Comparison of molecular responses and outcomes between BCR::ABL1 e14a2 and e13a2 transcripts in chronic myeloid leukemia

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Abstract
Several studies have compared the molecular responses between e14a2 and e13a2 BCR::ABL1 transcripts in chronic myeloid leukemia (CML) patients treated with frontline imatinib, but there were very limited studies on nilotinib or dasatinib-treated patients. We retrospectively analyzed the molecular responses in 1124 CML patients with the e14a2 or e13a2 transcript receiving frontline imatinib, nilotinib, or dasatinib treatment. Patients with the e14a2 transcript had higher optimal response rates than those with the e13a2 transcript at 12 months in the imatinib-treated group, and 6 and 12 months in the nilotinib-treated group. The optimal response rates were not significantly different between the two transcripts in the dasatinib-treated group at landmark molecular responses. With a median follow-up time of 48.4 months, higher cumulative incidences of BCR::ABL1 International Scale ≤1% and major molecular response were observed in patients with the e14a2 rather than the e13a2 transcript.
receiving front-line imatinib or nilotinib treatment, but not in dasatinib-treated patients. The progression-free survival and overall survival did not differ between the two transcripts in all three treatment groups. In view of the speed and depth of molecular responses, BCR::ABL1 transcript subtypes might provide helpful information in selecting a front-line tyrosine kinase inhibitor for individual young patients with future potential treatment-free remission.

KEYWORDS
chronic myeloid leukemia, molecular response, survival, transcript, tyrosine kinase inhibitor

1 | INTRODUCTION

Chronic myeloid leukemia (CML) is characterized by the presence of the BCR::ABL1 fusion gene, formed by a reciprocal t(9;22)(q34;q11.2) translocation between chromosomes 9 and 22. The BCR::ABL1 oncogene translates into an oncoprotein with constitutive tyrosine kinase activity, which is central to the pathogenesis of the disease. The location of breakpoints in the chromosome 22 cluster within a small (5.8 kb) region, spanning exons 12–16, known as the major breakpoint cluster region. The breakpoint in the ABL1 gene is usually located between exons a1 and a2. These breakpoints result in various BCR::ABL1 rearrangements. In the great majority of patients with CML, transcript variants of e14a2 and e13a2 are expressed. The two transcript subtypes differ in length by 75 bp (25 amino acids), which codes for a 210-kDa protein: p210.

Several studies have investigated the impact of BCR::ABL1 transcript subtypes on the outcomes of patients with CML who mainly received imatinib, but revealed conflicting results. Most studies showed that patients with the e14a2 transcript had faster and deeper response to imatinib than those with the e13a2 transcript, but one study reported no influence on molecular response.

Only limited data have been available for the impact of different BCR::ABL1 transcript subtypes on molecular responses and outcomes in patients with CML who were treated with second generation (2G) tyrosine kinase inhibitors (TKIs). To the best of our knowledge, only three studies have analyzed patients who received front-line nilotinib treatment, and only one study enrolled patients receiving front-line dasatinib treatment. However, no direct comparison between the two transcript subtypes was available. The GIMEMA CML Working Party reported a trend of lower major molecular response (MMR) rate by 12 months, molecular response 4.0 (MR4.0) achievement rate by 36 months, estimated cumulative incidences of MMR and MR4, and inferior progression-free survival (PFS) and overall survival (OS) among front-line nilotinib-treated patients carrying the e13a2 transcript compared with those with the e14a2 transcript. Genthon et al. reported similar findings to the GIMEMA CML Working Party: under nilotinib treatment, patients harboring the e14a2 transcript had significantly higher MMR rates at 12 months and faster molecular response (MR4.5) response achievement compared to those with the e13a2 transcript. The study from the MD Anderson Cancer Center enrolled CML patients treated with all three TKIs and found that the expression of the e14a2 transcript predicted improved probability of optimal response at 3, 6, and 12 months according to the European LeukemiaNet criteria and longer event-free survival and transformation-free survival than those of the e13a2 transcript in the multivariate model.

