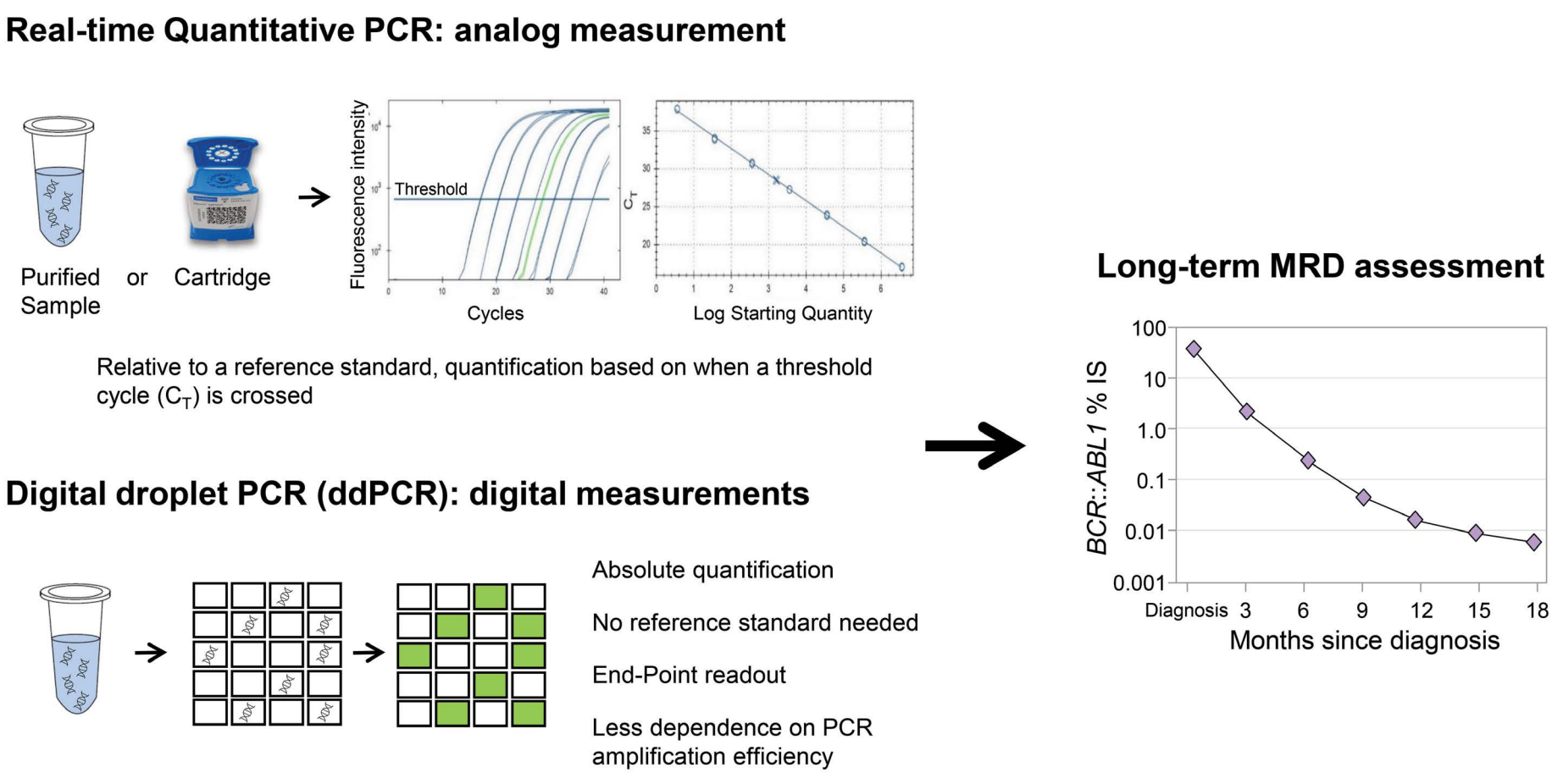


Figure 2. Measurement of residual disease during treatment. Measurable residual disease in chronic myeloid leukemia can be measured using real-time quantitative polymerase chain reaction or digital polymerase chain reaction: analog *versus* digital measurement. Slide courtesy of Dr Jerry Radich and Dr Daniel Chiu. PCR: polymerase chain reaction; MRD: measurable residual disease; IS: International Scale.

2019 study[14] stressed that ddPCR was not ready to be incorporated into the criteria for eligibility of a clinical trial of TKI cessation. The assay requires careful calibration of signal-to-noise ratio and standardization across laboratories. Furthermore, excluding patients on the basis of a ddPCR value at the time of considering TKI cessation would exclude a proportion of patients who would maintain TFR. A recent multicenter study has demonstrated the feasibility of using ddPCR to monitor treatment response.[65] Broader use of digital PCR would require a thorough demonstration of comparable results across laboratories and clinical applicability if it were to be used to direct treatment decisions. Toward this goal, the performance characteristics of the first US Food and Drug Administration approved ddPCR assay for *BCR::ABL1* monitoring have been published.[66] The study demonstrated reproducibility of results across laboratories. Attempts to increase the sensitivity of the methodology for better prediction of TFR included *BCR::ABL1* DNA PCR.[67] The method could not reliably predict TFR and is not currently recommended.

Molecular monitoring in resource-poor regions

Standardized molecular monitoring is not available in all regions due to economic constraints. Automated systems for measuring *BCR::ABL1*, such as the Cepheid GeneXpert,[68] relieve the burden of resource-intensive in-house method development, optimization, IS standardization and validation. The Cepheid technique incorporates a stand-alone microfluidic system in which all processes necessary to generate a standardized *BCR::ABL1* ratio occur within a disposable cartridge. Results are generated rapidly and the method requires minimal training. The system may be a viable option for monitoring patients with CML in resource-poor regions. Shipment of dried blood spots by regular mail has also been demonstrated as a means of extracting viable RNA and for generating reliable standardized *BCR::ABL1* ratios.[69] This process is a cost-effective alternative to shipment of samples to a central laboratory for testing.

The impact of genomic heterogeneity at the time of diagnosis for treatment response

Evidence has accumulated using various next-generation sequencing techniques (Figure 3) that mutation of cancer-related genes is associated with treatment failure and drug resistance in CML.[70-76] In patients selected for genomic analysis at diagnosis on the basis of their known response to therapy, mutation of cancer genes was associated with poor outcome.[71,73,74] Research on unselected cohorts of consecutively treated patients is required to establish the true predictive value of these mutations if present at diagnosis. However, it is known that some patients with cancer gene mutations at diagnosis can achieve optimal responses. For example, the most frequently mutated gene at diagnosis of CML is *ASXL1*[72] and some patients with mutated *ASXL1* can rapidly achieve MMR.[71] The long-term outcome for patients with additional mutations at diagnosis is unknown. However, a recent small study suggested that somatic mutation of epigenetic modifier genes within the leukemic clone at CML diagnosis may impact the chance of TFR.[77]

