Cytogenetic landscape and impact in blast phase of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy

Z Chen1,2, C Shao3, W Wang1, Z Zuo1, X Mou4, SJ Hu5, JA DiGiuseppe6, Y Zu7, LJ Medeiros1 and S Hu1

The landscape of additional chromosomal alterations (ACAs) and their impact in chronic myeloid leukemia, blast phase (CML-BP) treated with tyrosine kinase inhibitors (TKIs) have not been well studied. Here, we investigated a cohort of 354 CML-BP patients treated with TKIs. We identified +8, an extra Philadelphia chromosome (Ph), 3q26.2 rearrangement, −7 and isochromosome 17q (i(17q)) as the major-route changes with a frequency of over 10%. In addition, +21 and +19 had a frequency of over 5%. These ACAs demonstrated lineage specificity: +8, 3q26.2 rearrangement, i(17q) and +19 were significantly more common in myeloid BP, and −7 more common in lymphoid BP; +Ph and +21 were equally distributed between two groups. Pearson correlation analysis revealed clustering of common ACAs into two groups: 3q26.2 rearrangement, −7 and i(17q) formed one group, and other ACAs formed another group. The grouping correlated with risk stratification of ACAs in CML, chronic phase. Despite the overall negative prognostic impact of ACAs, stratification of ACAs into major vs minor-route changes provided no prognostic relevance in CML-BP. The emergence of 3q26.2 rearrangement as a major-route change in the TKI era correlated with a high frequency of ABL1 mutations, supporting a role for TKI resistance in the changing cytogenetic landscape in CML-BP.

Leukemia (2017) 31, 585–592; doi:10.1038/leu.2016.231

INTRODUCTION

Chronic myeloid leukemia (CML) is defined by the presence of BCR-ABL1 fusion gene resulting from t(9;22)(q34;q11.2), its variant translocations, or rarely, cytogenetically cryptic chromosomal rearrangements. Using conventional karyotyping analysis, t(9;22)/BCR-ABL1 is the sole chromosomal abnormality in over 90% of patients with CML in chronic phase (CP).1,2 With disease progression, additional chromosomal alterations (ACAs) emerge and ~60–80% of patients with CML in blast phase (BP) have ACAs.3,4

Studies of CML performed before tyrosine kinase inhibitor (TKI) therapy available showed that trisomy of chromosome 8 (+8), an extra Philadelphia chromosome (+Ph), isochromosome 17q (i(17q)) and trisomy of chromosome 19 (+19) were the most common ACAs in CML, with frequencies of 34%, 31%, 20% and 13%, respectively, in a large database review by Johansson et al.1 These ACAs with a frequency over 10% (% of all cases with ACAs) were designated as ‘major-route’ changes and occurred in over 90% of patients with CAs. Other ACAs with a frequency of <10% were designated as ‘minor-route’ changes.5,6 In terms of their prognostic impact, others have shown that CML patients with major-route ACAs had a worse outcome than those with minor-route changes.2,7 These studies, however, were based on CML patients in all disease stages, mostly in CP. Johansson et al.1 comprehensively reviewed published reports on CML treated in the pre-TKI era and found the ACA pattern in BP was similar to that in CP. According to their review, +8, +Ph, i(17q) and +19 had a frequency of over 10%, and thus were considered as major-route changes in BP.

In the era of TKI therapy, a large and comprehensive study on the cytogenetic landscape of CML in BP is lacking, largely owing to a drastic decrease in frequency of disease progression from CP to BP. Indeed, the long-term cumulative probability of progression from CP to BP is about 5% in the era of TKI therapy.8–10 Despite the marked therapeutic efficacy of TKIs, nevertheless, a minority of patients develop TKI resistance through BCR-ABL1-dependent or -independent mechanisms. BCR-ABL1 kinase domain mutations are detectable in ~50% of patients experiencing treatment failure and disease progression.11,12 These results raise the issue whether the change in treatment regimens to TKIs might affect the overall landscape of ACAs in BP. Several studies showed that the ACAs after TKI treatment followed the above-mentioned major-route changes.13–16 However, these results were compromised by the small number of cases of BP in each study. Although the probability of blastic transformation is low in the era of TKI therapy, the outcome of BP is dismal with a median survival of 6–10 months.17,18

To better understand the biology of CML-BP in the era of TKI therapy, in this study, we investigate the overall prevalence, complexity, lineage specificity and prognostic impact of ACAs in a large cohort of CML-BP patients treated with TKIs. We further explore the relationship between common ACAs as well as any potential relationship between ACAs and ABL1 mutations.

1Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 2Department of Hematology, Huashan Hospital, Fudan University, Shanghai, China; 3Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA; 4Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA; 5Department of Hematology, The University of Michigan, Ann Arbor, MI, USA; 6Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, TX, USA; Correspondence: Dr S Hu, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Box 85, Houston, TX 77030, USA. E-mail: shu1@mdanderson.org

Received 4 May 2016; revised 1 August 2016; accepted 4 August 2016; accepted article preview online 18 August 2016; advance online publication, 2 September 2016
MATERIALS AND METHODS

Patient selection

Cases of CML-BP that met the following selection criteria were included in the study: (1) diagnosis from 1999, the second year after TKI therapy was implemented in our hospital, to 2015; (2) presence of t(9;22)(q34;q11.2) or variant translocations detected by conventional karyotyping analysis; and (3) the patients who received TKIs before blastic transformation, if diagnosed initially in CP or AP (for accelerated phase), or received TKI treatment if diagnosed initially in BP. We excluded patients with CML in whom conventional cytogenetic analysis was negative for t(9;22), but BCR-ABL1 was detected by fluorescence in situ hybridization or molecular methods. We also excluded cases that presented with isolated myeloid sarcoma without concurrent BP in the bone marrow (BM) or peripheral blood, and cases of Ph-positive de novo acute leukemia. BP was defined as ≥20% blasts in the BM or peripheral blood. This study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center.

Conventional karyotyping and fluorescence in situ hybridization analyses

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from cultured BM aspirate specimens. For the diagnosis of CML and disease monitoring, karyotyping and fluorescence in situ hybridization analyses were performed in line with the European LeukemiaNet recommendation and NCCN guidelines. The clonality of an ACA was defined according to International System for Human Cytogenetic Nomenclature (2013).

Detection of BCR-ABL1 transcripts

Total RNA was extracted from BM aspirate or blood specimens using Trizol reagent (Gibco-BRL, Gaithersburg, MD, USA), followed by complementary DNA synthesis using random hexamers and Superscript II reverse transcription (Invitrogen, Life Technologies, Waltham, MA, USA). Multiplex real-time quantitative PCR for BCR-ABL1 fusion transcripts was performed according to standard instructions (ABI, Foster City, CA, USA). Size of fusion transcripts was determined by capillary electrophoresis.

Detection of ABL1 kinase domain mutations

Sanger sequencing of the ABL1 kinase domain was performed on BM or blood specimens in two separate PCRs that cover codons 221–380 and 350–500, respectively. Standard dideoxy chain-termination DNA sequencing was performed using Big Dye chain terminator reagents on an ABI3700 analyzer, and analyzed using Sequence Analysis software version 3.3 and the SeqScape software V2 (ABI). All mutations were confirmed by sequencing both forward and reverse strands, with a sensitivity of 10% mutation-bearing transcripts in the analyzed population. Separately, for quantitative mutation analysis of codons 311–317 and codons 250–255, pyrosequencing was performed using one biotin-tagged primer and single-stranded product isolated by avidin-Sepharose beads (GE Life Sciences, Piscataway, NJ, USA) on a HSP96 Pyrosequencer (Biotage, Uppsala, Sweden). The sensitivity of pyrosequencing was 1–5% mutation-bearing transcripts in the total RNA pool.

Pearson correlation analysis of common ACAs

Pearson correlation coefficients were calculated for each pair of the variables (common ACAs). The correlation coefficient between two variables x and y is defined by the following equation:

$$r_{xy} = \frac{n \sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\sqrt{n \sum_{i=1}^{n} x_i^2 - \left( \sum_{i=1}^{n} x_i \right)^2} \sqrt{n \sum_{i=1}^{n} y_i^2 - \left( \sum_{i=1}^{n} y_i \right)^2}}$$

where i is the patient index, n is the total number of patients, i = 1, ..., n, r_{xy} is a measure of linear correlation between variables x and y. The values of r_{xy} are always between −1 and 1. By computing the correlation coefficients for all possible pairwise combinations of variables, we obtained a correlation matrix, C. In the correlation matrix, C, the element at the intersection of jth row and kth column, is the correlation coefficient between variables j and k. When r_{jk} ≈ 0 and |r_{jk}| (absolute value) is large, it indicates that variables j and k are strongly positively correlated, or equivalently, they are likely to simultaneously occur; when r_{jk} < 0 and |r_{jk}| is large, it implies that the two variables are strongly negatively correlated, and they are not likely to occur at the same time; when r_{jk} is close to zero, it suggests that there is no obvious correlation between variables j and k.