In this study, we retrospectively reviewed the molecular responses of a large number of CML-chronic phase patients who received front-line imatinib, nilotinib, or dasatinib treatment with a long-term follow-up and compared the differences between the e14a2 and e13a2 transcripts in milestone molecular responses monitored in a single reference laboratory.

2 | MATERIALS AND METHODS

2.1 | Patients

Between June 2004 and November 2018, 1191 newly diagnosed adult patients with CML-chronic phase received different front-line TKIs, with molecular diagnosis and BCR::ABL1 monitoring performed at the central laboratory (Chang Gung Memorial Hospital at Linkou, Taiwan). These patients were all Asian and registered in the Taiwan CML Study. Written informed consent was obtained from each patient (IRB No. 96-0358B and No. 100-0927B). BCR::ABL1 transcript subtypes were determined at the time of diagnosis by reverse transcriptase-polymerase chain reaction (RT-PCR) or multiplex RT-PCR assay on cDNA synthesized from total leukocyte RNA. Only patients who carried BCR::ABL1 transcripts of either e14a2 (n = 767) or e13a2 (n = 357) type were enrolled; a combination of e14a2 and e13a2 (n = 53) or other rare subtypes of e14a3, e13a3, e19a2 and e1a2 (n = 14) were excluded from analysis. Of the 1124 patients with e14a2 or e13a2 subtype, 752 received imatinib, 195 received nilotinib, and 177 received dasatinib as front-line treatment. After 2012, choice of any of the three TKIs was at the doctor’s discretion, and all were reimbursed by the National Health Insurance in Taiwan (Figure S1). Five patients rapidly progressed to blast crisis after diagnosis within 12 months, ranging from 3.4 to 11 months. Four of these five patients were treated with imatinib and one with nilotinib. Following TKI therapy, BCR::ABL1 levels in the peripheral blood were measured by using the real-time quantitative RT-PCR with TaqMan assay every
3 months with 2-week interval variation allowed at the central laboratory, and results were expressed as International Scale (IS) with the laboratory-specific conversion factor, which was obtained from the reference laboratory at Adelaide, Australia, and we also retrospectively converted all the data examined before 2006 to IS. Optimal molecular response criteria based on the 2013 European LeukemiaNet were adopted: major cytogenetic response (IS < 10%) at 3 months, complete cytogenetic response (CCyR; IS < 1%) at 6 months, and MMR (IS ≤ 0.1%) at 12 months. IS > 10% at 6 months and > 1% from 12 months onward defined treatment failure. The deep molecular response was defined as follows: MR^4.0, detectable disease with < 0.01% and > 0.0032% BCR::ABL1IS or undetectable disease in cDNA with > 10,000 ABL1 transcripts, and MR^4.5, detectable disease with < 0.0032% BCR::ABL1IS or undetectable disease in cDNA with > 32,000 ABL1 transcripts in the same volume of cDNA used to test for BCR::ABL1. MR^4.0 and MR^4.5 were validated through a proficiency test of the College of American Pathologists and Secondary Reference Panel (Lyo panel) Study II. The TKI adherence was measured by using the ratio of the total days' supply of the index TKI across all prescription fills during the refill interval to the total number of days in that interval. According to previous study, the ratio less than 85% was considered as low adherence. PFS was measured from the date on which treatment was started to the date when the disease progressed to the accelerated phase or blast crisis. OS was calculated from the time of treatment initiation to the date of death from CML-related cause or the last molecular response follow-up date.

### 2.2 Statistics analysis

The baseline clinical data of patients and their disease characteristics were reviewed. The differences were analyzed using \( \chi^2 \) tests, Fisher's exact tests, and Wilcoxon's rank-sum test when appropriate. The optimal response rates at 3, 6, and 12 months were compared by using \( \chi^2 \) tests. Gray's test was used for estimate and comparison of cumulative incidences of CCyR, MMR, MR^4.0, and MR^4.5 under consideration of competing risks defined by TKI switch or death. PFS and OS were analyzed by using the Kaplan–Meier method, and the differences were compared using the log-rank test. All tests were two-tailed, and \( P < 0.05 \) was considered to represent a statistically significant difference. Statistical analysis was conducted by using IBM SPSS Statistics version 20 and the R4.1.2 software package.