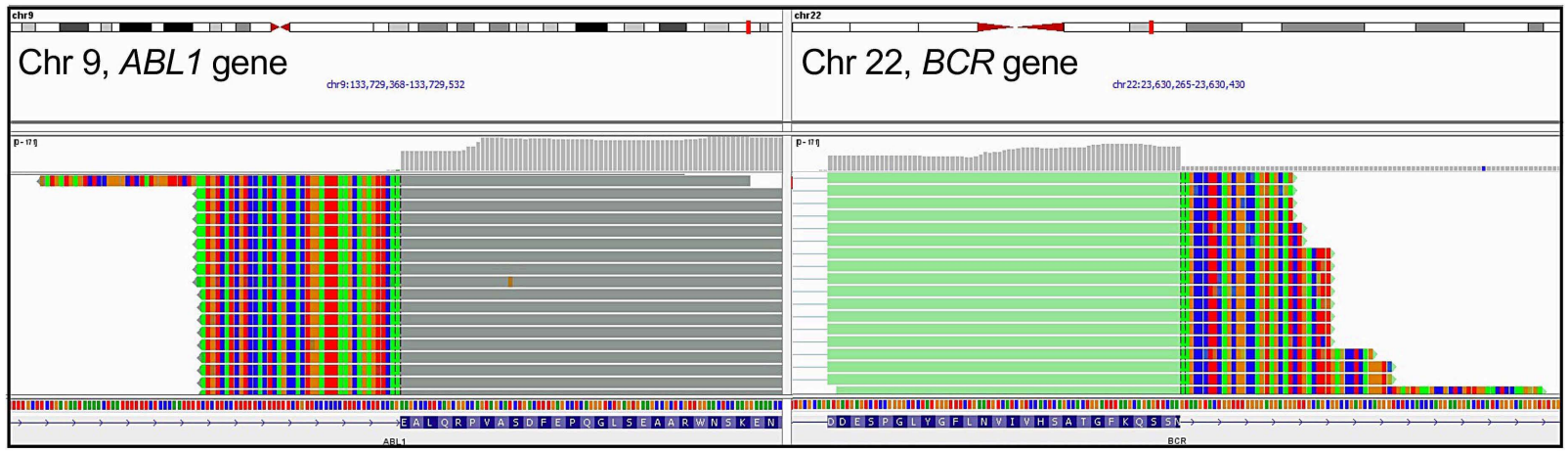
Importance of treatment adherence

Adherence to therapy is known to be a critical factor for achieving and maintaining response.[78-80] One study found that as few as 14% of patients were completely adherent in taking all TKI doses and a third of patients were classified as non-adherent.[78] In a UK population, 26% of patients had less than 90% adherence.[79] Factors associated with better adherence were older age, male sex, appropriate management of side effects, taking only one tablet per day, and feeling well informed and supported by the clinician.[81,82] Patients were also less adherent when more than 2 years from diagnosis.[82] Long-term, regular molecular monitoring can help to identify patients who are less adherent. Features of non-compliance include unexpected variations in *BCR::ABL1* ratios (usually in patients who have previously achieved at least MMR) and plateauing of response after prior steady declines in transcript levels.

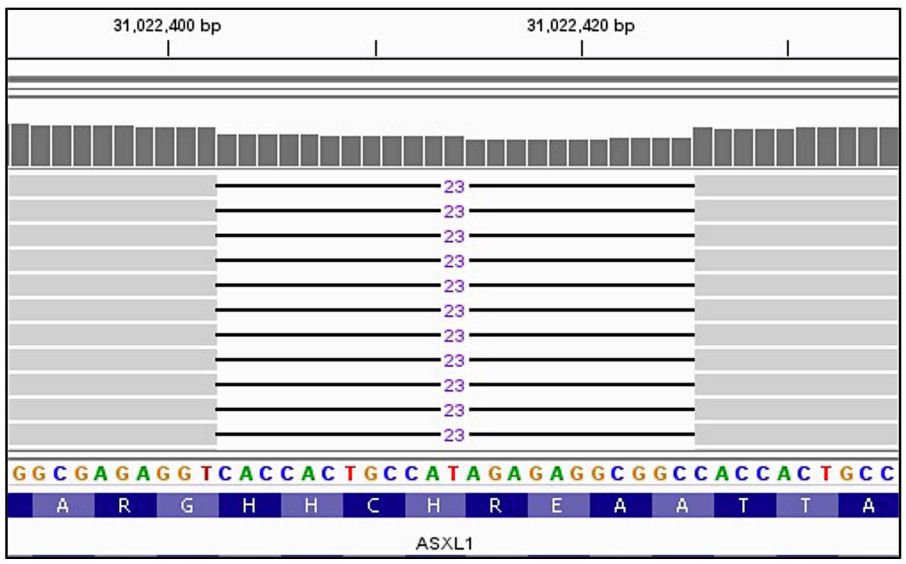
Enhanced detection of *BCR::ABL1* kinase domain mutations

BCR::ABL1 kinase domain mutations are the best-recognized mechanism of acquired resistance and signal treatment failure.[20,21] Early detection can allow timely therapeutic intervention. Since each leukemic cell has one copy of the *BCR::ABL1* gene fusion and one copy of normal *ABL1*, the mutated allele can be specifically isolated by positioning PCR amplification primers within *BCR*, just before the fusion junction, and in *ABL1*, immediately after the kinase domain sequence. This allows exquisite sensitivity to detect mutations in patients with MRD and kinase domain mutations can be detected using Sanger sequencing in patients with *BCR::ABL1* ratios <0.1%, prior to relapse.[30] However, the sensitivity of Sanger sequencing is limited to 10-20%.[83,84] The relevance of sensitive *BCR::ABL1* mutation detection using next-generation sequencing has been demonstrated for patients with a nonoptimal molecular response, when the actionable threshold of mutant detection was 3%.[83,84] In a prospective study that compared Sanger sequencing and next-generation sequencing, low-level TKI-resistant mutants detectable using next-generation sequencing invariably expanded over time if the patient was not switched to an appropriate alternate TKI.[84] Early detection of mutants at levels as low as 3% could warrant treatment intervention to curtail clonal expansion of the resistant clone and loss of response. The clinical

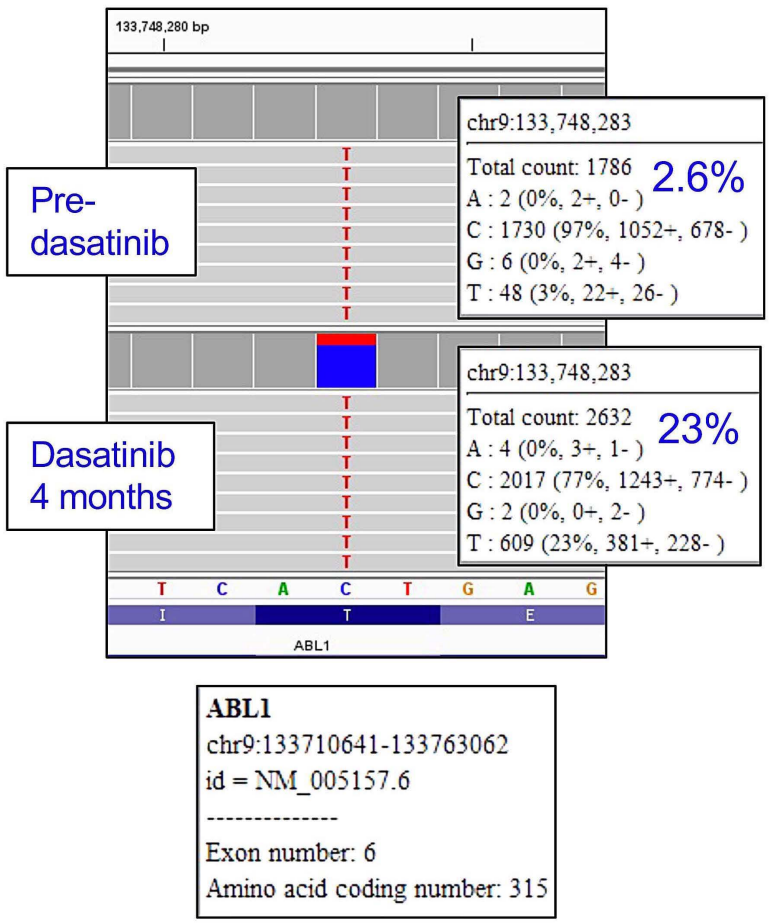
A *BCR*::*ABL1* fusion transcript



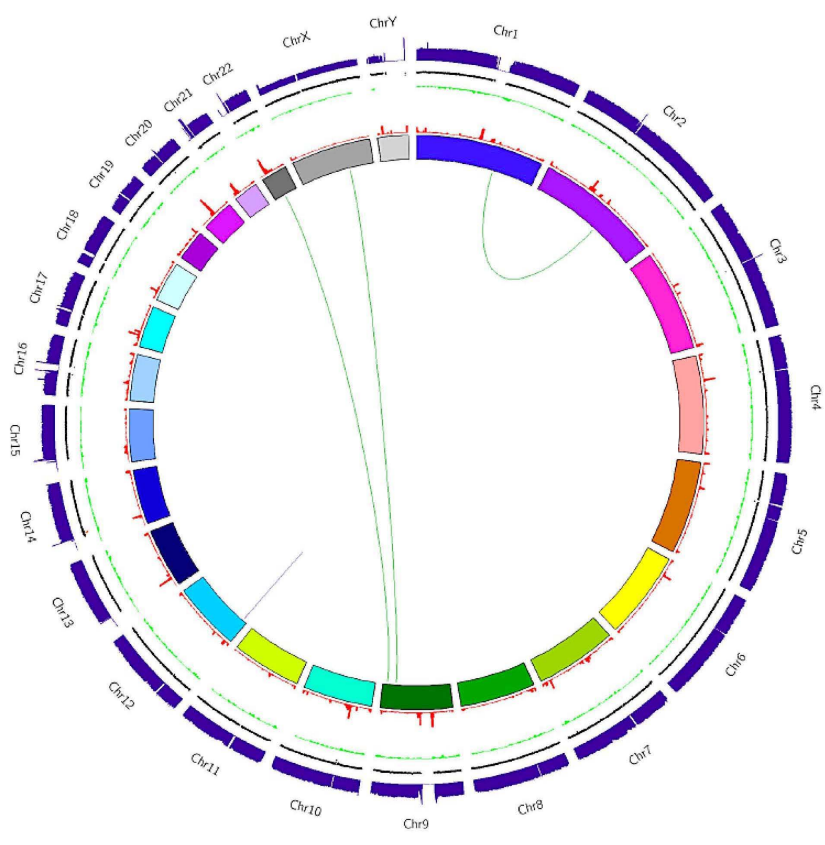
B *ASXL1* c.1900_1922del:p.(Glu635Argfs*15)
chr20(GRCh37):g.31022406



C *ABL1* c.944C>T:p.(Thr315Ile)
chr9(GRCh37):g.133748283



D Circos plot of a CML genome with a standard t(9;22)(q34;q11.2)



Continued on following page.