To visualize the pairwise correlation coefficients, we provided a correlation heat map. In the correlation heat map, each square subregion corresponds to an element in the correlation matrix. Different colors are used to represent different correlation strength.

Statistical analyses

Dichotomous variables were compared between groups using the χ² or Fisher’s exact tests. Non-normally distributed continuous variables (for example, age and interval time) between different groups were compared by Mann–Whitney test. Survival curves were built using the Kaplan–Meier method and differences in survival were evaluated by the log-rank test. Overall survival was calculated from the date of diagnosis of BP to the date of last follow-up or death.

RESULTS

Baseline clinical characteristics of the study cohort

A total of 354 CML-BP patients that met the selection criteria formed the study group, including 333 patients with CML diagnosed initially in CP or AP, and 21 with CML diagnosed initially in BP. There were 214 (60.5%) men and 140 (39.5%) women. The median age at diagnosis of BP was 52 years old (range 13–92 years old). The median interval from initial diagnosis of CML to the diagnosis of BP was 23 months (range, 0–232 months). By immunophenotype, 240 had myeloid BP (MyBP), 106 lymphoid BP (LyBP) and 8 mixed phenotype BP. All 333 patients with CML diagnosed initially in CP or AP received TKI therapy before blastic transformation, and all 21 patients diagnosed initially in BP received TKI therapy. During BP, overall 323 (91.2%) patients received TKIs, including 253 patients who also received chemotherapy, and 70 patients who were followed up at outside institutions and whose chemotherapy status was unknown. Thirty-one (8.8%) patients received chemotherapy only. The status (dead or alive) at last follow-up was known for all 354 patients and the treatment response was known in 339 (95.8%) patients.

Overall landscape of ACAs in CML-BP by frequency and lineage distribution

Two hundred and seventy patients (76.3%) had ACAs and 84 (23.7%) did not at the time of diagnosis of BP (Figure 1a). Sixty-eight (25.2%) patients had single ACAs and 202 (74.8%) had ≥2 ACAs (complex ACAs). Of patients with ACAs, the median number of ACAs was 2.0 (range, 1–27) and the mean was 3.58 ± 3.41. Stratified by immunophenotype, MyBP had a higher frequency of ACAs than LyBP (79.6% vs 69.8%, P = 0.048).

There were 29 different types of ACAs with a frequency of over 2%, and most of these were numerical abnormalities involving the loss or gain of an entire chromosome (Figure 1b). The most common ACAs with a frequency of over 10% included: +8 (76/270, 28.1%), +Ph (71/270, 26.3%), 3q26.2 rearrangement (41/270, 15.2%), −7 (36/270, 13.3%) and i(17q) (29/270, 10.7%), which met the criteria for major-route changes recommended in literature, and herein designated as a group ‘TKI-BP major-route’ ACAs. Three additional common ACAs, including +21, −Y and +19, had a frequency of over 5%. For comparison, we designated major-route changes (+8, i(17q), +Ph, +7, +19, +Ph) previously identified in pre-TKI era ‘traditional major-route’ ACAs in the following analyses.

The recurrent chromosomal abnormalities typical for acute myeloid leukemia were rare in CML-BP except inv(3)(t(3;3)). In this cohort, the frequencies for inv(16)(p13.1q22), t(9;11)(p22;q23) and t(15;17)(q22;q12) were 2.2% (6/270), 1.5% (4/270) and 0.4% (1/270), respectively. No cases with t(8;21)(q22;q22), t(6;9)(p23; q34) or t(1;22)(p13;q13) were identified.

Leukemia (2017) 585 – 592

Complexity and lineage specificity of common ACAs

We divided CML-BP cases with the most common ACAs into two groups: those with single versus complex ACAs (Figure 2a). Most of the common ACAs were associated with other chromosomal alterations. All cases with +19 had complex ACAs. In contrast, 39% of cases with 3q26.2 rearrangement had this abnormality as the sole ACA. The frequencies of being complex for +8, +Ph, −7, i(17q), +21 and −Y were 90.8%, 97.3%, 85.8%, 79.4%, 88.0% and 92.7%, respectively.