### 3 RESULTS

#### 3.1 Patient characteristics

The comparison of clinical characteristics at diagnosis among patients with different BCR::ABL1 transcript subtypes in the 3 TKI-treated groups is summarized in Table 1. The proportion of patients carrying the e14a2 transcript was higher than that of the e13a2 transcript in all three TKI-treated groups. There was no statistically significant difference in the median age, male to female ratio, TKI mean dose, adherence, Sokal risk groups, or median follow-up time between the two transcript subtypes in all three TKI-treated groups. Patients carrying the e13a2 transcript had a higher rate of disease progression and death than those with the e14a2 transcript in all TKI-treated groups, particularly in the imatinib group, but the difference was statistically nonsignificant in each subgroup.

#### 3.2 Molecular responses at treatment milestone

The comparison of optimal molecular response rates between the two transcripts at 3, 6, and 12 months in the three TKI-treated groups is illustrated in Figure 1. In the imatinib-treated group, patients with the e14a2 transcript had a higher optimal response rate at 12 months than those with the e13a2 subtype (38% vs. 27%, \( P = 0.010 \)) (Figure 1(A)). The optimal response rates were significantly higher in the nilotinib-treated patients with e14a2 than e13a2 at 6 months (78% vs. 60%, \( P = 0.020 \)) and 12 months (84% vs. 58%, \( P = 0.002 \)) (Figure 1(B)). There was no statistically significant difference in the optimal response rates between the two transcripts in the dasatinib-treated group at 3, 6, and 12 months (Figure 1(C)). A higher treatment failure rate was observed in the imatinib-treated patients with the e13a2 than the e14a2 transcript at 6 and 12 months (22% vs. 35%, \( P = 0.0002 \) and 33% vs. 46%, \( P = 0.003 \), respectively) (Figure 1(A)). By contrast, the failure rates at 6 and 12 months did not differ between the two transcripts in the nilotinib- and dasatinib-treated groups (Figure 1(B,C)). For the deep molecular responses of MR^4.0 and MR^4.5 (Figure S2), the response rates at 3, 6, and 12 months were comparable between the two transcripts in all three TKI groups.

#### 3.3 Cumulative incidences of molecular responses

The cumulative incidences of CCyR, MMR, MR^4.0, and MR^4.5 are shown in Figures 2 and 3. In the imatinib-treated group, those carrying the e14a2 transcript had a higher cumulative incidence of CCyR (\( P = 0.001 \)) and MMR (\( P = 0.002 \)) compared to patients with e13a2 (Figure 2(A,B)). A significantly higher cumulative incidence of CCyR (\( P = 0.041 \)) and MMR (\( P = 0.0006 \)) was also found in patients with e14a2 who were treated with nilotinib (Figure 2(C,D)), whereas the cumulative incidences of CCyR and MMR did not differ between the two transcripts in the dasatinib-treated patients (Figure 2(E,F)).

