Retrospective preliminary study on the magnitude of q-RT-PCR for BCR-ABL over FISH BCR-ABL in follow up of CML patient receiving First Line TKI Therapy

Mohammed Al-Sayegh1,2, Hani Al Hashmi1, Omar Abduljali1, Afra Al-Dayel1, Khalid Al Anezi1, Panagiotis Kalogiannidi, Dina Soliman1, Arwa Al Saber1, Ali Al Matrok3, Mahmoud M Kamel4, Heba N. Raslan3,4

1Adult Hemato-Oncology and BMT Unit King Fahad Specialist Hospital Dammam, KSA
2King Fahad Hospital Jeddah, KSA
3Laboratory Department King Fahad Specialist Hospital Dammam, KSA
4Laboratory Department National Cancer Institute Cairo University, Egypt

alsayegh59@yahoo.com, hebaraslan2012@gmail.com, mm.kamel@yahoo.com

Abstract: Background: The pathogenesis of Chronic Myelogenous leukaemia (CML) is driven by the BCR-ABL1 fusion oncoprotein, a dysregulated tyrosine kinase, that results from a reciprocal translocation (9:22) (q34; q11) or Philadelphia chromosome (Ph). Over the past decades, CML therapy has relied on specific tyrosine kinase inhibitors (TKIs) whose success has established CML as a model disease for targeted cancer treatment. The hallmark of CML diagnosis and the efficacy of TKI therapy are confirmed by cytogenetic and molecular investigations. Objective: Our study was to compare the molecular and cytogenetic results in a cohort CML patients receiving first line TKI therapy and determine whether molecular testing has a better predictive value over cytogenetic testing and to investigate whether molecular testing could be solely as sufficient to monitor CML with limiting cytogenetic testing used at to the diagnostic and resistance stages only. Methods: A total of 66 patients were included in the Imatinib treatment regimen, of which 63 Patients had molecular and/or cytogenetic data at at least one specific time point. Results and Conclusion: Molecular response could predict a subsequent cytogenetic response while a CCyR milestone was not an effective indicator of therapeutic progress in the patient cohort. Since a molecular response was sufficient to determine treatment outcome in our patient group, we propose to substitute the largely redundant cytogenetic testing with the more sensitive and reproducible molecular testing.

Key words: CML, Molecular and cytogenetic response, Philadelphia chromosome and TKI therapy

1. Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferativeneoplasm originating from the clonal expansion of hematopoietic cells carrying the BCR-ABL fusion oncogene. The latter, also called the Philadelphia Chromosome (Ph), is created due to the reciprocal translocation between the long arms of the chromosomes 9 and 22. The resulting BCR-ABL oncoprotein, a constitutively active tyrosine kinase (TK), is the main driver of leukaemogenesis owing to its upstream position in signalling pathways that affect the growth and proliferation of cells (Drucker, 2007; Melo, 2008).

The discovery of BCR-ABL revolutionized the targeted therapy of hematopoietic neoplasms with the synthesis of TK inhibitors (TKIs) that specifically targeted BCR-ABL affected clones. First and second generation TKIs such as Imatinib, Dasatinib and Nilotinibhave been successfully used over the last few years to improve the prognosis of CML patients (Natoli et al., 2010). The goal of TKI-based therapy is the normalization of blood counts, eliminationof Ph+ cells and the elimination of BCR-ABL transcripts. The response to TKI therapy is therefore evaluated as optimal, sub-optimal or failed through these haematological, cytogenetic and molecular indicators. The achievable treatment response of CML patients with the TKI has definitively validated this therapeutic strategy and established CML as a model neoplasm for targeted therapy.

Although Karyotyping is the gold standard for monitoring cytogenetic response (Baccarani, et al., 2009), this technique is often limited by low sensitivity, long turnaround time of the test and the complexities of obtaining bone marrow aspirates for the metaphase analysis. Furthermore, as reported by Zagaria et al (2004), 5-10% of CML patients do not even show the presence of the Ph+ chromosome. Nevertheless, a positive cytogenetic response (CyR) in terms of decreased Ph+ metaphase load has been associated with improved prognosis and survival following Imatinib treatment. The CyR is detected at 3, 6 and 12 months starting therapy using either chromosome banding or FISH (Baccarani, 2009;
ISCN, 2009). As per the guidelines of the European LeukaemiaNet (ELN) (Baccarani, et al., 2006), the goal of CML treatment is achieving a complete cytogenetic response (CCyR), defined as absence of Ph+ metaphases. ELN guidelines also define partial CyR (PCyR) as 1-35% Ph+ metaphases and no response (NR) as more than 95% Ph+ metaphases.