Figure 3. Next-generation sequencing for the detection of various mutation types. New technology can detect *BCR::ABL1* transcripts and kinase domain mutations, plus other clinically relevant variants. Some of these techniques enhance the detection of low level variants. (A) RNA-sequencing detection of e14a2 *BCR::ABL1* fusion transcripts. Sequence reads are mapped to the hg19 reference genome and visualized in the Integrative Genomics Viewer. 'View mate region in split screen' is selected. The multicolored region in the left panel at the junction of *ABL1* exon 2 indicates that the reads are derived from a different genomic location. The dark green color specifies that the reads are derived from chromosome 22. The multicolored region in the right panel at the junction of *BCR* exon 14 and the light green solid color indicate the reads are derived from chromosome 9 and map to *ABL1*. (B) Whole exome sequencing or targeted gene sequencing can detect clinically relevant variants in cancer-related genes at the time of diagnosis of chronic myeloid leukemia or at drug resistance. In this case a common *ASXL1* 23 base pair deletion was detected. (C) Next-generation sequencing of the *BCR::ABL1* kinase domain provides enhanced sensitivity. A low level T315I mutation was below the level of detection using Sanger sequencing but was retrospectively detected at 2.6% in a drug-resistant patient prior to commencing dasatinib (top panel). The dasatinib-resistant mutant rapidly expanded to 23% after commencing dasatinib (bottom panel). (D) Whole genome sequencing is not suitable for the detection of residual disease but can simultaneously detect multiple different mutation types, with the exception of fusion transcripts. A circos plot provides a snapshot of rearrangements and other variants. In this case, the standard 9;22 translocation was detected and indicated by the arc between chromosomes 9 and 22, plus other rearrangements. Chromosomes are indicated in the outermost track. Circos plot courtesy of Dr Philippi May, Imperial College Healthcare NHS Trust, in conjunction with the Genomics England 100,000 Genomes project. CML: chronic myeloid leukemia.

relevance of TKI-resistant mutations using next-generation sequencing that are below the current threshold of 3% has not been established. Furthermore, the introduction of next-generation sequencing for routine clinical monitoring requires appropriate validation according to international standards for diagnostic testing.[83] Importantly, there is no clinical need for *BCR::ABL1* mutation analysis at the time of diagnosis in CP using Sanger sequencing or sensitive next-generation sequencing.

Treatment decisions for patients with long-term measurable residual disease

A major goal for many patients is TFR but not all patients achieve the strict criteria for a trial of stopping therapy. Most patients face life-long TKI therapy, which can cause debilitating side effects. With the introduction of increasingly potent TKI, clinicians are faced with therapy-related dilemmas for patients on treatment for many years. Should a patient who is tolerating TKI therapy and has minimal or no side-effects, switch to a more potent TKI to achieve a DMR in order to qualify for a trial of drug cessation? Should prior TKI resistance influence decisions? What are the long-term vascular risks for patients treated with potent TKI over many years and should this influence treatment decisions? How should patients be managed when *BCR::ABL1* ratios remain relatively and stubbornly high without meeting the molecular criteria for treatment failure? Are these patients at risk of disease progression? We present theoretical cases in which these treatment dilemmas may arise and discuss the pros and cons of treatment options.

Case scenarios

Patient 1, a case of sudden blast phase

A 48-year-old male was diagnosed with CP CML: he had a high-risk EUTOS long-term survival (ELTS) score, expressed the e13a2 transcript, had no additional chromosome abnormalities and no mutations in cancer-related genes. He commenced imatinib 600 mg OD in the context of a clinical trial. Figure 4 shows the *BCR::ABL1* ratios measured over time. The *BCR::ABL1* ratio was 160% at diagnosis and 12% IS at 3 months. Early research suggested a *BCR::ABL1* ratio >10% at 3 months was sufficient to identify patients destined to fare poorly, thereby allowing early treatment intervention.[27] However, subsequent studies highlighted the importance of the trend of initial *BCR::ABL1* decline using multiple data points.[33,34] This strategy is recommended when interpreting the 3-month ratio.[20,21] There was a substantial *BCR::ABL1* decline at 3 months for this patient although the response fell into the warning/possible TKI resistance categories. A good mantra would be to try to avoid making decisions on any single result and always confirm an unexpected result. It is also important to consider treatment compliance and discuss with the patient the presence of side effects or any other reason why they might have missed any medication.[20,21] Assuming good compliance, continuing the initial therapy would be a perfectly reasonable treatment decision. In this case the trial protocol mandated a rapid treatment switch to nilotinib because of failure to achieve time-dependent molecular milestones at 3, 6 or 12 months

Treatment was therefore changed to nilotinib 400 mg BD at 5 months and a 12-month *BCR::ABL1* ratio compatible with CCyR was achieved. As can be seen from Figure 4, the MMR achieved at 2 years was not stable, perhaps again raising issues of compliance, which in turn may reflect the presence of troublesome side effects and/or problems associated with twice daily dosing. Consideration was given to performing allogeneic SCT at this time, and HLA-typing of the patient and siblings was requested. In the meantime treatment was changed to dasatinib. A stable MMR was maintained for a number of years but a DMR was never achieved.

