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Long-term Follow-up Results of Chronic Myeloid Leukemia by RQ-PCR Monitoring of BCR-ABL Transcripts in Imatinib Era - A Single Institutional Experience

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder associated with the Philadelphia chromosome and peripheral leukocytosis which prior to the imatinib era, eventually led to acute leukemia within 3-5 years. According to current treatment guidelines, the monitoring of molecular response by RQ-PCR has been considered an important part of management of patients on tyrosine kinase inhibitor (TKI) therapy. **Patients and Methods:** This retrospective study aimed to evaluate the characteristics and treatment outcomes of CML patients treated at our institution from July 2004 until February 2012. The molecular response was monitored by RQ-PCR, and the impact of early molecular response on overall survival (OS) and event free survival (EFS) was also analyzed. **Results:** A total of 50 patient records were reviewed. The mean age was 43.5 years. Forty patients (80%) were diagnosed as CML in CP, while 4 (8%) were in AP and 6 (12 %) in BC. Patients with CML in CP had significantly longer mean survival of 109.4 months, compared with 58.5 months in AP and 48.9 months in BC groups (p=0.001). There was no significant OS benefit associated with MMR at 12 months (p=0.86) and 18 months (p=0.69). Early reduction of more than 10% of BCR-ABL transcripts at 3 months was related to high probability of achieving MMR at 12 and 18-month landmarks. In addition, MMR at 18 months and 10% or greater BCR-ABL reduction at 3 months were significantly associated with durable EFS (p=0.011 and p=0.015 respectively). **Conclusions:** The current analysis in our cohort of patients from Taiwan confirmed the efficacy and safety of imatinib therapy seen in larger randomized trials in CML patients. Early achievement of molecular response improved durable EFS, but not OS.

Key words: chronic myeloid leukemia, imatinib, BCR-ABL transcript, RQ-PCR

INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by abnormal expansion of hematopoietic stem cells, and it results in marked peripheral leukocytosis, 30~50% incidence of thrombocytosis and basophilia.[1] The genetic hallmark is the Philadelphia chromosome (Ph) which arises from a reciprocal translocation, t(9;21)(q34;q11) and leads to the production of an active tyrosine kinase, the BCR-ABL fusion protein of 210 KDa in most CML cases within all age groups.[1,3] Based on different breakpoints and mRNA splicing, an isoform of 190 KDa is a more potent oncogene and associated with 15-30% of acute lymphoblastic leukemia (ALL), 2% of acute myelogenous leukemia (AML) and few CML.[1-3]

CML accounts for approximately 20% of all adult leukemia.[4] The age-adjusted annual incidence rate in the United States is 1.75 per 100,000 persons, and the median age is 53-66 years.[1,5] Although the typical symptoms include fatigue, anorexia and weight loss, about 40% of

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patients are asymptomatic. Ninety percent of patients are diagnosed during the indolent chronic phase (CP), which may progress to accelerated phase (AP) or even the rapidly fatal blast crisis (BC) within three to five years.[1,3] Until 12 years ago, the mainstay of treatment for CML included interferon-alfa, chemotherapy and allogeneic hematopoietic stem cell transplantation (alloHSCT), achieving 50-70% of 5-year survival rate in CP, but poorer efficacy in AP or BC.[1,6] Imatinib (Gleevec, Novartis, STI571), a relatively specific BCR-ABL tyrosine kinase inhibitor (TKI), has been approved for newly diagnosed CML in CP based on the results of The International Randomized Study of Interferon and STI571 (IRIS) trial in 2003.[3,6-8] Durable hematological and cytogenetic response, lower progression to AP/BC and superior OS rate of 86% were showed by 7-year follow-up of the IRIS trial.[9] In addition, imatinib and second-generation BCR-ABL TKIs are now also incorporated in the treatment of Ph+ acute leukemia. In the imatinib era, BCR-ABL transcripts monitored every 3 months by real-time quantitative polymerase chain reaction (RQ-PCR) demonstrate prognostic significance, especially when a more than 3-log reduction in BCR-ABL below baseline is detected at 18 months.[9-11] The treatment response of CML by RQ-PCR has been recommended by European Leukemia Net since 2009.[12]

This study aimed to evaluate the clinical characteristics, prognostic factors, molecular response and overall treatment outcomes of CML patients treated at our institution from July 2004 until February 2012.