Further comparison of the cumulative incidence of MMR among the three TKI-treated groups by transcript subtypes is illustrated in Figure 2(G,H). Higher cumulative incidences of MMR at 12, 24, 36, and 60 months were observed in 2G TKI treatment compared with imatinib-treated patients in both subtypes (\( P < 0.01 \)). The cumulative incidences of MMR at these time points were not significantly different between nilotinib- and dasatinib-treated patients in both transcript subtypes.
| Characteristics                      | Imatinib (N = 752) | Nilotinib (N = 195) | Dasatinib (N = 177) |
|-------------------------------------|--------------------|---------------------|---------------------|
|                                     | e14a2  | e13a2  | P value | e14a2  | e13a2  | P value | e14a2  | e13a2  | P value |
| Patient numbers                     | 499     | 253     | 0.672   | 143     | 52     | 0.572   | 125     | 52     | 0.603   |
| Median age (range) (years)          | 47.7 (18–88) | 46.8 (18–87) | 0.697   | 49.5 (20–89) | 46.2 (21–80) | 0.418   | 45.2 (18–76) | 43.6 (19–92) | 0.869   |
| Male/female                         | 279 (56%) /220 (44%) | 146 (58%) /107 (42%) | 0.672   | 66 (46%) /77 (54%) | 28 (54%) /24 (46%) | 0.572   | 65 (56%) /38 (44%) | 25 (58%) /17 (42%) | 0.569   |
| Mean dose (mg/day)                  | 389     | 406     | 0.124   | 555     | 564     | 0.565   | 94      | 89      | 0.569   |
| Low adherence                       | 29/363 (8.0%) | 8/187 (4.2%) | 0.109   | 3/112 (2.7%) | 1/36 (2.8%) | 1.000   | 2/101 (2.0%) | 1/41 (2.4%) | 1.000   |
| Sokal risk group                    |                     |                     |         |                     |                     |         |                     |                     |         |
| Low                                 | 141 (39.6%) | 76 (40.9%) | 0.961   | 25 (19.2%) | 12 (25.0%) | 0.701   | 27 (25.2%) | 12 (26.7%) | 0.572   |
| Intermediate                        | 119 (33.4%) | 61 (32.8%) |         | 56 (43.1%) | 19 (39.5%) |         | 45 (42.1%) | 15 (33.3%) |         |
| High                                | 96 (27.0%) | 49 (26.3%) |         | 49 (37.7%) | 17 (35.4%) |         | 35 (32.7%) | 18 (40.0%) |         |
| Median follow-up time (range) (months) | 52.2 (3.1-168.0) | 49.2 (3.4-162.6) | --      | 36.3 (6.1-112.6) | 35.1 (3.2-117.2) | --      | 39.2 (6.0-102.2) | 36.2 (3.4-121.3) | --      |
| Median time to MMR (range) (months) | 11.8 (2.3-70.0) | 13.0 (3.7-79.6) | 0.527   | 5.9 (3.0-44.3) | 7.0 (2.9-28.7) | 0.189   | 6.4 (2.6-29.4) | 6.4 (2.8-23.7) | 0.975   |
| Median time to MR^4.0^ (range) (months) | 22.0 (3.7-96.0) | 20.4 (5.3-96.3) | 0.642   | 11.6 (3.2-59.8) | 10.8 (5.3-56.6) | 0.453   | 10.6 (4.3-77.7) | 14.2 (5.5-65.2) | 0.326   |
| Median time to MR^4.5^ (range) (months) | 33.0 (3.7-92.0) | 36.0 (5.3-87.0) | 0.571   | 14.8 (3.2-69.1) | 17.3 (5.8-59.4) | 0.372   | 19.8 (5.5-65.1) | 19.1 (5.5-47.5) | 0.726   |
| AP/BC progression                   | 25 (5.0%) | 22 (8.7%) | 0.056   | 3 (2.1%) | 2 (3.8%) | 0.611   | 3 (2.4%) | 3 (5.8%) | 0.361   |
| Death (%)                           | 18 (3.6%) | 16 (6.3%) | 0.097   | 3 (2.1%) | 2 (3.8%) | 0.611   | 2 (1.6%) | 3 (5.7%) | 0.152   |

Abbreviations: AP, accelerated phase; BC, blast crisis; MMR, major molecular response.
The median time to achieve MMR, MR<sup>4.0</sup>, and MR<sup>4.5</sup> and the cumulative incidences of MR<sup>4.0</sup> and MR<sup>4.5</sup> were not statistically different between the two transcripts in all TKI-treated groups (Table 1 and Figure 3).