A rapid rise in the *BCR::ABL1* ratio occurred at 7 years. This

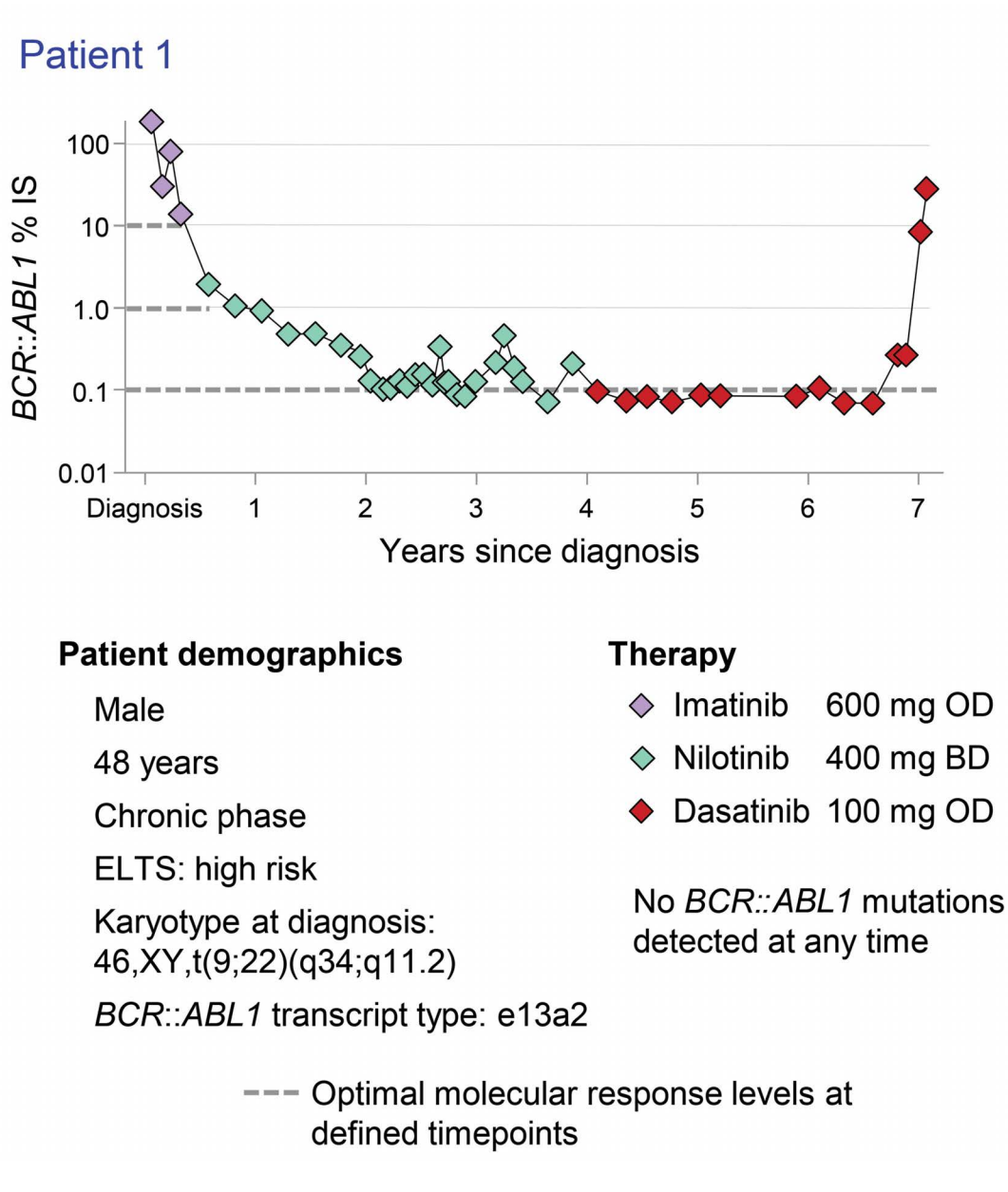


Figure 4. A rare case of sudden blast phase. Measurement of *BCR::ABL1* ratios using real-time quantitative polymerase chain reaction revealed that the patient never achieved a deep molecular response despite switching to second-generation tyrosine kinase inhibitors. The very rapid *BCR::ABL1* rise at 7 years after diagnosis heralded blast phase. IS: International Scale; ELTS: EUTOS long-term survival score.

triggered a kinase domain mutation analysis, which was negative. Prior studies had determined that the average rate of a *BCR::ABL1* rise after stopping TKI corresponds to a *BCR::ABL1* doubling time of 8-9 days.[38,85] The *BCR::ABL1* doubling time for Patient 1 was 11 days, which is rapid and consistent with complete lack of kinase inhibition. This could indicate complete non-adherence to TKI therapy or could portend a more dangerous scenario for the patient: progression to BP. In a study of 12 CP patients with *BCR::ABL1* <10% IS who relapsed into BP, the median *BCR::ABL1* doubling time was 9 days.[38] In contrast, the *BCR::ABL1* doubling time was significantly longer (median 48 days) for 30 patients who acquired *BCR::ABL1* mutations but maintained CP.[38]

Issues of compliance were addressed and the patient denied missing his drugs. Unfortunately the patient had also lost complete hematologic response and subsequently progressed to BP within a month. He was treated with two courses of AML-like chemotherapy and achieved a second CP. He has recently undergone a sibling allogeneic SCT and his RT-qPCR confirms undetectable disease. Could we have predicted this tragic turn of events? Progression to advanced phase after the establishment of a durable MMR is unusual but has been described.[86,87] Although the achievement of MMR has been termed a 'safe haven', Claudiani and colleagues showed that attainment of DMR, sustained for at least 12 months, was associated with a remarkably low probability of losing MMR in the absence of other events such as a trial of treatment discontinuation, lack of compliance, or reduced drug dosing.[88] The mechanism of treatment failure in Patient 1 is unknown. Additional chromosome abnormalities were not detected but broader genomic analysis would likely identify mutated cancer-related genes at the time of BP.

Patient 2, a case of non-adherence and late acquisition of BCR::ABL1 mutations

A 28-year-old male was diagnosed in CP in 2003: he had a low risk ELTS score, expressed e14a2 and had no additional chromosome abnormalities. He was treated with imatinib 400 mg OD. Figure 5 shows his *BCR::ABL1* ratios measured over time. The patient met the current criteria for treatment failure at 6 and 12 months, but maintained CP. A slow *BCR::ABL1* rise occurred in the third year of imatinib treatment, when the doubling time was slow at 53 days. A *BCR::ABL1* kinase domain mutation was not detected. The rise was attributed to non-adherence to therapy and the dynamics of the rise were consistent with intermittent imatinib dosing.[38] MMR was achieved at 6 years but was somewhat unstable, again attributed to

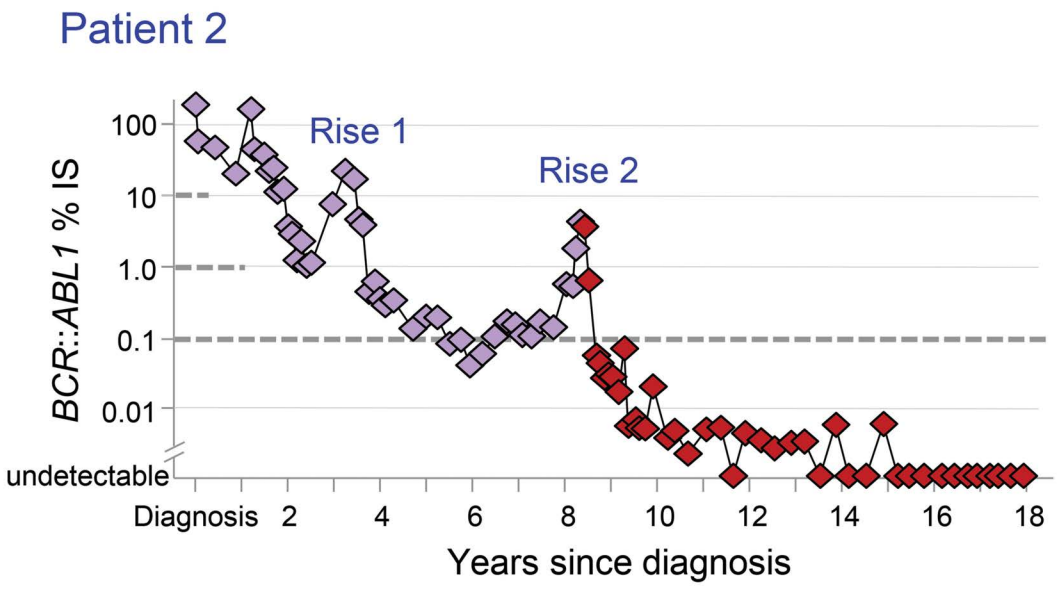


Figure 5. A case of non-adherence and late acquisition of *BCR::ABL1* kinase domain mutations. Increases in *BCR::ABL1* ratio over the first 3 years after diagnosis for this patient were associated with non-adherence to therapy. A deep molecular response on imatinib was never attained and a slow *BCR::ABL1* rise at 8 years was accompanied by the detection of *BCR::ABL1* mutations and failure of imatinib. This case demonstrates the importance of regular and sustained molecular monitoring. The rise prompted *BCR::ABL1* mutation analysis, which confirmed imatinib resistance rather than non-adherence and allowed timely therapeutic intervention. IS: International Scale; ELTS: EUTOS long-term survival score.

non-compliance which was admitted by the patient. This did not seem to be related to any particular side-effect that might have been best treated by a switch of TKI. Unfortunately the patient experienced a significant *BCR::ABL1* rise at year 8. At that time two imatinib-resistant *BCR::ABL1* mutations were detected using Sanger sequencing: E275K and E459K. *BCR::ABL1* mutations are mostly acquired within the first few years of first-line TKI therapy in resistant patients so this was a late occurrence. Whether the persistent, relatively high levels of *BCR::ABL1* contributed to an environment conducive to DNA damage and the acquisition of mutations is unknown. Both of the mutations are sensitive to second-generation TKI and a switch to dasatinib reinstated and indeed deepened the response such that the patient is now in stable DMR.