Apart from cytogenetic assays, molecular monitoring of CML also gained importance over the years owing to the deeper responses elicited by the first and second generation TKIs. The initial use of molecular tests in CML diagnostics was limited to detection of disease relapse following bone marrow transplantation and were qualitative in nature – giving basic information as to the presence or absence of BCR-ABL1 fusion gene (Cross, 1993). Subsequent development of sensitive and reproducible real time PCR procedures made the molecular monitoring of CML more routine and enabled the sequential quantification of BCR-ABL1 in response to therapy and also made possible the detection of residual disease that remained even after a CCyR. The International Randomized Study of Interferon and STI571 (IRIS) study (Hughes et al, 2003) was not only the first to prove the higher efficacy of imatinib over interferon based treatment but also the definitive study that established the importance of molecular response in TKI therapy.

The level of BCR-ABL1 transcript in CML patient samples was measured relative to the control BCR gene and the results from three different treatment centres were normalized to this standardized baseline (Hughes, et al., 2003). The IRIS scale formed the template for the international scale (IS) of BCR-ABL1 measurement. The IS expresses a detectable disease as a percentage, with 100 % BCR-ABL1 defined as the IRIS-standardized baseline and 10%, 1% and 0.1 % BCR-ABL1 corresponding to different levels of molecular response (Hughes, et al., 2006; Branford, 2008).

Subsequently, the guidelines of ELN were re-defined to include a regular molecular monitoring every 3 months starting therapy - the molecular response is determined by assessing the percentage reduction in the BCR-ABL transcripts to a standardized baseline. The first level of molecular response, defined as an early molecular response (EMR), is characterized by 1-2 log reduction in transcript levels from the baseline (1-10% on the IS) and BCR-ABL1 levels of 1% IS roughly corresponds to a CCyR. A further 3-log reduction in BCR-ABL transcripts (≤0.1% on the IS) is indicative of a major molecular response (MMR), which in turn has been associated with improved prognosis and decreased risk of progression to blast crisis (Press, et al., 2006). Currently, the ELN recommends the milestones of ≤10% BCR-ABL transcripts at 3 months, ≤1% at 6 months and ≤0.1% or an MMR at 12 months to be counted as an optimal response to treatment (Baccarani, et al., 2009, 2013).

DOI 10.1007/s11899-016-0303-8

Studies have shown that conventional cytogenetic tests and RT-PCR are largely correlated, yet Cytogenetic testing: Karyotyping and FISH are still extensively used for the initial evaluation of a patient suspected of CML as it is needed for detection of the variant translocations that involve a third or fourth chromosome in addition to chromosome 9 and 22, or have a cryptic translocation of 9q34 and 22q11.2 that cannot be identified by routine cytogenetic analysis, together with the site of the breakpoint in the BCR gene which produce different fusion proteins, in majority of the cases the breakpoint in BCR is in the major breakpoint cluster region M-BCR, spanning exon 12-16, (previously known as b1-b5) and an abnormal fusion protein P210 is formed, rarely the breakpoint in the BCR gene occurs in the μ-BCR region, spanning exons 17-20 (previously known as c1-c5) and a larger fusion protein 230 is encoded, the third break is in the minor breakpoint region m-BCR (BCR-exons 1-2) leads to a shorter fusion protein P190, for this the value of Cytogenetic analysis exist; however the technique is labour intensive, time consuming and requires the invasive and painful procedure of collecting bone marrow aspirates as it is essential to ensure sufficient material for a complete Karyotype, and FISH analysis.

On the other hand the accuracy, sensitivity and technical feasibility of performing RT-PCR on peripheral blood samples, this approach can detect virtually all kinds of BCR-ABL1 rearrangements, cryptic translocation and minimal residual disease (Yeung et al, 2016).

On the other hand, BCR-ABL1 PCR is not informative regarding bone marrow morphologic findings suggestive of disease acceleration (including localized blasts collections, megakaryocytic sheets, etc) and in cases where additional cytogenetic abnormalities are present (Ross, et al, 2006). The clinical recommendations of ELN therefore encompass both cytogenetic and molecular milestones to evaluate an accurate response to TKI therapy. According to the latest ELN guidelines published in 2013 (Baccarani, 2013), the molecular response must be followed every three months till an MMR is achieved and then every 3-6 months while the cytogenetic response has to be followed (also at 3 month intervals) till a CCyR occurs and then every 12 months thereafter.