To exclude the possibility that more recent patients were prone to receive 2G TKIs, which might affect the results, we reanalyzed imatinib-treated patients diagnosed after 2012. As shown in Figure S3(A,B), a similar pattern of cumulative incidences of CCyR
and MMR was observed in the whole cohort of patients treated with imatinib from 2004 onwards (n = 752) and after 2012 (n = 248). Likewise, the cumulative incidence of MMR was significantly lower in imatinib-treated patients compared with that of nilotinib- or dasatinib-treated patients between the two transcript subtypes (Figure S3(C,D)).

### 3.4 Long-term outcomes

The 5-year PFS between the two transcript subtypes was not significantly different in all three TKI-treated groups (P = 0.119 for imatinib, P = 0.445 for nilotinib, and P = 0.245 for dasatinib; Figure 4(A–C)). Five-year OS also did not significantly differ between the two transcripts in all three TKI-treated groups (P = 0.136 for imatinib, P = 0.454 for nilotinib, and P = 0.104 for dasatinib; Figure 4(D–F)).

### 3.5 Molecular responses and outcomes by Sokal risk group

Patients receiving different TKI treatments were stratified according to the Sokal risk group (Table 1). We compared the molecular responses and outcomes between the patients with two transcript subtypes in different Sokal risk groups (Table S1). The optimal response rate at 3 months was not significantly different between the two transcripts for all three Sokal risk groups in the three TKI-treated patients. The 2-year MMR rates were significantly higher in the e14a2 than in the e13a2 transcript in low and intermediate Sokal risk groups (low: 61.0% vs. 44.3%, P = 0.024; intermediate: 55.5% vs. 37.7%, P = 0.028) in the imatinib-treated patients. The 2-year MMR rate was significantly higher in the high risk group carrying the e14a2 rather than the e13a2 transcript (66.1% vs. 43.2%, P = 0.031) in patients treated with front-line nilotinib but not in those treated with dasatinib. There were no statistically significant differences in the 3-year MMR, 5-year PFS, or OS between the two transcripts regardless of the Sokal risk groups across all three TKI-treated patients.

### 4 DISCUSSION

A recent CML study of an international cohort from 45 countries (n = 45,503), including our Taiwanese cohort, reported that the proportion of transcript subtypes might differ to some extent across ethnicities. To the best of our knowledge, the present study is the...
first report on nationwide multicenter research in Asia comparing the difference in molecular responses and outcomes between the e14a2 and e13a2 transcripts in patients with CML who were treated with different TKIs. In our study, more patients carried the e14a2 transcript than the e13a2 transcript in all three TKI-treated groups. Our findings were in line with the most previous reports. In our study, the optimal response rates at 3 and 6 months were comparable between e14a2 and e13a2 patients who received front-line imatinib treatment, but the difference became statistically significant at 12 months. A significantly higher optimal molecular response rate at 3 and 6 months was observed in patients with e14a2 compared to those with e13a2 in the nilotinib-treated group, whereas the response rates were not significantly different between the two transcript subtypes in dasatinib-treated patients. Previous studies showed that patients who carried the e14a2 transcript receiving front-line imatinib treatment had a higher optimal response rate at landmark monitoring (Table S2). A higher MMR rate at 12 months was also observed in patients with the e14a2 transcript in the two previous studies of front-line nilotinib-treated patients, but Gentthon et al. reported the optimal response rates were not significantly different between the two transcripts at 3 and 6 months. We did not find any statistically significant difference in optimal response rates at 3, 6, and 12 months between the two transcript subtypes in front-line dasatinib treatment as had been reported by Jain et al., in which it should be noted that more patients carried the e13a2 transcript than the e14a2 transcript, which contrasts with our cohort and all other series. The distribution of Sokal risk group was different in different study groups (Table S2). Thus, ethnic differences and different patient characteristics might contribute to the conflicting results.