What next for this patient who has been on dasatinib for >10 years and maintained a DMR for 3 years? Does the patient qualify for a trial of treatment cessation? Could residual leukemic cells carry the *BCR::ABL1* mutations and does this influence decisions regarding treatment cessation? *BCR::ABL1* mutations can be selected and deselected.[89,90] In some cases the mutants persist at undetectable levels for many years and even reappear at molecular relapse upon treatment cessation.[91] Some *BCR::ABL1* mutants may have a proliferative advantage.[92] Prior TKI resistance is among the current ELN exclusion criteria for a treatment-cessation trial.[20] However, recent versions of the NCCN guidelines for treatment cessation no longer exclude prior detection of *BCR::ABL1* mutations in patients who maintained CP.[21] Claudiani and colleagues assessed ten patients with previous *BCR::ABL1* mutations who stopped TKI due to intolerance.[93] All had maintained MR4 for at least 1 year prior to stopping TKI (median 6.3 years) and the median duration of TKI therapy before stopping was 13 years. The molecular relapse-free survival for the ten patients was 50% with 1 to 4.7 years of follow-up. Two of the patients who maintained TFR had prior T315I mutations. The rate of TFR for the ten patients with prior resistance is consistent with the rate reported in clinical trials of TKI cessation that excluded patients with prior resistance.[94] The authors speculated that if a patient with a *BCR::ABL1* mutation promptly receives an effective alternative TKI and a DMR is achieved and maintained, the adverse outcomes associated with *BCR::ABL1* mutations can be overcome.[93]

Patient 3, a case of early treatment failure

A 37-year-old female was diagnosed with CML in 2013 and received nilotinib as first-line therapy in a clinical trial. The transcript was e14a2/e13a2, the ELTS score was intermediate and there were no additional chromosome abnormalities at diagnosis. Exploratory genomic analysis at diagnosis revealed an *ASXL1* nonsense mutation. Figure 6 shows the *BCR::ABL1* ratios measured over time. The pa-

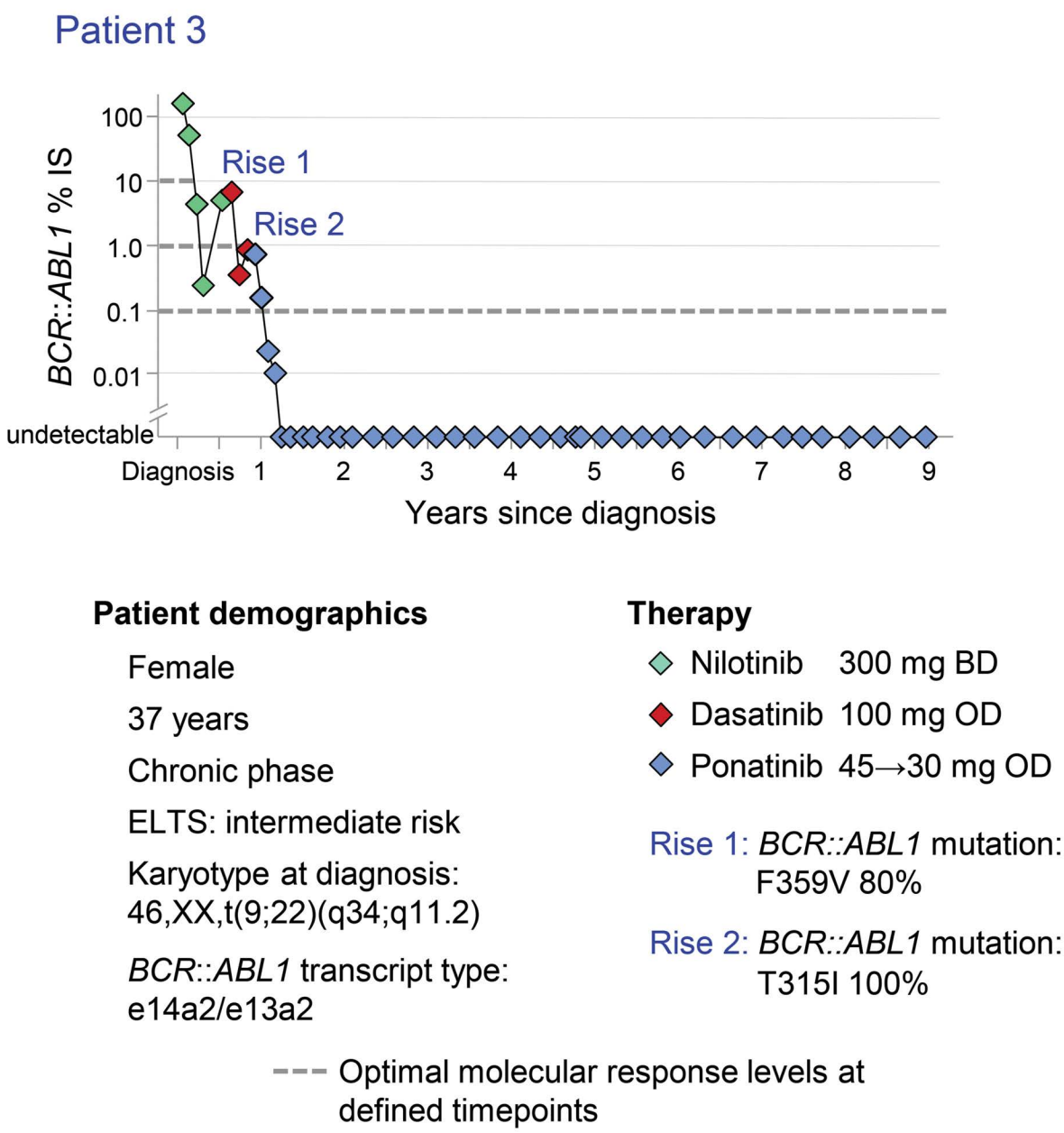


Figure 6. A case of early treatment failure. Despite an initial rapid response to first-line nilotinib the patient failed to benefit from nilotinib and subsequent dasatinib therapy, as indicated by the acquisition of successive *BCR::ABL1* mutations. T315I detected at the second *BCR::ABL1* rise prompted a switch to the third-generation inhibitor, ponatinib. The rapid achievement of a deep molecular response, sustained for many years, indicates that the T315I-mutant leukemic cells were highly sensitive to ponatinib. IS: International Scale; ELTS: EUTOS long-term survival score.

tient rapidly developed a nilotinib-resistant mutation, F359V, despite a good initial response. A switch to dasatinib was swiftly followed by the acquisition of the T315I mutation, which is resistant to imatinib and the second-generation TKI nilotinib, dasatinib and bosutinib. T315I was acquired in an independent clone, which was indicated by its clonal dominance and disappearance of the F359V clone. T315I is sensitive to ponatinib and the patient rapidly achieved and maintained a DMR after commencing treatment with ponatinib. The dose of ponatinib at the start of treatment was 45 mg OD and this was reduced to 30 mg OD within 1 month. Recent results from the OPTIC study, in which patients were randomized to one of three doses of ponatinib (45, 30 or 15 mg) and instructed to dose reduce to, or continue on, 15 mg, once the RT-qPCR fell below 1% IS, would suggest that she could now be safely reduced to 15 mg daily. Probably given the length of time she has been on 30 mg, she is not at high risk of arterial thrombotic events but minimizing the dose while maintaining response is a reasonable goal for all patients.[95] Monitoring general health is recommended and interventions should be made where necessary. Asciminib is a BCR::ABL1 inhibitor recently approved by the US Food and Drug Administration for patients in whom prior TKI therapy has failed.[96,97] Early data suggest that asciminib, at a higher dose of 200 mg twice daily, has efficacy against T315I and may be better tolerated than ponatinib. However, the follow-up was short. The other possibility is a trial of treatment discontinuation but as discussed above, data are sparse as to the safety of this approach in patients with kinase domain mutations, particularly T315I.

Biomarkers at diagnosis cannot predict the early acquisition of TKI-resistant *BCR::ABL1* kinase domain mutations. Why did this patient acquire a resistant mutation within months of commencing treatment, whereas Patient 2 only acquired resistant mutations after 8 years? Patient 3 had an *ASXL1* mutation at diagnosis, whereas the mutation status of Patient 2 at diagnosis was unknown. Ongoing genomic studies of cohorts of unselected patients may provide further evidence for enhanced risk stratification on the basis of a cancer gene mutation at diagnosis. Mutated *ASXL1* is not only the most frequently detected mutation at diagnosis of CML, but is also among the most frequently observed in BP CML.[72] In the largest study of genomic heterogeneity in BP CML, *ASXL1* mutations were associated with a poorer outcome, even in this very poor risk setting.[76]