MATERIAL AND METHODS

Patients and Study design

This retrospective analysis enrolled a total of 50 patients with Ph+ CML documented by cytogenetic analysis or positive BCR-ABL transcripts in the non-quantitative PCR assay at Tri-Service General Hospital between July 2004 and February 2012. The standard RQ-PCR method for peripheral blood or bone marrow material was performed at the time of diagnosis for baseline measurements and repeated every 3 to 6 months. The clinical data collected from medical records included: age and disease status at diagnosis, sex, treatment patterns, survival duration, mutational analysis, and current molecular response to CML treatment. Adverse effects were documented in patients receiving imatinib therapy. In order to evaluate the impact of early molecular response on overall and event free survival outcomes, we also analyzed patients in CP with or without at least 10% reduction of BCR-ABL transcripts at 3 months and the achievement of MMR at 12 months and 18 months. The EFS was defined as the time from the start of treatment until the occurrence any of the following events: (1) loss of complete hematological response (CHR). (2) loss of major cytogenetic response (MCR). (3) progression to AP/BC. (4) death due to any cause.[9]

Definition of treatment response and ELN time-based landmarks[10,12-14]

The initial level of response and the earliest monitoring point is the CHR, defined as the normalization of peripheral blood with absence of splenomegaly. Complete cytogenetic remission (CCyR) indicates no Ph+ cells in bone marrow, which is approximately equivalent to a 2-log BCR-ABL reduction below the baseline. MCR includes CCyR and partial cytogenetic response (pCyR), achieving 1-2 log transcript reduction. More than 3-log reduction of transcripts below baseline is classified as major molecular response (MMR). A reduction of more than 4.5 logs is considered as complete molecular response (CMR). Optimal and suboptimal treatment response of CML were evaluated as per recommendations by the European Leukemia Net.

The RQ-PCR methodology

A total of 10 ml whole blood sample was collected into an EDTA-containing tube for RNA extraction by commercial kits (RNeasy; Qiagen, Hilden, Germany). We used two-step reverse transcription polymerase chain reaction procedure according to the manufacturer's guidelines (Applied Biosystems, USA). For all RQ-PCR reactions, the K562 cell line for positive control and water for negative control were amplified simultaneously to exclude any false-positive and false-negative results. RQ-PCR was performed in duplicate using LightCycler TaqMan Master (Roche, Mannheim, Germany), and LightCycler software 2.0. PCR was performed under the following cycling conditions: denaturation at 95℃ for 10 min, 45 cycles at 95℃ for 5 sec, 60℃ for 30 sec, and 72℃ for 2 sec. All primers and probe sequences were chosen as per previous literature.[15,16] In order to construct a standard curve, commercial mutant plasmid DNA (Roche, TIB, Germany) were used in real-time PCR, while the number of target molecules in each sample was calculated by reference to this curve. The quantity of BCR-ABL transcripts was normalized to the ABL expression level, and the result was expressed as the ratio of BCR-ABL copy number to ABL copy number.

Table 1 Baseline characteristics, treatment and Survival of CML

Characteristics	Patients (N=50)
Mean age (yrs)±s.d[1]	43.5±16.1
Current status (n,%)	
Alive	35 (70%)
Death	15 (30%)
Sex (n,%)	
Male	31 (62%)
Female	19 (38%)
Status at diagnosis (n,%)	
Chronic phase	40 (80%)
Accelerated phase	4 (8%)
Blast crisis	6 (12%)
Variants of CML	
P190	0
P210	50 (100%)
Treatment (n,%)	
Imatinib	47 (94%)
Nilotinib	6 (12%)
Dasatinib	5 (10%)
Chemotherapy	3 (6%)
HSCT[2]	3 (6%)
Mean imatinib duration (m)±s.d	44.8±31.2
Mean duration of shifting to 2nd TKIs (m)±s.d	30.0±28.4
Molecular response to treatment (n,%)	
CHR	16 (32%)
MCR	6 (12%)
CCyR	10 (20%)
MMR	7 (14%)
CMR	5 (10%)
Others[3]	6 (12%)
Mean Overall survival (m)±s.d	93.9±7.8
Chronic phase	109.9±7.4
Accelerated phase	58.5±12.3
Blast crisis/ acute leukemia	48.9±18.0

1.s.d=standard deviation

2.Hematopoietic stem cell transplantation

3.Loss of CHR or leukemia

Statistical analysis

Data was analyzed by using the software of SPSS. In addition to descriptive and frequency statistics, multivariate analysis was used to evaluate the significance of the various factors contributing to clinical outcomes in CML patients. Survival curves and EFS were analyzed by the Kaplan-Meier method and compared using log-rank test with $P<0.05$ being defined as statistically significant.