Our study showed that the cumulative incidences of CCyR and MMR at 12, 24, and 36 months were higher in patients with the e14a2 transcript than patients with e13a2 receiving front-line imatinib or nilotinib treatment, but the cumulative incidences were not significantly different between the two transcript subtypes in dasatinib-treated patients. Achieving an MMR predicts a CML-specific survival close to 100% as disease progression is uncommon once this level of molecular response has been achieved. The probability of MMR achievement was lowest in patients

![FIGURE 4 Kaplan–Meier assessment of progression-free survival and overall survival in front-line imatinib-treated patients (A, B), nilotinib-treated patients (C, D), and dasatinib-treated patients (E, F).]
treated with imatinib compared with the 2G TKI treatment, irrespective of transcript type. The cumulative incidence of MMR was not statistically significant different between nilotinib- and dasatinib-treated patients. We observed that patients with the e14a2 transcript reached an optimal response earlier than patients with e13a2, but the long term PFS and OS were not statistically significant different between the two transcripts, as described by other investigators (Table S2).7–12,14 All these studies were based on retrospective analyses. Clinicians might change TKIs if treatment responses were suboptimal, thus PFS and OS will not be affected by the transcript type.

The mean daily doses of the three TKIs and adherence rates in the present study were not statistically significant different between the two transcripts in each treatment group. The underlying mechanism of the difference in the molecular responses between the two transcript subtypes was unclear. The possible explanation might be the different biological features of the two transcripts. The p210 BCR::ABL1 oncoprotein isoforms, e14a2 and e13a2, differ in size, with a 25 amino acid insertion coded by the e14 exon and a Glu903Asp substitution between e14a2 and e13a2. The secondary structural elements of the two proteins show differences in α-helices and β-strands which relate to differences in the Src homology 1 (SH1)-, SH2-, SH3-, and DNA-binding domains of the p210 protein. In addition, the modification in the SH3 region of ABL1 affects BCR::ABL1 catalytic efficiency and leukemogenic ability, probably resulting in the different roles of the two isoforms in mediating signal transduction.24,25 In addition, Lucas et al. discovered phosphorylation of the CT10 regulator of kinase-like adaptor protein (CrKL), an adaptor protein that consists of an SH2 domain and two tandem SH3 domains in the absence of a catalytic domain. The pCrKL/CrKL ratio was used to measure BCR::ABL1 tyrosine kinase activity,26 and a higher level was found in patients with e13a2 at diagnosis.5 These findings were based on imatinib-treated patients, and investigation focusing on 2G TKIs remains to be explored.

Other investigators showed the conformation differences of the BCR::ABL1/TKI complex.27 The imatinib and nilotinib bind to the inactive conformation of ABL1. On the contrary, the affinity of dasatinib for BCR::ABL1 drastically decreases in the order active>alternative>inactive as a result of differential contributions from the single residues lining the kinase binding pocket.28 The difference in the BCR::ABL1 conformation and TKI affinity might partially explain why the molecular responses between the two transcript subtypes existed in the nilotinib-treated group but not in the patients receiving dasatinib treatment.

Previous study found that JAK/STAT, PI3K/AKT, and Ras/MEK signaling proteins are at the forefront of pathogenic signaling via BCR::ABL1.29 Dasatinib has been developed as a dual SRC/ABL inhibitor, and is 325 times as potent as imatinib in vitro with much higher number of protein kinases interaction.30 This might explain in part the better treatment response in e13a2 patients receiving dasatinib treatment than the other two TKIs. Clark et al.31 reported that the e14a2 junctional sequences KQSSKALQR and GFKQSSKAL are bound to HLA-A3/A11 and HLA*B8, respectively, and these peptides induced cytotoxic T-cell responses, which might kill HLA-matched CML leukemic cells. Dasatinib could inhibit T-cell activation and proliferation as well as cytokine production and degranulation.32,33 We hypothesized that the molecular responses were comparable between the two transcripts in dasatinib-treated patients because the cytotoxic T-cell responses to HLA-matched CML leukemic cells were inhibited by the e14a2 transcript, which in turn might abolish its advantage. However, all these hypotheses merit further investigation.