When analyzed by lineage distribution, +8, 3q26.2 rearrangement, i(17q) and +19 were significantly more common in MyBP (Figure 2b). In contrast, −7 was significantly more common in LyBP, whereas +Ph and +21 were approximately equally distributed between MyBP and LyBP. In our cohort, 3q26.2 rearrangement was not observed in any case of LyBP.

Prognostic impact of traditional major-route vs minor-route ACAs

Previous studies have indicated that traditional major-route changes are associated with poor prognosis. The major-route but not the minor-route changes acquired during therapy were included as one of the criteria for AP in the most recent version of European LeukemiaNet recommendations for the management of CML patients.21 We then asked whether the traditional major-route or minor-route changes had any prognostic impact in BP in the TKI era. Of 270 patients with ACAs, 125 had traditional major-route and 145 had traditional minor-route ACAs. Sixteen of them had single traditional major-route ACAs and 52 had single traditional minor-route ACAs. There was no significant difference in patient survival.
between those with traditional major-route vs those with minor-route ACAs regardless of the complexity of ACAs (Figures 3a and b).

Prognostic impact of TKI-BP major-route vs minor-route ACAs
Of 270 CML-BP patients with ACAs, 176 had TKI-BP major-route and 94 TKI-BP minor-route ACAs. The clinical characteristics of these two groups are shown in Table 1. Compared with patients with TKI-BP minor-route ACAs, those with TKI-BP major-route changes tended to have a longer interval from the initial diagnosis of CML to the diagnosis of BP (24.8 vs 16.4 months, \( P = 0.02 \)) and a higher rate of being complex (140/176, 79.5% vs 62/94, 66.0%, \( P = 0.014 \)). There was no significant difference in age at diagnosis of BP, gender and treatment response between these two groups. Correspondingly, there was no significant difference in patient survival between those with TKI-BP major-route ACAs vs those with TKI-BP minor-route ACAs regardless of the complexity of ACAs (Figures 3c and d). Similarly, when further stratified into MyBP and LyBP subgroups, there was no significant difference in survival between patients with TKI-BP major-route vs those with TKI-BP minor-route ACAs (data not shown), which is consistent with our previous study.4

Linkage relationship between common ACAs revealed by Pearson correlation analysis
We performed Pearson product-moment correlation analysis to determine the potential relationship between common ACAs with a frequency over 5% (Figure 4). \(-Y\) was not included in this analysis owing to its questionable clinical significance and it being limited to male patients. In total, there were 186 cases of BP with one or more of these 7 common ACAs. Correlation analysis revealed clustering of these ACAs into two groups. Group A included 3q26.2, \(-7\) and \(i(17q)\), and Group B included +8, +19, +21 and +Ph. The ACAs in Group A were negatively correlated with each other within the group and with ACAs in Group B. In contrast, ACAs in Group B were positively correlated with each other within the group, but negatively correlated with those in Group A. In other words, ACAs in Group A tended to emerge independently from other common ACAs in both groups, whereas the ACAs in Group B tended to coexist with other ACAs in the same group, but not with ACAs in Group A. The only exception was the positive correlation between \(i(17q)\) and +8. On the basis of the prognostic impact and treatment response in a recent study, 3q26.2, \(-7\) and \(i(17q)\) were clustered together as a group conferring dismal impact in CP, whereas +8, +Ph and other single ACAs formed a group with relatively better impact.22

ABL1 mutation in CML-BP with or without 3q26.2 rearrangement
Regardless of the disease stage, 3q26.2 rearrangement in CML was very rare in the pre-TKI era. However, it emerged as one of most common ACAs with a frequency of 15.2% in our study of patients who received TKI therapy. We previously have shown that CML patients with 3q26.2 rearrangement emerging in CP or AP had a high rate of progression to BP. Patients with 3q26.2 rearrangement had a marginal response to TKI treatment.23 We then asked whether the high frequency of 3q26.2 rearrangement in TKI era correlated with ABL1 mutation status.

Of the entire study cohort, 199 patients were tested and 103 (103/199, 51.8%) had ABL1 mutations at diagnosis of BP. Of all patients with ACAs, 155 were tested and 78 (78/155, 50.3%) had ABL1 mutations. Of all patients without ACAs, 44 were tested and 25 (56.8%) had ABL1 mutations. There was no significant
difference in the frequency of ABL1 mutations in patients with vs without ACAs ($P = 0.45$).

