The significance of major and stable molecular responses in chronic myeloid leukemia in the tyrosine kinase inhibitor era

Ilana Zalcberg Renault1
Vanessa Scholl1
Rocio Hassan1
Paola Capelleti1
Marcos de Lima2
Jorge Cortes2

1 Molecular Biology Laboratory, Bone Marrow Transplantation Center – CEMO, Instituto Nacional de Cáncer – INCA, Rio de Janeiro, RJ, Brazil
2 MD Anderson Cancer Center – MDACC, Houston, Texas, USA

Tyrosine kinase inhibitors have changed the management and outcomes of chronic myeloid leukemia patients. Quantitative polymerase chain reaction is used to monitor molecular responses to tyrosine kinase inhibitors. Molecular monitoring represents the most sensitive tool to judge chronic myeloid leukemia disease course and allows early detection of relapse. Evidence of achieving molecular response is important for several reasons: 1. early molecular response is associated with major molecular response rates at 18-24 months; 2. patients achieving major molecular response are less likely to lose their complete cytogenetic response; 3. a durable, stable major molecular response is associated with increased progression-free survival. However, standardization of molecular techniques is still challenging.

Keywords: Leukemia, myelogenous, chronic, BCR-ABL positive; Cytogenetic; Monitoring; Mutation; Polymerase chain reaction

Introduction

Chronic myeloid leukemia (CML) is most frequently diagnosed in the chronic phase (CP) and, if left untreated, the disease will progress to the accelerated phase (AP) and, eventually within 3-5 years, to the terminal blast phase (BP).

CML is characterized by a reciprocal translocation between chromosomes 9 and 22, which creates the BCR-ABL fusion gene. Breakpoints in the ABL gene are often upstream of exon 2 (ABL a2 fusion type) and rarely downstream of exon 2 (ABL a3). Breakpoints in the BCR gene are more variable; the most frequent are downstream of either exon 13 or exon 14 (e13 and e14 previously referred to as exons b2 and b3), leading to e13a2 or e14a2 mRNA fusion subtypes, respectively. These isoforms are found in approximately 98% of CML patients and are referred to as the major BCR-ABL fusion subtype. Both BCR-ABL mRNA molecules encode a 210 KDa constitutively active kinase protein, which is essential for leukemic transformation in CML. Most BCR-ABL breakpoints in CML patients are accounted by a variety of fusions, which involve BCR exons 6, 8 or 19 (e6a2, e8a2 or e19a2) and ABL exon 3 (e13a3 or e14a3), which encodes the p210 KDa protein. Less than 1% of CML show a breakpoint downstream of BCR exon 1 (e1a2) resulting in an mRNA fusion encoding the p190 KDa oncoprotein.(1)

International Randomized Study of Interferon and STI571 (IRIS) trial and European Leukemia Net criteria to monitor response in chronic myeloid leukemia

CML treatment was revolutionized by the advent of imatinib mesylate (IM), a tyrosine kinase inhibitor (TKI).(2) IM has become the treatment of choice for newly diagnosed patients in CP-CML based on the results of the International Randomized Study of Interferon and STI571 (IRIS) trial and confirmed by the recent 8-year update.(3,4) However, whilst the vast majority of newly diagnosed CP-CML patients in the IRIS trial treated with IM have a sustained benefit at 8 years, many patients do not reach what is considered an optimal response. Approximately 35% of CML patients initially treated with IM fail due to refractoriness (primary resistance), loss of response after an initial response (secondary resistance), or intolerance.(5) In addition, 12-33% of patients meet criteria for suboptimal response at any given time.(6) Unless the disease can be controlled with further therapy, these individuals invariably progress to the blastic phase that is usually terminal. With the aim of improving the results of IM therapy, second-generation TKIs, Dasatinib and Nilotinib, have been introduced to treat patients with IM resistance
or intolerance. To date, their use in Brazil is limited mainly to these situations.