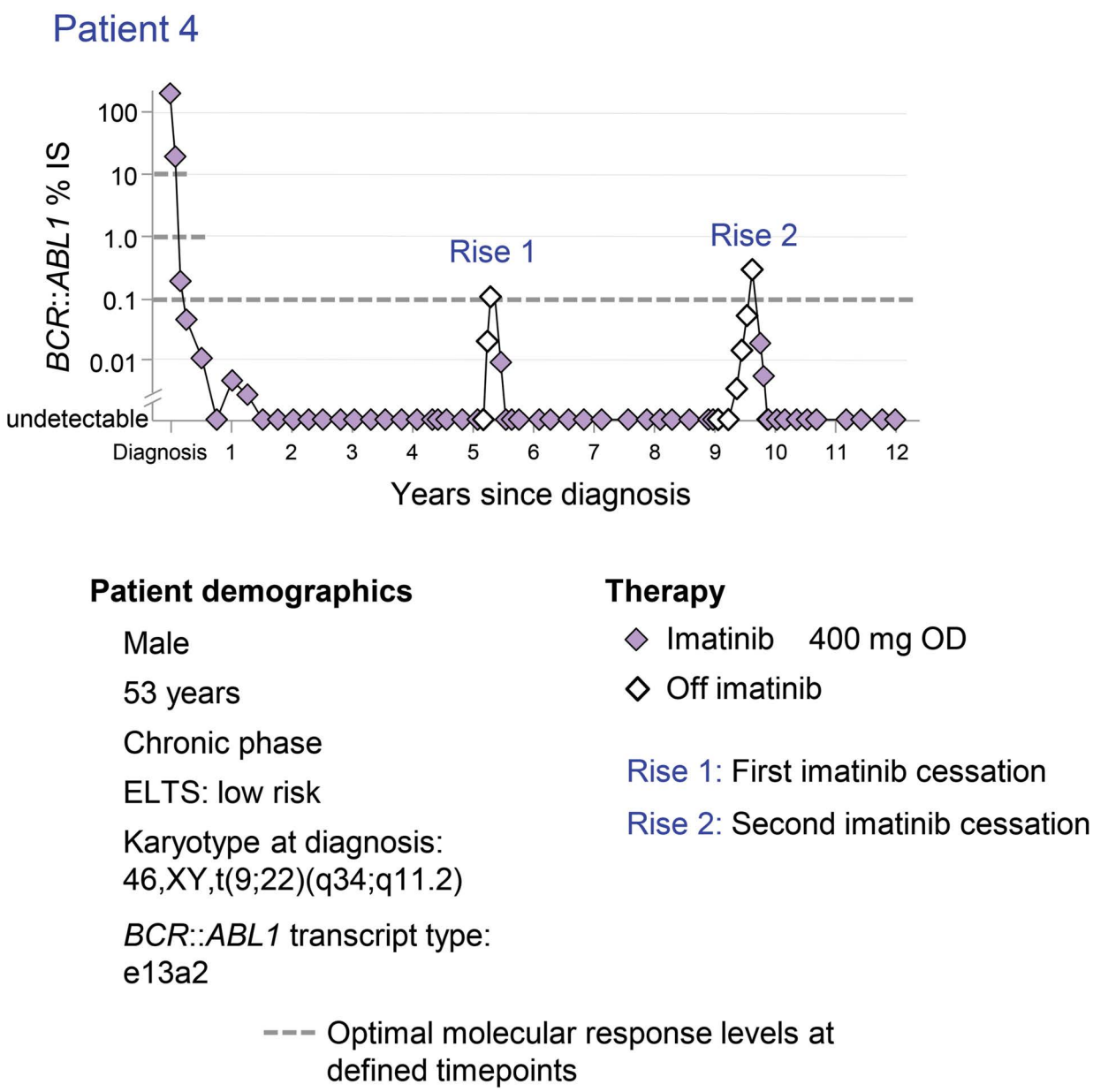


Figure 7. A case of two failed attempts to achieve treatment-free remission. The patient achieved the optimal milestone *BCR::ABL1* ratios and sustained a deep molecular response for more than 5 years before imatinib discontinuation in an attempt to achieve treatment-free remission. Molecular relapse was rapid at both cessation attempts. It is not known why some patients are unsuccessful in multiple attempts to sustain treatment-free remission and the reasons for molecular relapse could be multifactorial. Life-long tyrosine kinase inhibitor therapy may be required for this patient. Regular molecular monitoring could be critical to monitor for potential episodes over time of non-adherence to therapy. A rapid *BCR::ABL1* rise associated with non-adherence could potentially lead to loss of complete hematologic response, unless detected promptly by the clinician through molecular monitoring. IS: International Scale; ELTS: EUTOS long-term survival score.

Patient 4, a case of treatment-free remission attempts

A 53-year-old male was diagnosed in CP in 2010 and commenced imatinib 400 mg OD. The transcript was e13a2, the ELTS score was low and there were no additional chromosome abnormalities at diagnosis. Exploratory genomic analysis at diagnosis revealed an *ASXL1* frameshift mutation. Figure 7 shows the *BCR::ABL1* ratios over time. All *BCR::ABL1* optimal milestones were achieved. MR4.5 was maintained for 4.5 years before imatinib was discontinued for a trial of TFR. A rapid rise that commenced at 1 month after cessation prompted imatinib restart and MR4.5 was rapidly regained. Imatinib was ceased for a second attempt at TFR after a further 3.5 years of DMR, but relapse was again rapid.

The chance of TFR is approximately 50% for patients who attempt TFR. Longer treatment and DMR durations were associated with an increased probability of maintaining TFR at 6 months in the EURO-SKI study, which was the largest TKI cessation trial.[94] The optimal cut-offs were 5.8 years on therapy and 3.1 years of DMR. Patient 4 was on imatinib for 5 years before attempting TFR and the chances of success may have increased with longer time on imatinib. However, the EURO-SKI study determined that the duration of DMR was the most important factor affecting the probability of TFR. Patients with e13a2, as in this case, may have an inferior probability of TFR.[62,64] TFR is achievable after a second TKI cessation attempt for some patients.[98] The French RE-STIM study of 70 patients reported a TFR rate of 42% at 24 months after cessation. The relapse pattern at the first cessation attempt was the only factor significantly associated with TFR at the second attempt. Patients who relapsed after 3 months had a significantly higher rate of TFR at the second attempt: 72% *versus* 36% at 24 months. Patient 4 had a very rapid relapse at the first attempt. This patient had mutated *ASXL1* at diagnosis which was not detectable in remission and a recent small study has found an association between mutations in epigenetic modifier genes at diagnosis and a lower rate of TFR.[77]

What next for this patient? He is now 12 years after diagnosis and has received only imatinib to which he has responded deeply and durably. He has tolerated the imatinib well and could remain on the drug life-long. After two unsuccessful attempts at treatment discontinuation of imatinib it seems unlikely that further treatment cessation will achieve a better result. If TFR is an important goal for this patient then re-starting treatment using a more potent TKI would be an entirely reasonable

approach but the chance of successful discontinuation must be balanced against the increased risk of side effects with a new drug. This is an excellent example of the need for honest and transparent dialogue between patient and physician.

Conclusion

In 2022 it is virtually impossible to imagine managing any patient with CML without accurate molecular monitoring. The technology accurately identifies patients who are responding well and who might be future candidates for treatment discontinuation. Conversely patients with primary and secondary resistance can be recognized promptly and treatment switched in an attempt to induce response and prolong survival. If the change in therapy is unsuccessful the patient can be referred for allogeneic SCT while still in CP and thereby maximize their chance of a good outcome. But accurate monitoring can also highlight issues of compliance, which can then be addressed and the patient supported to adhere to treatment and deepen their response. The methodology continues to evolve and can be adapted to suit most clinical situations and resources. There is little doubt that the efficacy of the TKI in CML has been complemented by the ability to accurately measure residual disease and modify treatment accordingly to optimize outcome.

Disclosures

SB is a member of advisory boards for Qiagen, Novartis and Cepheid; has received honoraria from Qiagen, Novartis, Bristol-Myers Squibb and Cepheid, and has received research support from Novartis and Cepheid. JFA is a member of advisory boards for Incyte and Novartis; has received honoraria from Incyte, Novartis and Pfizer; and has received research support from Incyte, Novartis and Pfizer.

Contributions

SB conducted the literature review, prepared the figures, wrote and reviewed the manuscript. JFA conducted the literature review, designed the original layout, wrote and reviewed the manuscript. The authors approved the final version of the manuscript.

Acknowledgments

SB is supported by National Health and Medical Research Council of Australia: APP1117718. JFA is an emeritus senior investigator for the National Institute of Health Research (NIHR) and acknowledges the support of the NIHR Imperial College Biomedical Research Center.