The aim of our study is to determine, from retrospectively collected data, whether molecular
testing can replace cytogenetic (FISH) testing during the monitoring phase. The proposed notion is to limit the cytogenetic analysis to be performed as baseline at the time of CML diagnosis and to rely on the molecular responses to predict the cytogenetic response to TKI therapy. We proceeded by correlating the molecular and cytogenetic responses of individual patients at different time intervals post Imatinib therapy and to find out which of the two techniques had a better predictive value in determining a response at a future time point.

2. Methods

Patients:
A total of 102 patients diagnosed as CML in King Fahad hospital-Dammam from 2007-2015. A total of 66 patients were included in the Imatinib treatment regimen, of which 63 Patients had molecular and/or cytogenetic data at at-least one specific time point. The patients excluded had either incomplete/unavailable data or had missed follow-up or died during this time period.

Assessment of cytogenetic and molecular responses:
In our retrospective study, we followed the ELN guidelines and considered the following targets as the optimal response during the 12-month treatment period: a partial cytogenetic response (1-35% Ph+ metaphases) and 10% BCR/ABL-1 transcripts at 3 months, a CCyR (0% Ph+ metaphases) and 1% BCR/ABL-1 at 6 months and an MMR or <0.1% BCR/ABL-1 with sustained CCyR at 12 months.

FISH:
Bone marrow samples were collected for chromosomal analysis and FISH. The BM cells were cultured (short term) and analysed by light microscopy. The FISH analysis was carried out on interphase cells of the patients using Vysis BCR/ABL1/ASS tricolour dual fusion FISH probe.

Quantitative reverse transcriptase polymerasechainreaction RT- PCR:
Blood /bone marrow aspirates (200μl) were lysed according to the manufacturer's protocol. RNA extraction, reverse transcription and Q-PCR were performed with the lysates using the automated Gene Xpert assay/instrument. Wild-type ABL transcripts were used as internal controls and the difference between BCR/ABL-1ct (cycle threshold) and ABLct - ct (Δct) was calculated by the instrument's software. For positive specimens, the %BCR/ABL-1/ABL was calculated as EΔct*(Δct) *100*Conversion factor (EΔct defined as the efficiency of the BCR/ABL-1 to ABL RQ-PCR reaction for a given lot of reagents). For specimens negative for the BCR/ABL-1 transcripts, %BCR/ABL-1/ABL was calculated as EΔct*ABLct. Following a thorough validation, we replaced the IS provided by the manufacturer with the Mayo Clinic IS of 2.57(Raslan, 2013).

3. Results

3.1 Diagnostic status of patients before treatment:
A total of 63 patients had evaluable data at diagnosis.
- Out of the 50 patients who were tested for the Ph chromosome, 90% (n=45) were Ph+ and amongst the Ph+ cohort, 44 had >35% Ph+ and only 1 had 1-35% Ph+ load.
- Forty-five (45) patients had molecular data available – 34 (75%) had >10%, 2 (4%) had 1-10%, 3 (6.7%) had 0.1-1% and 6 (13.3%) BCR/ABL-1 transcripts.
- We classified the patients with >35% Ph+ metaphase load on the basis of the BCR/ABL-1 levels and found that 25 (57%) had >10%, 3 (7%) had >0.1-10% and 2 (5%) had <0.1% BCR/ABL-1 transcript while 14 (31%) did not have any molecular data available. Only 3 out of the 61 patients had <0.1% BCR/ABL-1 and also lacked any Ph+ metaphases (table 1).

3.2 Correlation between BCR/ABL-1 mRNA levels and Ph+ metaphase load in individual patients:
To determine the correlation between the molecular and cytogenetic response in each patient at specific time points during treatment, a regression analysis was performed between the two variables. The degree of correlation was determined by calculating the r squared (r²) value. A value closer to 1 would indicate stronger correlation and imply similar specificity of the two techniques vis-à-vis CML response. Baseline testing, before start of treatment, the r² was 0.1376 indicating a weak positive correlation (figure 1a). As the therapy progressed, the r² value increased steadily through0.4953 (3 months, figure 1b), 0.6659 (6 months, figure 1c) to 0.9815 at 12 months (p<0.001, figure 1d) signifying an equivalent response by both approaches.