The effectiveness of TKIs is evaluated by cytogenetic and molecular responses at defined time-points. In this scenario, tumor burden reflected by these responses is a predictive parameter of individual response to TKIs and is considered the main biomarker to test the efficacy of new drugs in clinical studies. The concepts that the therapeutic benefit of a TKI can be predicted by the speed of reduction in tumor burden (the number of Philadelphia + or BCR-ABL + cells) and that disease response is the major parameter for estimating the probability of progression-free survival (PFS) and overall survival (OS), are now well established. Conversely, acquisition of additional abnormalities is associated with resistance.

Achievement of complete cytogenetic response (CCyR) has remained the gold standard for an optimal outcome in CML because it is associated with an improved probability of survival. After achievement of CCyR, the main parameter for response assessment, is the molecular response (MR), measured by the reduction in BCR-ABL transcripts using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and classified according to a uniform scale referred to as the International Reporting Scale (IS) that is valid for RT-qPCR and classified according to a uniform scale referred to as the International Reporting Scale (IS) that is valid for any patient and attributes an absolute value of 0.1% to a 3 log reduction from a hypothetical 100% BCR-ABL value at diagnosis.

Molecular biology for BCR-ABL monitoring: measuring BCR-ABL transcripts

The IS resulted from recent effort to create an international standard, where laboratory-specific values of the BCR-ABL constitutive gene for each patient sample obtained by RT-qPCR are converted to an IS score applying a derived laboratory-specific correction factor validated by a reference laboratory. According to the recommendations of the European Leukemia Net (ELN), optimal responders should achieve a major molecular response (MMR), a ≤ 0.1% value on the IS, by 18 months of therapy.

Reaching a proficient approach to reliably monitor molecular responses in BCR-ABL + patients requires several steps, including the standardization of a stable and controlled assay according to the Minimum Information for Publication of Quantitative Real-Time Polymerase Chain Reaction Experiments (MIQE) guidelines, the internal validation in a clinical setting, and international validation with a reference laboratory, in order to obtain a correction factor to convert BCR-ABL levels according to the IS. This process, including the different levels of validation based on the experience of the Molecular Biology laboratory at the Instituto Nacional de Câncer (INCA), is shown in Figure 1. Since 2006, 661 patients with CML treated at INCA and 18 centers throughout Brazil were investigated and successfully monitored.

The work flow for CML molecular monitoring in our laboratory starts with the definition of the BCR-ABL transcript type by a qualitative multiplex assay in pretreatment samples. This procedure is performed to assure that RT-qPCR monitoring will target the correct fusion type and false negative results occurring due to the presence of atypical transcripts (fusions involving exon 6 BCR e6a2, e8a2, e19a2 and exon 3 ABL e13a3 or e14a3 fusions) not contemplated in the quantitative assay will be safely excluded. If the fusion subtype is not identified by qualitative polymerase chain reaction (PCR) prior to starting treatment, qRT-PCR false negative results cannot be excluded.

Molecular responses are assessed by absolute quantification via RT-qPCR assays at diagnosis and then approximately every 3 months, independently on the type of cytogenetic response of the patient. A multiplex RT-qPCR, including hydrolysis probes and primers for both e13a2 and e14a2 transcripts, is run with ABL as the control gene. For absolute quantification purposes, every RT-qPCR run includes standard curves prepared using plasmids, as well as "low" and "high" copy quality controls, as recommended (Figure 1). Each BCR-ABL/ABL value is converted to the IS applying our laboratory-specific conversion factor which was validated by the Australian Molecular Laboratory at Adelaide. For this, a significant number of samples, including patients in various disease phases, were tested at different times using different RT-qPCR runs, both in the INCA laboratory and at the validating center in Adelaide.

BCR-ABL transcripts are undetectable with the reference molecular methodology at a sensibility of 4.5 log. Definition of a complete molecular response (CMR) requires an undetectable BCR-ABL transcript level by RT-qPCR confirmed by negative nested-PCR, where the lowest limit of detection is 10⁻⁴.