References

1. Baccarani M, Castagnetti F, Gugliotta G, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia. 2019;33(5):1173-1183.
2. Zaccaria A, Rosti G, Sessarego M, et al. Relapse after allogeneic bone marrow transplantation for Philadelphia chromosome positive chronic myeloid leukemia: cytogenetic analysis of 24 patients. Bone Marrow Transplant. 1988;3(5):413-423.
3. Kawasaki ES, Clark SS, Coyne MY, et al. Diagnosis of chronic myeloid and acute lymphocytic leukemias by detection of leukemia-specific mRNA sequences amplified in vitro. Proc Natl Acad Sci U S A. 1988;85(15):5698-5702.
4. Sawyers CL, Timson L, Kawasaki ES, Clark SS, Witte ON, Champlin R. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci U S A. 1990;87(2):563-567.
5. Morgan GJ, Hughes T, Janssen JW, et al. Polymerase chain reaction for detection of residual leukaemia. Lancet. 1989;1(8644):928-929.
6. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990;76(12):2462-2465.
7. van Rhee F, Lin F, Cullis JO, et al. Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. Blood. 1994;83(11):3377-3383.
8. Cross NC, Hughes TP, Feng L, et al. Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: correlations with acute graft-versus-host disease and relapse. Br J Haematol. 1993;84(1):67-74.
9. Cross NC, Feng L, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood. 1993;82(6):1929-1936.
10. Goh HG, Lin M, Fukushima T, et al. Sensitive quantitation of minimal residual disease in chronic myeloid leukemia using nanofluidic digital polymerase chain reaction assay. Leuk Lymphoma. 2011;52(5):896-904.
11. Mori S, Vagge E, le Coutre P, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910-914.
12. Lee SE, Choi SY, Song HY, et al. Imatinib withdrawal syndrome and longer duration of imatinib have a close association with a lower molecular relapse after treatment discontinuation: the KID study. Haematologica. 2016;101(6):717-723.
13. Alikian M, Ellery P, Forbes M, et al. Next-generation sequencing-assisted DNA-based digital PCR for a personalized approach to the detection and quantification of residual disease in chronic myeloid leukemia patients. J Mol Diagn. 2016;18(2):176-189.
14. Nicolini FE, Dulucq S, Boureau L, et al. Evaluation of residual disease and TKI duration are critical predictive factors for molecular recurrence after stopping imatinib first-line in chronic phase CML patients. Clin Cancer Res. 2019;25(22):6606-6613.
15. Bernardi S, Malagola M, Zanaglio C, et al. Digital PCR improves

the quantitation of DMR and the selection of CML candidates to TKIs discontinuation. Cancer Med. 2019;8(5):2041-2055.
16. Franke GN, Maier J, Wildenberger K, et al. Comparison of real-time quantitative PCR and digital droplet PCR for BCR-ABL1 monitoring in patients with chronic myeloid leukemia. J Mol Diagn. 2020;22(1):81-89.
17. Salmon M, White HE, Cross NCP, Hochhaus A. Standardization of molecular monitoring for chronic myeloid leukemia: 2021 update. In: Hehlmann R, ed. Chronic Myeloid Leukemia. Cham: Springer International Publishing; 2021:105-117.
18. Jovanovski A, Petiti J, Giugliano E, et al. Standardization of BCR-ABL1 p210 monitoring: from nested to digital PCR. Cancers (Basel). 2020;12(11):3287.
19. Radich J, Yeung C, Wu D. New approaches to molecular monitoring in CML (and other diseases). Blood. 2019;134(19):1578-1584.
20. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966-984.
21. NCCN Clinical Practice Guidelines in Oncology: Chronic Myeloid Leukemia. Version 3.2022, Accessed 24th March 2022.
22. Merx K, Muller MC, Kreil S, et al. Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib after failure of interferon alpha. Leukemia. 2002;16(9):1579-1583.
23. Wang L, Pearson K, Ferguson JE, Clark RE. The early molecular response to imatinib predicts cytogenetic and clinical outcome in chronic myeloid leukaemia. Br J Haematol. 2003;120(6):990-999.
24. Branford S, Rudzki Z, Harper A, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia. 2003;17(12):2401-2409.
25. Hughes TP, Hochhaus A, Branford S, et al. Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). Blood. 2010;116(19):3758-3765.
26. Hanfstein B, Muller MC, Hehlmann R, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26(9):2096-2102.
27. Marin D, Ibrahim AR, Lucas C, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30(3):232-238.
28. Branford S, Kim D-W, Soverini S, et al. Initial molecular response at 3 months may predict both response and event-free survival at 24 months in imatinib-resistant or -intolerant patients with Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase treated with nilotinib. J Clin Oncol. 2012;30(35):4323-4329.
29. Marin D, Hedgley C, Clark RE, et al. Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first-line dasatinib. Blood. 2012;120(2):291-294.
30. Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28-37.
31. Branford S, Fletcher L, Cross NCP, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood. 2008;112(8):3330-3338.
32. Huet S, Cony-Makhoul P, Heiblig M, et al. Major molecular response achievement in CML patients can be predicted by BCR-ABL1/ABL1 or BCR-ABL1/GUS ratio at an earlier time point of follow-up than currently recommended. PLoS One. 2014;9(9):e106250.
33. Hanfstein B, Shlyakhto V, Lauseker M, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988-1992.
34. Branford S, Yeung DT, Parker WT, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014;124(4):511-518.
35. Pennisi MS, Stella S, Vitale SR, et al. BCR-ABL1 doubling-times and halving-times may predict CML response to tyrosine kinase Inhibitors. Front Oncol. 2019;9:764.
36. Karpurmath SV, Seshachalam A, Selvaraj K, et al. Halving time of BCR-ABL1 in chronic myeloid leukemia, is it a better bet than day 90 value – multicenter study from south India. Clin Lymphoma Myeloma Leuk. 2020;20(5):e205-e211.
37. Stuckey R, Casado LF, Colomer D, et al. Early prediction of subsequent molecular response to nilotinib in patients with chronic myeloid leukemia: comparison of the quantification of BCR-ABL1 ratios using ABL1 or GUSB control genes. J Mol Diagn. 2020;22(10):1217-1224.
38. Branford S, Yeung DT, Prime JA, et al. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. Blood. 2012;119(18):4264-4271.
39. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-884.
40. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med. 2003;349(15):1423-1432.
41. Branford S, Hughes TP, Rudzki Z. Dual transcription of b2a2 and b3a2 BCR-ABL transcripts in chronic myeloid leukaemia is confined to patients with a linked polymorphism within the BCR gene. Br J Haematol. 2002;117(4):875-877.
42. Sharplin K, Altamura H, Taylor K, Wellwood J, Taylor D, Branford S. Chronic myeloid leukaemia: the dangers of not knowing your BCR-ABL1 transcript. Leuk Res. 2019;87:106231.
43. Mills KI, MacKenzie ED, Birnie GD. The site of the breakpoint within the bcr is a prognostic factor in Philadelphia-positive CML patients. Blood. 1988;72(4):1237-1241.
44. Grossman A, Silver RT, Arlin Z, et al. Fine mapping of chromosome 22 breakpoints within the breakpoint cluster region (bcr) implies a role for bcr exon 3 in determining disease duration in chronic myeloid leukemia. Am J Hum Genet. 1989;45(5):729-738.
45. Shtalrid M, Talpaz M, Kurzrock R, et al. Analysis of breakpoints within the bcr gene and their correlation with the clinical course of Philadelphia-positive chronic myelogenous leukemia. Blood. 1988;72(2):485-490.
46. Dreazen O, Berman M, Gale R. Molecular abnormalities of bcr and c-abl in chronic myelogenous leukemia associated with a long chronic phase. Blood. 1988;71(3):797-799.
47. Zaccaria A, Martinelli G, Buzzi M, et al. M-BCR breakpoint