3.3 Predictive value of early molecular response (EMR) and complete cytogenetic response (CCyR):
In order to compare the efficacy of molecular and cytogenetic predictive responses relative to each other, the predictive value of each was determined. At the 3-month time-point, 64.7% of the 51 evaluable patients had achieved an EMR with BCR/ABL-1 transcript level <10% (IS) (figure 2a). To determine whether achieving an EMR could predict a later cytogenetic response at 6 months, we next looked at the Ph+ metaphase load in these patients at that time point. Both the EMR+ and EMR- groups had 5 patients each with evaluable cytogenetic data at 6 months. All patients who achieved an EMR had also achieved a CCyR with no detectable Ph+ chromosomes, whereas none of the EMR- patients had reached the CCyR.
This clearly indicated that an EMR at 3 months was predictive of a subsequent cytogenetic response at 6 months. Similarly, we also tested whether attaining a CCyR at 6 months could predict a major molecular response (MMR) at 12 months. A total of 14 patients were available who were tested for Ph+ metaphases at 6 months and also had molecular data available at 12 months; out of these 14, 8 (57.14%) were inCCyR+ and 6 (42.86%) were CCyR- at 6 months. Only 50% of the CCyR+ patients had also achieved an MMR at 12 months whereas 100% of the CCyR- patients failed to reach an MMR. Therefore, a CCyR at 6 months was not a reliable predictor of the 12 month MMR milestone.

3.4 Cytogenetic testing at 6 months has no added value when the molecular response was known at 12 months:

At 12 months post treatment, 49% of the 51 evaluable patients had achieved an MMR while the remaining 51% failed an MMR (figure 3a). In order to determine the extent to which the cytogenetic response coincided with the molecular status, the proportion of patients which had reached a CCyR at 12 months was determined in the MMR+ and MMR- groups. A total of 6 MMR+ and 10 MMR- patients were also tested for a cytogenetic response at 12 months. All the MMR+ patients and 60% of the MMR- patients also showed a CCyR 12 months (figure 3b). Therefore, a CCyR at 12 months is not only redundant when an MMR has been achieved, it can also falsely predict a positive outcome.

3.5 An early molecular response predicts a major molecular response at 12 months:

To investigate the correlation between EMR at its predictive power of MMR, the molecular status at 12 months was compared for patients that reached EMR at 3 months to those that reached the same in 6 months. Achieving an EMR at 3 months correlated with a higher rate of MMR at 12 months compared to attaining the EMR at 6 months (figure 4). Around two-thirds of the patients (69%) that achieved an EMR at 3 months also reached an MMR at 12 months, while 22% of failed to achieve MMR and 9% lost the molecular response attained between 3-6 months. On the other hand, only 16% of the patients that reached the EMR milestone at 6 months could also attain an MMR at 12 months. The majority of these patients (67%) never reached an MMR and 17% lost the 6-month molecular response.

4. Discussion

The primary goal of CML therapy is the elimination of the fusion tyrosine kinase BCR/ABL-1 at the cytogenetics as well as the molecular level – the former is gauged by monitoring the Philadelphia (Ph+) chromosome load and the latter by measuring the BCR/ABL-1 transcript levels. Cytogenetic analysis has long been the gold standard of CML diagnostics with a complete cytogenetic response (CCyR) or absence of Ph+ chromosomes correlating with improved prognosis and survival (Talpaz, 1987; Kantarjian, 1995; Baccarani, et al 2009). With the advent of the tyrosine kinase inhibitor (TKI) based therapies, 80-85% of the patients treated with the TKIs were able to achieve a CCyR (Kantarjian, 2008). However, a state of CCyR can still harbour minimal residual disease in the form of circulating BCR/ABL-1+ cells (Baccarani, 2008) that can only be detected through molecular approaches.

The molecular response to TKI therapy is defined as the progressive reduction in the levels of the BCR/ABL-1 mRNA levels relative to an internationally accepted baseline standard or international scale (IS) (Baccarani, 2006; Branford, 2008; Hughes, 2006) as detected by the highly sensitive real time PCR. In the landmark IRIS trial (Hughes, 2003), patients treated with Imatinib displayed a gradual decrease in leukemic burden starting with a normalization of blood counts, followed by a CCyR and finally a major molecular response (MMR), characterized by a 3-log reduction (<0.1% on the IS) in BCR/ABL-1 mRNA (Hughes, 2003; Hughes, 2006).