Nowadays, most newly diagnosed CML patients have normal life expectancy under front-line TKI treatment. The identification of patients with the potential of discontinuation of TKI treatment is a rapidly developing research field. Young female CML patients may consider starting a family and planning for pregnancy. The teratogenicity of TKIs is related to off-target, most likely platelet-derived growth factor receptor (PDGFR), inhibition during organogenesis. All TKIs are contraindicated throughout pregnancy.34 We found no impact on molecular responses among different age groups. Despite this, the BCR::ABL1 transcript type could serve as one of the parameters for selecting TKIs in newly diagnosed CML patients. For young female patients who are planning pregnancy and intend to discontinue TKI treatment, our results suggest that patients harboring the e13a2 transcript might benefit from dasatinib therapy to achieve rapid deep molecular response with shorter treatment duration. However, the impact of transcript subtype on the discontinuation of TKI therapy requires further prospective studies in a large number of patients across different ethnicities for confirmation.

In summary, the present study showed that BCR::ABL1 transcript subtypes affected the optimal responses at milestone landmarks in imatinib- and nilotinib-treated patients, but no significant difference was observed in dasatinib-treated patients and there was no influence on long-term outcomes in all three TKI-treated groups. In view of the different speed and depth of molecular responses among various TKIs, our results might provide helpful information in selecting the most appropriate front-line TKI for individual young CML patients at initial diagnosis, especially for the future potential of treatment-free remission.

**AUTHOR CONTRIBUTIONS**

L.Y.S. designed the study and conducted the research. M.C.K., T.Y.C., M.C.W., Y.S.Y., M.C.M., T.L.L., H.C., C.L.J.T., P.C.H., C.C.C., P.N.W., and L.Y.S. provided patient samples and their clinical data. M.C.K., T.H.L., and L.Y.S. collected and assembled data. L.Y.S., Y.J.S., M.C.K., and T.H.L. analyzed and interpreted the data. Y.J.S. and L.Y.S. wrote the manuscript. All authors read and approved the final manuscript.

**ACKNOWLEDGMENTS**

The authors would like to thank the following clinicians for recruiting patients: Chang Gung Memorial Hospital at Kaohsiung, Kaohsiung, Taiwan: Ching-Yuan Kuo, MD; E-Da Hospital, Kaohsiung, Taiwan: Sung-Nan Pei, MD; Chang Gung Memorial Hospital at Keelung,
Keelung, Taiwan: Yen-Ming Huang, MD; Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan: Jin-Hou Wu, MD, Yu-Shin Hung, MD, Hsiao-Wen Kao, MD; Taichung Veterans General Hospital, Taichung, Taiwan: Tseng-Hsi Lin, MD; Ditmanson Medical Foundation Chia-Yi Christian Hospital, Chia-Yi, Taiwan: Ming-Yang Li, MD; Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan: Ming-Sun Yu, MD; Mackay Memorial Hospital, Hsinchu, Taiwan: Hung-I Cheng, MD.

FUNDING INFORMATION
This work was supported by grants from Chang Gung Memorial Hospital, Taiwan (CMRPG350071 and XMRPG1A0081) and partly supported by MOST108-2314-B-182-006 and MOHW109-TDU-B-212-134011, Taiwan.

DISCLOSURE
Lee-Yung Shih received research support from Novartis (Taiwan) Co., Ltd. The other authors disclosed no conflict of interest related to this work.

ETHICS STATEMENT
Approval of the research protocol by an Institutional Reviewer Board: Chang Gung Memorial Hospital at Linkou, committees of ethics of human research (IRB No. 96-0358B and No. 100-0927B).

INFORMED CONSENT
Written informed consent was provided by all participants.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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**How to cite this article:** Su Y-J, Kuo M-C, Chen T-Y, et al. Comparison of molecular responses and outcomes between BCR::ABL1 e14a2 and e13a2 transcripts in chronic myeloid leukemia. *Cancer Sci*. 2022;113:3518-3527. doi: 10.1111/cas.15501