location does not predict survival in Philadelphia chromosome-positive chronic myeloid leukemia. Haematologica. 1992;77(1):16-20.
48. Shepherd P, Suffolk R, Halsey J, Allan N. Analysis of molecular breakpoint and m-RNA transcripts in a prospective randomized trial of interferon in chronic myeloid leukaemia: no correlation with clinical features, cytogenetic response, duration of chronic phase, or survival. Br J Haematol. 1995;89(3):546-554.
49. Hanfstein B, Lauseker M, Hehlmann R, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014;99(9):1441-1447.
50. Castagnetti F, Gugliotta G, Breccia M, et al. The BCR-ABL1 transcript type influences response and outcome in Philadelphia chromosome-positive chronic myeloid leukemia patients treated frontline with imatinib. Am J Hematol. 2017;92(8):797-805.
51. Dmytrenko IV, Fedorenko VG, Shlyakhtychenko TY, et al. Assessment of response to imatinib therapy in patients with chronic myeloid leukemia with e13a2 and e14a2 transcripts of BCR/ABL1 gene. Probl Radiac Med Radiobiol. 2015;20:328-340.
52. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269-1275.
53. Greenfield G, McMullan R, Robson N, McGimpsey J, Catherwood M, McMullin MF. Response to imatinib therapy is inferior for e13a2 BCR-ABL1 transcript type in comparison to e14a2 transcript type in chronic myeloid leukaemia. BMC Hematol. 2019;19:7.
54. Marce S, Xicoy B, Garcia O, et al. Impact of BCR-ABL1 transcript type on response, treatment-free remission rate and survival in chronic myeloid leukemia patients treated with imatinib. J Clin Med. 2021;10(14):3146.
55. Lucas CM, Harris RJ, Giannoudis A, et al. Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. Haematologica. 2009;94(10):1362-1367.
56. Sharma P, Kumar L, Mohanty S, Kochupillai V. Response to imatinib mesylate in chronic myeloid leukemia patients with variant BCR-ABL fusion transcripts. Ann Hematol. 2010;89(3):241-247.
57. Kjaer L, Skov V, Andersen MT, et al. Variant-specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay. Eur J Haematol. 2019;103(1):26-34.
58. Bernardi S, Bonifacio M, Iurlo A, et al. "Variant-specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay." Is dPCR the key? Eur J Haematol. 2019;103(3):272-273.
59. Machova Polakova K, Salmon M, Zizkova H, et al. Individual molecular response evaluation on both DNA and mRNA BCR-ABL1 level diminished differences in time to molecular response achievement between CML patients with E13A2 vs E14A2 transcript type. Hemasphere. 2020;4(1):351.
60. Dominy KM, Claudiani S, O'Hare M, et al. Assessment of quantitative PCR for BCR::ABL1 transcripts in CML: are improved outcomes in patients with e14a2 transcripts an artefact of technology? Br J Haematol. 2022;197(1):52-62.
61. Clark RE, Polydoros F, Apperley JF, et al. De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. Lancet Haematol. 2019;6(7):e375-e383.
62. Claudiani S, Apperley JF, Gale RP, et al. e14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in individuals with chronic myeloid leukemia after stopping tyrosine kinase inhibitor therapy. Haematologica. 2017;102(8):e297-e299.
63. D'Adda M, Farina M, Schieppati F, et al. The e13a2 BCR-ABL transcript negatively affects sustained deep molecular response and the achievement of treatment-free remission in patients with chronic myeloid leukemia who receive tyrosine kinase inhibitors. Cancer. 2019;125(10):1674-1682.
64. Shanmuganathan N, Pagani IS, Ross DM, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. Blood. 2021;137(9):1196-1207.
65. Bochicchio MT, Petiti J, Berchialla P, et al. Droplet digital PCR for BCR-ABL1 monitoring in diagnostic routine: ready to start? Cancers (Basel). 2021;13(21):5470.
66. Shelton DN, Bhagavatula P, Sepulveda N, et al. Performance characteristics of the first Food and Drug Administration (FDA)-cleared digital droplet PCR (ddPCR) assay for BCR::ABL1 monitoring in chronic myelogenous leukemia. PLoS One. 2022;17(3):e0265278.
67. Ross DM, Branford S, Seymour JF, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. Leukemia. 2010;24(10):1719-1724.
68. Winn-Deen ES, Helton B, Van Atta R, et al. Development of an integrated assay for detection of BCR-ABL RNA. Clin Chem. 2007;53(9):1593-1600.
69. Sala Torra O, Beppu L, Smith JL, et al. Paper or plastic? BCR-ABL1 quantitation and mutation detection from dried blood spots. Blood. 2016;127(22):2773-2774.
70. Kim T, Tyndel MS, Kim HJ, et al. Spectrum of somatic mutation dynamics in chronic myeloid leukemia following tyrosine kinase inhibitor therapy. Blood. 2017;129(1):38-47.
71. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948-961.
72. Branford S, Kim DDH, Apperley JF, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835-1850.
73. Nteliopoulos G, Bazeos A, Claudiani S, et al. Somatic variants in epigenetic modifiers can predict failure of response to imatinib but not to second-generation tyrosine kinase inhibitors. Haematologica. 2019;104(12):2400-2409.
74. Adnan Awad S, Kankainen M, Ojala T, et al. Mutation accumulation in cancer genes relates to nonoptimal outcome in chronic myeloid leukemia. Blood Adv. 2020;4(3):546-559.
75. Ko TK, Javed A, Lee KL, et al. An integrative model of pathway convergence in genetically heterogeneous blast crisis chronic myeloid leukemia. Blood. 2020;135(26):2337-2353.
76. Ochi Y, Yoshida K, Huang Y-J, et al. Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. Nat Commun. 2021;12(1):2833.
77. Adnan Awad S, Bruck O, Shanmuganathan N, et al. Epigenetic modifier gene mutations in chronic myeloid leukemia (CML) at diagnosis are associated with risk of relapse upon treatment discontinuation. Blood Cancer J. 2022;12(4):69.
78. Noens L, van Lierde MA, De Bock R, et al. Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. Blood. 2009;113(22):5401-5411.
79. Marin D, Bazeos A, Mahon F-X, et al. Adherence is the critical factor for achieving molecular responses in patients with

chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. J Clin Oncol. 2010;28(14):2381-2388.
80. Noens L, Hensen M, Kucmin-Bemelmans I, Lofgren C, Gilloteau I, Vrijens B. Measurement of adherence to BCR-ABL inhibitor therapy in chronic myeloid leukemia: current situation and future challenges. Haematologica. 2014;99(3):437-447.
81. Rychter A, Jerzmanowski P, Holub A, et al. Treatment adherence in chronic myeloid leukaemia patients receiving tyrosine kinase inhibitors. Med Oncol. 2017;34(6):104.
82. Geissler J, Sharf G, Bombaci F, et al. Factors influencing adherence in CML and ways to improvement: results of a patient-driven survey of 2546 patients in 63 countries. J Cancer Res Clin Oncol. 2017;143(7):1167-1176.
83. Kizilors A, Crisà E, Lea N, 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-e284.
84. Soverini S, Bavaro L, De Benedittis C, et al. Prospective assessment of NGS-detectable mutations in CML patients with nonoptimal response: the NEXT-in-CML study. Blood. 2020;135(8):534-541.
85. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005;435(7046):1267-1270.
86. Jabbour E, Kantarjian H, O'Brien S, et al. Sudden blastic transformation in patients with chronic myeloid leukemia treated with imatinib mesylate. Blood. 2006;107(2):480-482.
87. Okada Y, Sato K, Kobayashi S, et al. Sudden blast phase in chronic myeloid leukemia developed during nilotinib therapy after major molecular response was achieved. Int J Hematol. 2018;107(4):495-497.
88. Claudiani S, Gatenby A, Szydlo R, et al. MR4 sustained for 12 months is associated with stable deep molecular responses in chronic myeloid leukemia. Haematologica. 2019;104(11):2206-2214.
89. Hanfstein B, Muller MC, Kreil S, et al. Dynamics of mutant BCR-ABL-positive clones after cessation of tyrosine kinase inhibitor therapy. Haematologica. 2011;96(3):360-366.
90. Gruber FXE, Lamark T, Anonli A, et al. Selecting and deselecting imatinib-resistant clones: observations made by longitudinal, quantitative monitoring of mutated BCR-ABL. Leukemia. 2005;19(12):2159-2165.
91. Parker WT, Yeoman AL, Jamison BA, et al. BCR-ABL1 kinase domain mutations may persist at very low levels for many years and lead to subsequent TKI resistance. Br J Cancer. 2013;109(6):1593-1598.
92. Griswold IJ, MacPartlin M, Bumm T, et al. Kinase domain mutants of Bcr-Abl exhibit altered transformation potency, kinase activity, and substrate utilization, irrespective of sensitivity to imatinib. Mol Cell Biol. 2006;26(16):6082-6093.
93. Claudiani S, Apperley J, Khan A, Khorashad J, Milojkovic D. Prolonged treatment-free remission in chronic myeloid leukemia patients with previous BCR-ABL1 kinase domain mutations. Haematologica. 2020;105(5):e225-e227.
94. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747-757.
95. Valent P, Hadzijusufovic E, Schernthaner G-H, Wolf D, Rea D, le Coutre P. Vascular safety issues in CML patients treated with BCR/ABL1 kinase inhibitors. Blood. 2015;125(6):901-906.
96. Hughes TP, Mauro MJ, Cortes JE, et al. Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315-2326.
97. Réa D, Mauro MJ, Boquimpani C, et al. A phase 3, open-label, randomized study of asciminib, a STAMP inhibitor, vs bosutinib in CML after 2 or more prior TKIs. Blood. 2021;138(21):2031-2041.
98. Legros L, Nicolini FE, Etienne G, et al. Second tyrosine kinase inhibitor discontinuation attempt in patients with chronic myeloid leukemia. Cancer. 2017;123(22):4403-4410.