Patients achieving an MMR had a negligible risk of disease progression and/or relapse in the following 12 months (Press, 2006). Furthermore, the molecular responses correlated with the cytogenetic responses – a 1-log reduction in BCR/ABL-1 levels (10% IS) was equivalent to a partial cytogenetic response (PCyR) – 1-35% Ph+ chromosomes) while 2-log reduction (1% IS) to the CCyR. The higher sensitivity of molecular testing called into question the need for continued cytogenetic testing as Ross et al (2006) reported in a study that Imatinib treated patients that reached an MMR did not show any cytogenetic abnormality; the clinical value of cytogenetic testing was therefore limited to patients that did not achieve MMR or who lost it subsequently.

The aim of our retrospective study was to examine the possibility of substituting FISH analysis with molecular testing only at specific time points post treatment (as per the guidelines of the ELN) and limit cytogenetic testing at diagnosis only. Reliable CML monitoring with RT-PCR alone would definitely avoid redundancy of unnecessary laboratory testing that are of limited added value and would drastically cut down the around time and cost of treatment follow-up. In addition, it will be more convenient for patients who will not go through frequent bone marrow invasive procedures.
The first step to answer this question was to estimate the correlation between the cytogenetic and molecular status of the disease in individual patients. With this objective, we performed a regression analysis between the BCR/ABL-1 levels and Ph+ chromosome load in individual patients at the start of the treatment and at different time points post treatment (figure 1a). The baseline $r^2$ value of 0.138 implied a weak correlation between the molecular and cytogenetic status at the start of treatment. Most of the patients who were tested for BCR/ABL-1 mRNA and Ph+ metaphases showed the presence of disease in terms of both the cytogenetic and molecular markers; the leukemic load however, was diagnosed at different levels by the cytogenetic and molecular parameters in the same patient (table1). For instance, there were 5 patients that had a high load of Ph+ metaphase (>35%) but tested for <10% BCR/ABL-1 mRNA levels (there is no clear explanation for such findings).

This strongly argues for the need of both molecular and cytogenetic tests at diagnosis to correctly determine the treatment course. The correlation improved as the treatment progressed, with $r^2 = 0.495$ and 0.666 after 3 and 6 months respectively and the strongest correlation was seen after 12 months ($r^2 = 0.981$) (figures 1b, c, d). This indicated that the response to TKI therapy at the stipulated time points was progressing at both cytogenetic and molecular levels and that both the techniques had similar specificity. A positive correlation between the molecular and cytogenetic responses however, alone is not sufficient to substitute cytogenetic monitoring with molecular testing.

Therefore, it was important to determine which of the two approaches had a better chance to predict treatment response and outcome. To this end, we first looked at the cytogenetic response of patients that had achieved an early molecular response (EMR) at 3 months (<10% BCR/ABL-1). All the patients that had achieved an EMR, and for whom cytogenetic data was available, showed a CCyR at 6 months. On the other hand, patients without an EMR also did not achieve a CCyR at 6 months (figure 2a). This showed that an EMR was 100% predictive of a complete cytogenetic response.

Conversely, attaining a CCyR at 6 months did not guarantee a major molecular response, with only 50% of the patients with a CCyR progressing to an MMR at 12 months after treatment. Lack of a cytogenetic response is however correlated with a failed MMR at 12 months (figure 2b). A similar observation was made by Quintas-Cardama (2009) who reported that patients who failed to reach a CCyR and a significantly reduced chance of attaining a molecular response 6 and 12 months in the first year of Imatinib therapy.

To further investigate the added value, if any, of cytogenetic testing at the 12 month milestone, we looked at the cytogenetic status of patients that had achieved an MMR against those who failed to reach an MMR. Overall, 48% of the patients had achieved an MMR after 12 months of TKI therapy and all the MMR+ patients who were also tested for Ph+ chromosome at 12 months had also achieved a CCyR (figure 3a). In contrast, 60% of the cytogenetically evaluable patients who failed to reach MMR had a CCyR (figure 3b), indicating the failure of the latter to predict the expected milestone. Taken together, our data pointed to an improved cytogenetic status of patients that had successfully achieved a molecular response.

This is in accordance with a study published by Pashka et al (2003) where patients with a major molecular response to imatinib did not lose the CCyR while 43% of the patients that failed to reach an MMR also lost their CCyR to imatinib. Similar trends were reported by Cortes et al (2005), Marin et al (2008) and Press et al (2007) in later imatinib treatment trials wherein the probability of losing a CCyR during a median follow up between 12-60 months became significantly higher with the failure to reach an MMR at 12 months.

It has been well established that the extent of an early molecular response can predict the later achievement of an MMR. In a study by Branford et al (2003), patients under imatinib treatment who displayed a 2-log reduction in BCR/ABL-1 levels had significantly better chance of achieving an MMR at 12-24 months compared to the patients who had less than 2-log reduction or who failed to respond altogether at the molecular level. Furthermore, attaining a molecular response at early time points during imatinib treatment is associated with higher rates of progression free survival (PFS) (Wang et al, 2003).