Table 2 Adverse effects of imatinib treatment

Adverse effect	Patients (n,%) Total 47 patients
None	17 (36.2%)
Cytopenia[1]	17 (36.2%)
Arthralgia and soreness	8 (17.0%)
Gastrointestinal upset	5 (10.6%)
Edema	3 (6.4%)
Fatigue	3 (6.4%)
Skin rash	3 (6.4%)
Liver function impairment	2 (4.3%)
Neurological symptoms	2 (4.3%)
Others[2]	2 (4.3%)

1.Anemia, thrombocytopenia or neutropenia

2.Others included one having chronic conjunctivitis and the other one having nontuberculous Mycobacterial infection

RESULTS

Patient characteristics and treatment

From July 2004 onward, a total of 50 CML patients have been recruited in this study, with the mean age of 43.5 years and OS duration of 93.9 months. On average CML affected males more frequently with a male/female ratio of 1.63. Table 1 summarizes the baseline characteristics, treatment types and survival duration of these patients. Forty patients (80%) were diagnosed as having CML in CP at initial presentation, while 4 (8%) were found to have AP and 6 (12 %) BC. BCR-ABL fusion protein of 210 KDa was detected in all CML patients in this study. A total of 47 participants (94%) received imatinib therapy for a mean duration of 44.8 months, with some undergoing chemotherapy (6%), HSCT (6%), or second-generation TKIs such as nilotinib (12%) or dasatinib (10%) for treatment failure or disease progression to AP/BC and acute leukemia. Nine patients received dose escalation of imatinib from standard 400mg once daily to 600mg because of suboptimal response in 5 patients (55.6%) and disease progression in 4 patients (44.4%) respectively. Dose reductions to 200mg or 300mg once daily were observed in 8 patients due to side effects of imatinib. The adverse effects included cytopenias (36.2%), followed by arthralgia and soreness (17.0%) and dyspepsia (10. 6%) (Table 2). The mean duration from imatinib treatment shifting to second-generation of TKIs was 30 months because of disease progression in 6 patients (27.2 months), treatment failure in 2 patients (40.5 months) and intolerance to side effects in 1 patient (26 months) respectively. Dasatinib was mostly common used for disease progression in our analysis. Three of the

Table 3 Clinical factors impacting Survival

Contributing factors	Significance (P value)
	Survival
Status at diagnosis	0.97
Age at diagnosis	0.23
Sex	0.58
Imatinib duration	0.002*
Nilotinib	0.34
Dasatinib	0.62
HSCT[2]	0.96
MMR at 12 months	0.48
MMR at 18 months	0.023*
<10% reduction at 3months	0.73

1. *, P<0.05

2. Hematopoietic stem cell transplantation

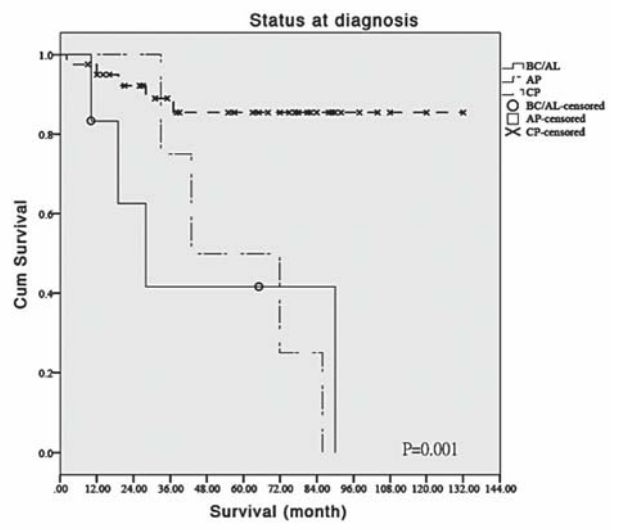

Fig. 1 Overall Survival of CML based on clinical status at diagnosis: patients with CML in chronic phase (CP) at diagnosis had significantly longer mean survival than those in accelerated phase (AP) or in blast crisis (BC).

four non-responding patients had CML mutation analysis, but only one had E255V mutation, which was highly resistant to imatinib.[13] However, twenty-eight patients (56%) had loss of CCyR achievement in current status of molecular response despite treatment although their diseases remained stable conditions and CHR (Table 1).