Thus, molecular responses are reported according to BCR-ABL transcript levels as compared to a standardized baseline used as an IS. MMR is a 3-log reduction from the baseline or the absolute value of ≤ 0.1% on the IS. Accordingly, a 2-log reduction ranges from 1% to 0.1% in the IS; a 1-log reduction from 10% to 1% in the IS and non-significant reduction is equivalent to values of more than 10% in the IS.

The adoption of these laboratory procedures has overcome the clinician’s lack of confidence in molecular test interpretation related to differences in protocols that can result in greatly varying end-points. This was important for several reasons as it: (i) provides an early tool to predict patient response; (ii) allows the use of BCR-ABL/ABL values to guide clinical decisions; (iii) facilitates patient mobility between different clinical facilities and (iv) facilitates interpretation of clinical research among different reference centers in the world, since MMR is the primary end-point of various clinical trials of ABL-inhibitors.
The significance of major and stable molecular responses in chronic myeloid leukemia in the tyrosine kinase inhibitor era

The work-flow for chronic myeloid leukemia (CML) molecular monitoring in our laboratory starts with the definition of BCR-ABL transcript type by qualitative multiplex assay in pre-treatment samples.

A: Standardization of a stable and controlled absolute quantification assay according to Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines. Plasmid standards with a BCR-ABL insert are prepared twice annually by sub-cloning an original plasmid kindly provided by Dr. NCP Cross. Multiplex reverse transcription quantitative polymerase chain reaction (RT-qPCR) with hydrolysis probes and primers for both e13a2 and e14a2 transcripts are run with ABL as the control gene. The same plasmid is used to quantify BCR-ABL and ABL, which should control any variation in plasmid quantification efficiency. Standard curves are prepared to cover a dynamic range of 10^6-10^1, and the assay limit of detection is 10. Together with the standards, in each RT-qPCR assay a NTC (non-template control, with DNA sample), NAC (non-amplification control, with a negative BCR-ABL cDNA), Blank (only reaction buffer) and “low” and “high” copy quality controls are included. The quality control stocks are prepared with cell dilutions of K562 and Kasumi cell lines. The RT-qPCR results are considered reliable if the ABL copy number is ≥30,000 and RT-qPCR negativity is defined as undetectable BCR-ABL transcripts only when a sample meets acceptable criteria for the ABL copy number. Definition of a complete molecular response (CMR) requires an undetectable BCR-ABL transcript level by RT-qPCR confirmed by negative nested-polymerase chain reaction (PCR), whose lowest limit of detection is 10^-5.

B: Conversion factor (CF) calculation and validation. Each BCR-ABL/ABL value is converted to the IS applying our derived laboratory-specific CF as validated by the Australian Molecular Laboratory at Adelaide. For that, an expressive number of samples, including patients in various disease phases, were tested in parallel at INCA laboratory and at the validating Center at Adelaide. Bias plot of the data generated at INCA for calculation of the CF (i) and the same data with converted validation data (ii). If there were no difference in the estimated mean bias, the dotted line would be at 0, thus, the CF was the antilog of the mean bias. The CF was validated by subsequent sample exchange.

C: The plots show an example of how the application of our CF reduces coefficient of variation in 70 of our samples at the specific values of 0.01%, 0.1%, 1%, and 10%.

What is the importance of MMR after complete cytogenetic response?