We looked for similar trends in our patient cohort and checked whether duration to attaining an EMR (at least 1-log reduction in BCR/ABL-1 or <10% on the IS) influenced a subsequent MMR. Among the patients that attained an EMR at 3 months, 70% also reached an MMR at 12 months in contrast, only 17% of the patients that achieved the EMR at 6 months could also achieve an MMR. Furthermore, none of the patients that failed an MR at 6 months were able to attain an MMR (figure 4). This finding is significant in light of a recent study at a Singapore medical centre (Bee et al, 2016) wherein the OS and EFS (event free survival) in an imatinib treated cohort depended majorly on achieving a BCR/ABL-1 of <10% at 6 months or less. Bee et al concluded that patients with an EMR had a significantly better OS and EFS compared to those who failed an EMR; interestingly,
attaining a CCyR at 12 months and an MMR at 18 months offered no survival advantage to the patients.

Taking all our data together, we concluded that molecular testing has a better predictive value and can be sufficient to monitor TKI therapy response. Our study however, has several limitations. For one, our patient cohort size was limited to 63, of which even fewer patients had complete data available for molecular and cytogenetic status at specified time points. Secondly, the patients needed to be followed after the 12 month milestone in order to assess whether the molecular response in these patients would translate into long term overall survival (OS) and PFS. For instance, in a recent meta-analysis conducted by Oriana et al (2016), CCyR and MMR observed after 12 months of therapy was successfully used to predict long time survival in the patients. In the IRIS trial also, patients with an MMR had a significantly higher rate of PFS compared to those that did not achieve any MMR at 12 months (Hughes et al., 2003); while the rate of OS was similar in both groups in a longer 8-year follow-up (Deininger, 2009).

There are however several caveats to relying only on the molecular response to monitor TKI therapy. Novel leukemic clones with chromosomal abnormalities other than the BCR/ABL-1 rearrangement may appear which would be undetectable by the RT-PCR approach (Ross, 2006). In fact studies have shown that TKI therapy itself can trigger chromosomal breakages and other instabilities (Chakraborty, 2012). Furthermore, in two separate single institute studies conducted in the USA
(Kantarjian, et al., 2008; de Lavallade, 2008) the investigators found no clinically significant difference in the OS of patients receiving imatinib therapy regardless of whether they achieved an MMR or not – this apparent lack of a prognostic effect of molecular response could possibly be the loss of a CCyR later in the therapy.

In another study of Kantarjian et al (2009), the increase in the levels of BCR/ABL-1 transcript levels was not clinically relevant in the presence of a sustained cytogenetic response. Taking cognizance of these findings, we would recommend that cytogenetic testing could be used as base line testing and in cases where MMR is never achieved or is lost (depicted by >1-log increase in BCR/ABL-1 levels).

In conclusion, a molecular response could predict a subsequent cytogenetic response while a CCyR milestone was not an effective indicator of therapeutic progress in the patient cohort. Since a molecular response was sufficient to determine treatment outcome in our patient group, we propose to substitute the largely redundant cytogenetic testing with the more sensitive and reproducible molecular testing.
Table 1: Table showing the number of patients with the BCR-ABL1 mRNA levels (IS) and the % of Ph+ metaphases at the start of imatinib treatment.

<table>
<thead>
<tr>
<th>Percentage of BCR-ABL1 mRNA (IS)</th>
<th>N/A</th>
<th>&gt;10%</th>
<th>1-10%</th>
<th>0.1-1%</th>
<th>&lt;0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;35%</td>
<td>14</td>
<td>25</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1-35%</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 3: The cytogenetic response of patients demarcated by major molecular response at 12 months post treatment.

(a) From a total of 51 patients with evaluable molecular response at 12 months, 49% showed an MMR while 51% did not achieve any MMR. (b) In the MMR+ cohort, 5 patients had cytogenetic data available and all tested positive for a CCyR. Amongst the MMR+ patients, 60% of the cytogenetically evaluable patients showed a CCyR at 12 months while 40% were negative for a CCyR.
Conflict of interest:
The authors declare no conflict of interest.

References
4. Bee PC, Sekaran V, Ng R, Kweh YT, Gan GG. The predictive value of early molecular response in chronic myeloid leukaemia patients treated with imatinib in a single real-world medical