Effect of response on survival outcome

In the multivariate analysis (Table 3), longer treatment duration of imatinib and achievement of MMR at 18 months significantly correlated with event free survival benefit (p=0.002 and p=0.023 respectively). Patients with

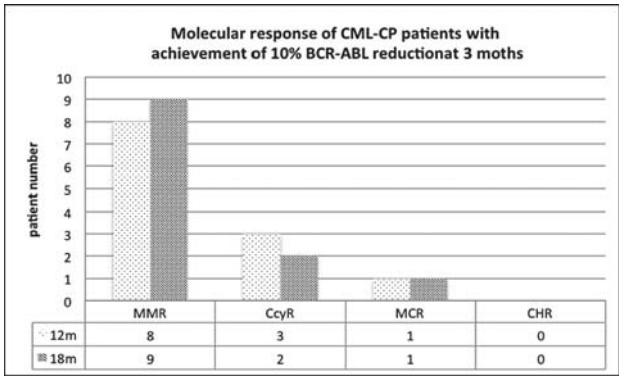

Fig. 2a Early reduction to more than 10% of BCR-ABL transcripts at 3 months was associated with high probability of achieving MMR at 12 and 18-month landmarks.

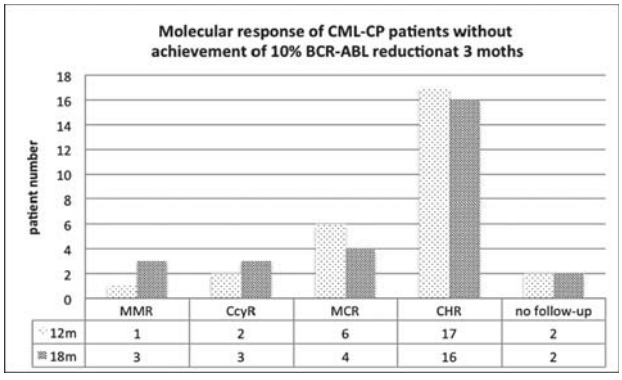

Fig. 2b Patients with less than 10% BCR/ABL reduction at 3 months had low probability of achieving MMR at 12 and 18-month landmarks.

CML in CP at diagnosis had significantly longer mean survival of 109.4 months, compared with 58.5 months in AP and 48.9 months in BC groups (p=0.001; Figure 1). The CP group also demonstrated excellent 5-year survival rate of 81.5% and 8-year survival rate of 77%. A total of 40 cases were finally recruited to evaluate the effect of early molecular response by imatinib in CP. Three cases were excluded due to no BCR-ABL measurements at 12 or 18-month landmarks. There was no significant survival difference between patients associated with the achievement of MMR at 12 months (p=0.86). At 18 months on the other hand, there was improved 5-year survival rate of 90.9% in patients who achieved MMR, compared with 86.2% in those that did not. However, the overall comparison between two groups showed no statistical difference (p=0.69). Also, early reduction to more than 10% of BCR-ABL transcripts at 3 months was related to high probability of achieving MMR at 12 and 18-month landmarks (Figure 2a; 2b), although no overall