As most CML patients treated with a TKI will reach a CCyR, quantification of residual BCR-ABL transcripts by RT-qPCR is the only available tool to further monitor response kinetics. It is assumed that the amount of BCR-ABL transcripts mirrors the number of residual BCR-ABL Ph+ cells. A close agreement between the absolute value of BCR-ABL transcripts given as 1% in the IS and a CCyR can be demonstrated. The ELN recently updated its recommendations to standardize treatment for patients with CML. It recommends that a patient should be seen as an optimal responder to IM when a CCyR is achieved within the first 12 months of treatment. Attainment of a MMR within 18 months is associated with an improved probability of prolonged event-free survival. Conversely, failure to achieve MMR at 18 months is regarded as a suboptimal response. The time required for a patient to reach a CCyR and a MMR is considered an early predictor of therapeutic response with a direct correlation with clinical outcome. However, clinical trials do not necessarily reproduce "real" clinical practice. While most randomized clinical studies in the world were carried out with newly diagnosed CP-CML patients, our experience was built on the responses of patients treated with IM as second-line therapy after treatment with interferon-alpha because Brazil public health care centers were not allowed to use IM as front-line therapy until 2008. Thus, a late responder group, compared to the ELN recommendations for CP-CML patients treated with first-line IM, was identified. Late responder patients represented approximately 40% of the patients that did not achieve optimal responses. Attainment of CCyR at any time was associated with a better OS. The results of this study show that an early predictor marker able to discriminate between the competing possibilities of achieving an optimum response versus progression is still missing.
The group of patients with CCyR was heterogeneous, consisting of those who had CCyR associated to MMR or not. The individual value of MMR was demonstrated in several studies. A recent work established an association between MMR and loosing CCyR. After 18 months of IM treatment, the probability of loosing CCyR when associated to MMR was 0% compared to 25% for patients with a CCyR but no MMR. Patients who achieve MMR at 6, 12 or 18 months after initiating IM treatment have a probabilities of 93%, 69% and 37%, respectively, to evolve with a lasting CCyR. Thus, achievement (and speed to achieve) MMR is a predictive factor for a durable CCyR. Rapidity in obtaining a MMR correlates with a higher probability of achieving a CMR. Patients achieving a MMR at 9 months have a 93% of probability of evolving towards a CMR. Also, achievement of a MMR within 12 months of starting the IM regimen was associated with a better PFS. However, other studies have not confirmed significant differences in OS and PFS in CCy responders in respect to the level of molecular response achieved. The additive effect of MMR associated to CCyR highlights the importance of a better understanding of the role of duration, extension and stability of a MMR in respect to clinical evolution.

Once achieved, does the major molecular response become more stable over time?

Besides the prognostic value of an early MMR, whether a stable MMR occurring during the course of a stable CCyR is an additive parameter to predict a better clinical evolution is a matter of discussion. A stable molecular response is associated with a lasting CCyR. MMR has been subdivided as stable (persistent MMR), unstable (occasionally less than MMR) and never reached MMR. A significant difference was observed, in terms of duration of CCyR and PFS between patients with stable versus unstable MMR and patients who never reached MMR. After a long follow-up from the first assessment of a cytogenetic response, 96% of patients with stable MMR maintain a CCyR compared with 79% of patients with unstable molecular response. Similar results were reported by the MD Anderson Cancer Center (MDACC) group of 276 CML patients treated with high-dose IM therapy; a durable and stable MMR (continuous MMR lasting for a period > 12 months) was associated with a longer PFS.

**What is the meaning of fluctuating BCR-ABL levels?**

Clinicians should be aware of inherent assay variations within a laboratory. Therefore, values may fluctuate, but without clinical significance, on occasions. What constitutes a significant change varies between laboratories. Different patterns of molecular responses based in fluctuations of BCR-ABL levels have been reported in the literature. Molecular monitoring may allow early recognition of acquired resistance. Increasing levels of BCR-ABL transcripts or suboptimal molecular responses (fluctuations at high BCR-ABL levels, i.e. 10%-1% on the IS at 18 months) are associated with increased risk of resistance due to mutations and loss of CCyR. In our experience, an increase of the BCR-ABL load to higher than 1% on any occasion is correlated with a loss of CCyR. In contrast, fluctuations at low BCR-ABL levels of from ≤ 0.1% IS values to undetectable levels, were associated with maintenance of the molecular response. Whether the stability and clinical value of an absolute clearance of BCR-ABL+ cells as detected by the most sensitive methodology (4.5 log by RT-qPCR) is the same as fluctuations of BCR-ABL at lower levels is still to be seen.

Are molecular remissions all the same?

MMR comprises those patients with tumor burden decreases to the range of 0.1% - 0.001% on the IS. The latter (MMR ≤ 0.001%) includes patients with undetectable levels by the most sensitive techniques available today (Nested-PCR and RT-qPCR) which are controversially classified as CMR. This difference between MMR and CMR is important because there is still a debate regarding the possibility of stopping IM in patients with a stable CMR. A French study showed that half of the patients who achieved and maintained CMR for a period of 2 years remained in CMR after IM discontinuation. The primary endpoint of the Stop Imatinib Trial (STIM study) is the evaluation of the persistence of molecular remission after IM discontinuation. Although 50% of patients who suspended IM are reported in CMR, the discontinuation of the drug is not recommended outside clinical trials.

**Resistance to tyrosine kinase inhibitors**

Selection or evolution of resistant clones is a major cause of treatment failure in targeted therapy for CML. The most common identified cause of resistance in TKI-treated patients is the acquisition of point mutations in the tyrosine kinase domain of BCR-ABL that could (i) interfere with binding of IM to the ATP pocket; (ii) prevent the adoption of the conformation required for optimal TKI binding or (iii) stabilize the enzyme in a conformation that is inaccessible to the action of TKI.

Mutation analysis remains an important tool in TKI resistance assessment. The current recommendation for mutation analysis is on IM failure or suboptimal response. The latter includes failure to achieve a CHR or cytogenetic response (CyR) (95% Ph+) by 3 months; major cytogenetic response (MCyR) by 6 months; CCyR by 12 months, and MMR by 18 months. Among these parameters, the association between MMR at 18 months and acquisition of a resistant phenotype due to a point mutation in the BCR-ABL kinase domain is the least well defined.
Among the cohort of 661 patients in this study, 125 were retrospectively selected for mutation analyses using direct sequencing, according to their timing of cytogenetic and molecular response achievement and extent of molecular response. Point mutations involving 17 different amino acids were detected in 25 patients. A double mutation was observed in three patients. Table 1 depicts position and frequency of BCR-ABL mutations found in IM treated patients. No published report of the V268M mutation was found in the literature.

### Table 1 - BCR-ABL mutations detected in imatinib-treated chronic myeloid leukemia patients, selected according to cytogenetic and molecular parameters

| BCR-ABL mutation | Location | N° of patients carrying the mutation | Frequency (%) |
|------------------|----------|------------------------------------|---------------|
| M244V            | -        | 1                                  | 4             |
| G250E            | p-loop   | 2                                  | 8             |
| Y253H            | p-loop   | 2                                  | 8             |
| E255K            | p-loop   | 3                                  | 12            |
| V268M            | -        | 1                                  | 4             |
| L298V            | -        | 1                                  | 4             |
| T315I            | Contact site | 4                              | 16            |
| F317L            | Contact site | 3                              | 12            |
| M351T            | kinase domain | 1                              | 4             |
| E355G            | kinase domain | 1                              | 4             |
| F359V            | kinase domain | 3                              | 12            |
| E450G            | -        | 1                                  | 4             |
| E453A            | -        | 1                                  | 4             |
| E456K            | -        | 1                                  | 4             |
| M458V            | -        | 1                                  | 4             |
| F486S            | -        | 2                                  | 8             |

Twenty-five patients were positive for BCR-ABL mutations; the frequency is higher than 100% as three patients in the same sample had two mutations.

Different increments in BCR-ABL levels (2 fold, 0.5-log and 1-log) assessed in at least two different samples were defined as parameters for mutation screening. Screening for mutant variants of BCR-ABL is frequently performed by Sanger's direct sequencing. The sensitivity of this method is of approximately 10-20% of total BCR-ABL⁺ cells; this sensitivity is assumed to be effective for detecting and characterizing mutations throughout the kinase domain.

There may be a role for more sensitive mutation analysis, particularly when considering the lack of TKI effectiveness against T315I mutated clones(27) and when considering therapeutic options after IM resistance. However this has not yet been established. Due to the existence of different mechanisms accounting for the resistant phenotype, different point mutations will differentially impact specific TKIs. Mutation analysis is always recommended before changing to another inhibitor after resistance. The use of dasatinib will be preferred for CML patients resistant to IM carrying a Y253H, E255V/K or F359C/V mutation, while nilotinib should be indicated in the presence of the V299L or F317L mutations.(28,29) To date, the T315I mutation confers resistance to all available TKIs.