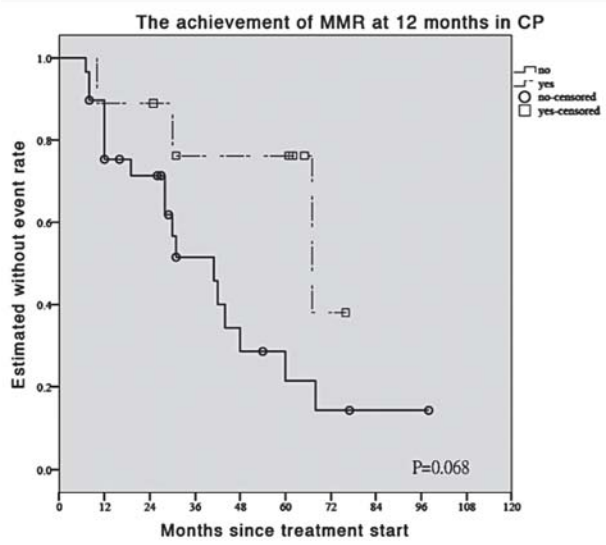

Fig. 3a The impact of MMR by 12m on event free survival in CML-CP patients

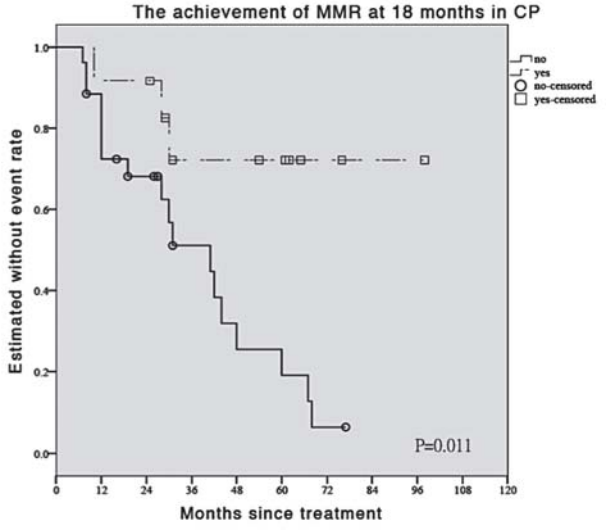

Fig. 3b The impact of MMR by 18m on event free survival in CML-CP patients

survival difference was observed in the analysis (p=0.54).

In analysis of EFS, a total of 21 cases were recorded with the first event occurring during treatment, including 19 with loss of MCR and 2 with progression to AP/BC. At the 12-month landmark, the achievement of MMR seemed to have only a trend toward increased EFS, compared with those without MMR (p=0.068; Figure 3a). However, Figure 3b and 3c show that the achievement of MMR at 18 months and 10% BCR-ABL reduction at 3

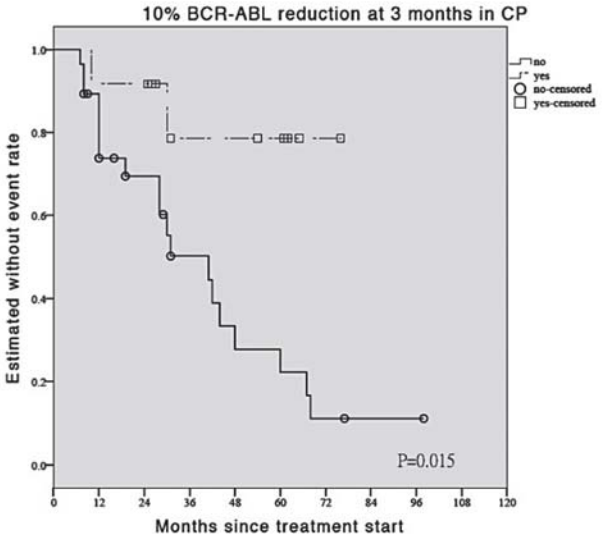

Fig. 3c The impact of more than 10% BCR-ABL reduction at 3 months on event free survival in CML-CP patients

months were significantly associated with durable event free survival benefit (p=0.011 and p=0.015 respectively).

DISCUSSION

CML is a commonly diagnosed hematologic malignancy in Asia, and appears to have an average annual incidence of 0.8~1.1 cases per 100,000 persons, which is lower than the 1.75 cases per 100,000 reported in the USA.[17,18] At our institution, CML patients had a younger mean age of 43.5 years than Caucasians, and was similar to the age of 45 years reported in another Asian study.[18] A similar gender distribution with a male/female ratio 1.63 was also noted. Since 2004, the technique of monitoring CML treatment by RQ-PCR was developed at our hematological laboratory to assist in the decision making of clinical physicians, though some CML patients did not receive RQ-PCR every 3 months in this study. Notably, only 64% of medical centers do repeat RQ-PCR routinely in Asia.[18]