### Conclusions

Detection of biomarkers that allow early prediction of response to TKIs is a major challenge. In an era of targeted TKI therapy, the key point is to select the appropriate agent at the appropriate juncture for each patient, with the aim of achieving long-term, durable responses with minimal toxicity. The rapidity of responses obtained by 2nd generation TKIs as compared to IM will most likely modify the management of patients with newly diagnosed CP-CML, but economic factors may influence treatment decisions.

### Acknowledgement

Role of the funding source: This work was supported by the Brazilian public agencies Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ-PPSUS) and INCT para Controle do Câncer (Grants CNPq 573805/2008-0 and FAPERJ E26/170.026/2008). The funding sources have had no involvement neither in the study design, collection, analysis and interpretation of data; nor in the writing of the manuscript or in the decision to submit the manuscript for publication.

### References

1. Lucas CM, Harris RJ, Giannoudis A, Davies A, Knight K., Watmough SJ, 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):1362-7. Comment in: Haematologica. 2010;95(5):852-3.
2. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood. 2008;112(13):4808-17.
3. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994-1004. Comment in: Curr Hematol Rep. 2004;3(1):37-8. N Engl J Med. 2003;348 (11):1048-50. Clin Lab Haematol. 2005;27(6):416-7.
4. Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up. Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. ASH Annual Meeting Abstracts 2009;114:1126.
5. Hochhaus A, O'Brien SG, Guilhot F, BJ Druker, S Branford, L Foroni, et al. Six-year follow- up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia. 2009;23(6):1054-61.
6. Alvarado Y, Kantarjian H, O'Brien S, Faderl S, Borthakur G, Burger J, et al. Significance of suboptimal response to imatinib, as defined by the European LeukemiaNet, in the long-term outcome of patients with early chronic myeloid leukemia in chronic phase Cancer. 2009;115(16):3709-18.
7. Kantarjian HM, O'Brien S, Cortes JE, Shan J, Giles FJ, Rios MB, et al. Complete cytogenetic and molecular responses to interferon-alpha-based therapy for chronic myelogenous leukemia are

Rev Bras Hematol Hemoter. 2011;33(6):455-60
associated with excellent long-term prognosis. Cancer. 2003;97 (4):1033-41.

8. Branco S. Chronic myeloid leukemia: molecular monitoring in clinical practice Hematology Am Soc Hematol Educ Program. 2007;376:83.

9. Foroni L, Wilson G, Gerrard G, Mason J, Grimmwade D, White HE, et al. Guidelines for the measurement of BCR-ABL1 transcripts in chronic myeloid leukaemia Br J Haematol. 2011;153:179-90. DOI: 10.1111/j.1365-2141.2011.08603.x.

10. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55(4):611-22. Comment in: Clin Chem. 2011 Jun;57 (6):919-21.

11. Otazú IB, Zaleberg I, Tabak DG, Dobbin J, Seuánez HN. Detection of BCR-ABL transcripts by multiplex and nested PCR in different haematological disorders. Leuk Lymphoma. 2000;37(1-2):205-11.

12. Hochhaus A, Reiter A, Skladny, H, Melo JV, Sick C, Berger U, et al. A novel BCR-ABL fusion gene (e6a2) in a patient with Philadelphia chromosome-negative chronic myelogenous leukemia. Blood. 1996;88(6):2236-40.

13. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88(7):2375-84. Comment in: Blood. 1997;89(10):3889. Comment on: Blood. 1996;88(7):2410-4.

14. Branford S, Hughes T. Diagnosis and monitoring of chronic myeloid leukemia by qualitative and quantitative RT-PCR. Methods Mol Med. 2006;125:69-92.

15. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, Cervantes F, Deininger M, Gratwohl A, Guilhot F, Hochhaus A, Horowitz M, Hughes T, Kantarjian H, Larson R, Radich J, Simonsson B, Silver RT, Goldman J, Hehlmann R; European LeukemiaNet. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041-51. Comment in: J Clin Oncol. 2010;28(18):e310; author reply e311.

16. Paschka P, Müller MC, Merx K, Kreil S, Schoch C, Lahaye T, et al. Molecular monitoring of response to imatinib (Glivec) in CML patients pretreated with interferon alpha. Low levels of residual disease are associated with continuous remission. Leukemia. 2003; 17(9):1687-94.

17. Cortes J, Talmaz M, O’Brien S, Jones D, Luthra R, Shan J, et al. Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. Clin Cancer Res. 2005;11(9):3425-32.

18. Iacobucci I, Saglio G, Rosti G, Testoni N, Pane F, Amabile M, Poerio A, Soverini S, Bassi S, Cilloni D, Bassan R, Brecchia M, Lauria F, Izzo B, Merante S, Frassoni F, Paolini S, Montefusco E, Baccarani M, Martinelli G; GIMEMA Working Party on Chronic Myeloid Leukemia. Achieving a major molecular response at the time of a complete cytogenetic response (CCgR) predicts a better duration of CCgR in imatinib-treated chronic myeloid leukemia patients. Clin Cancer Res. 2006;12(10):3037-42.

19. Press RD, Galderisi C, Yang R, Rempfer C, Willis SG, Mauro MJ, et al. A half-log increase in BCR-ABL RNA predicts a higher risk of relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. Clin Cancer Res. 2007;13 (20):6136-43.

20. Palandri F, Iacobucci I, Soverini S, Castagnetti F, Poerio A, Testoni N, et al. Treatment of Philadelphia-positive chronic myeloid leukemia with imatinib: importance of a stable molecular response. Clin Cancer Res. 2009;15(3):1059-63.

21. Marin D, Milojkovic D, Olavarria E, Khorashad JS, de Lavallade H, Reid AM, et al. European LeukemiaNet criteria for failure of suboptimal response reliably identify patients with CML in early chronic phase treated with imatinib whose eventual outcome is poor. Blood. 2008;112(12):4437-44.

22. Quintás-Cardama A, Kantarjian H, Jones D, Shan J, Borthakur G, Thomas D, et al. Delayed achievement of cytogenetic and molecular response is associated with increased risk of progression among patients with chronic myeloid leukemia in early chronic phase receiving high-dose or standard-dose imatinib therapy. Blood. 2009;113(25):6315-21.

23. Kantarjian H, Siffer J, Jones D, Cortes J. Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods. Blood. 2008;111(4):1774-80.

24. Palandri F, Iacobucci I, Soverini S, Castagnetti F, Poerio A, Testoni N, et al. Treatment of Philadelphia-positive chronic myeloid leukemia with imatinib: importance of a stable molecular response. Cancer. 2006;113(25):6315-21.

25. Mahon FX, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselet P; Intergroupe Français des Leucèmies Myéloïdes Chroniques. Intergroupe Français des Leucémies Myéloïdes Chroniques. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029-35. Comment in: Lancet Oncol. 2010;11(11):1010-1. Nat Rev Clin Oncol. 2011;8(3):127-8. Lancet Oncol. 2011;12(2):118.

26. O’Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, et al. In vitro activity of BCR-ABL inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res. 2005;65(11):4500-5.

27. Manrique G, Scholl V, Perez V, Bittencourt R, Moellmann A, Hassan R, et al. Rapid and sensitive allele-specific (AS)-RT-PCR assay for detection of T315I mutation in chronic myeloid leukemia patients treated with tyrosine-kinase inhibitors. Clin Exp Med. 2011;11(1):55-9.

28. La Rosée P, Deininger M. Resistance to Imatinib: Mutations and course of chronic myeloid leukemia. Semin Hematol. 2010;47(1):1033-41.

29. Foroni L, Wilson G, Gerrard G, Mason J, Grimmwade D, White HE, et al. Guidelines for the measurement of BCR-ABL1 transcripts in chronic myeloid leukaemia Br J Haematol. 2011;153:179-90. DOI: 10.1111/j.1365-2141.2011.08603.x.