This retrospective study presented the unique experience of long-term follow-up results in CML patients from a single institution. We noted a favorable result with 8-year OS rates of 77% in CP, although it was inferior to the result of 85% reported at 8-year follow-up of IRIS study.[19] There are several potential reasons for this. First, we enrolled not only newly diagnosed CML-CP patients, but also 6 cases that were refractory to interferon and

hydroxyurea in this study. Kantarjian *et al* reported 68% 10-year OS in CML patients treated by imatinib after interferon therapy.[20] One study showed that around 31% of CML patients had mutations with imatinib resistance after interferon failure, which contributes to poor prognosis and disease progression to AP/BC.[21] Second, nine patients (22.5%) had poor drug compliance documented in their charts and eight patients (20%) received reduced doses of imatinib below 400mg once daily due to side effects of imatinib. Nevertheless, the incidence of the adverse effects in our patients, such as cytopenias and arthralgias, were lower than in IRIS study. Third, a total of 28 cases (70%) in CP were treated without the use of the monitoring guidelines recommended by European Leukemia Net or NCCN to achieve optimal response, including a CHR at 3 months, a pCyR at 6 months, a CCyR at 12 months and a MMR at 18 months.[12] Therefore, only 35% of CML-CP patients achieved more than CCyR at 12 months and 30% had MMR at 18 months. Although only 10 cases were diagnosed as having AP/BC, it was notable that the median OS of 43 months in AP group was the same as that in STI517 0109 trial, but the BC group had longer median OS of 28 months as compared to 7 months in the STI571 0110 trial.[22] Longer duration of survival in these patients may be due to treatment with second-generation TKIs (dasatinib or nilotinib), best supportive care and high dose chemotherapy followed by HSCT, which has been shown to improve survival outcomes.[23,24]

The IRIS trial concluded that failure to achieve MMR at 12 months or 18 months of treatment initiation was shown to decrease the probability of EFS and freedom from progression to AP/BC, but had no significant impact on OS in a 7-year follow-up.[9,13] In addition, Cortes et al. found that patients who had less than 1-log reduction after 3 month of imitinb therapy had a 55% chance of achieving a MMR at 2 years, compared to patients with more than 1-log or more than 2-log reduction, in whom a MMR was achieved in 84% and 95% respectively.[25] More durable cytogenetic response reduced the possibility of disease progression, which was considered to reduce the probability of drug resistance to second-line TKIs or even chemotherapy followed by HSCT. The same results were also observed in the subgroup analysis in our study. However, due to the limitations inherent in the retrospective design of our study, one has to be very careful in interpreting these survival results because of uneven case numbers, heterogeneity of patients, and different dosages of imatinib.

BCR-ABL kinase domain mutation is the main mechanism associated with resistance to TKIs in CML patients. The sites of mutations are mostly clustered within the nine amino acid positions including T315I, Y253H/F, M351T, G250E, E255K/V, F359V, and H396R with varying sensitivities to imatinib. The frequency of mutations in TKI-exposed cases varied from 33% to 63% in an Asian series, and were more common than in newly diagnosed CML patients who had an incidence of 0-20%.[26] In our study, mutation analyses were performed in only 3 CML patients, and one with E255V mutation died because of leukemic transformation, which was refractory to imatinib, dasatinib and high-dose chemotherapy. However, the mutation tests were not routinely used in our institute, even when (1) BCR-ABL transcripts were elevated. (2) disease progression occurred on therapy or (3) the second-generation TKIs were used. Existence of unknown mutations could obviously affect molecular response and treatment outcomes. In addition to the mutation issue, there are several questions that have not been adequately answered, including optimal initial imatinib dose, the efficacy of frontline treatment with second-generation TKIs and timing of discontinuation of TKIs. Randomized prospective studies and long-term follow up are needed to bring better clarity to these important management issues.

CONCLUSION

In summary, the current analysis confirmed the efficacy and safety of continued imatinib therapy in Taiwanese patients with CML at our institution, with favorable 8-year OS of 77%. Early achievement of molecular response improved EFS and cytogenetic response, although there was no effect on OS in our subgroup analysis. Regular follow-up of BCR-ABL transcripts by RQ-PCR helped us identify treatment response at various benchmark time points thereby guiding mutational testing and the decision to switch to second-generation TKIs.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

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