Of 41 patients of CML-BP with 3q26.2 rearrangement, 16 of 22 tested (72.7%) had ABL1 mutations. This frequency was significantly higher than that of all CML-BP patients without 3q26.2 rearrangement (87/177 or 49.2%, $P = 0.04$) and that of CML-BP patients with ACAs other than 3q26.2 rearrangement (62/133 or 46.6%, $P = 0.04$; Figure 5). The frequencies of ABL1 mutations in patients with other common ACAs were 43.5% (10/23), 52.4% (11/21), 42.2% (19/45), 20.0% (2/10), 46.2% (6/13) and 37.8% (17/45) for −7, i(17q), +8, +19, +21 and +Ph, respectively. The frequency of ABL1 mutations associated with 3q26.2 rearrangement was significantly higher than that associated with non-3q26.2 high-risk ACAs (−7 and i(17q)) as a group and low-risk ACAs (+8, +19, +21 and +Ph) as a group ($P = 0.05$, $P = 0.03$, respectively).

**Table 1.** Clinical characteristics of CML-BP patients with TKI-BP major vs minor-route ACAs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TKI-BP major (N = 176)</th>
<th>TKI-BP minor (N = 94)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of BP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MyBP (N, %)</td>
<td>132 (75.0%)</td>
<td>59 (62.8%)</td>
<td>0.11</td>
</tr>
<tr>
<td>LyBP (N, %)</td>
<td>41 (23.3%)</td>
<td>33 (35.1%)</td>
<td></td>
</tr>
<tr>
<td>Mixed phenotype (N, %)</td>
<td>3 (1.7%)</td>
<td>2 (2.1%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (N, %)</td>
<td>114 (64.8%)</td>
<td>58 (61.7%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Female (N, %)</td>
<td>62 (35.2%)</td>
<td>36 (38.3%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>52.4</td>
<td>51.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Range</td>
<td>13.2–92.4</td>
<td>13.3–82.7</td>
<td></td>
</tr>
<tr>
<td>Interval (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>24.8</td>
<td>16.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Range</td>
<td>0–232.1</td>
<td>0–208.9</td>
<td></td>
</tr>
<tr>
<td>Complexity of ACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>36 (20.5%)</td>
<td>32 (34.0%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Complex</td>
<td>140 (79.5%)</td>
<td>62 (66.0%)</td>
<td></td>
</tr>
<tr>
<td>Status at last F/U</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive (N, %)</td>
<td>43 (24.4%)</td>
<td>30 (31.9%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Dead (N, %)</td>
<td>133 (75.6%)</td>
<td>64 (68.1%)</td>
<td></td>
</tr>
<tr>
<td>Treatment response$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>⩾ HR (N, %)</td>
<td>91 (53.8%)</td>
<td>46 (52.9%)</td>
<td>0.88</td>
</tr>
<tr>
<td>⩾ CCyR (N, %)</td>
<td>51 (30.2%)</td>
<td>28 (32.2%)</td>
<td>0.74</td>
</tr>
<tr>
<td>⩾ MMR (N, %)</td>
<td>35 (20.7%)</td>
<td>24 (27.6%)</td>
<td>0.21</td>
</tr>
<tr>
<td>MUL (N, %)</td>
<td>29 (17.1%)</td>
<td>19 (21.8%)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Abbreviations: ACAs, additional cytogenetic abnormalities; Age, age at diagnosis of BP; BP, blast phase; ⩾ CCyR, complete cytogenetic response or deeper; CML, chronic myeloid leukemia; F/U, follow-up; ⩾ HR, hematologic response or deeper; Interval, interval time from initial diagnosis of CML to the diagnosis of BP; LyBP, lymphoid BP; ⩾ MMR, major molecular response or deeper; MUL, molecularly undetectable leukemia; MyBP, myeloid BP. $^a$ A total of 256/270 (94.8%) patients with ACAs had treatment response information available.
The type of ABL1 mutations and their corresponding karyotypes are listed in Supplementary Table 1. The most common mutations include T315I (n = 25), compound mutations (n = 13), E255K/V/M (n = 12) and F317L (n = 9). Patients with 3q26.2 rearrangement had a slightly (but not statistically significantly) higher frequency of T315I or compound mutations than those without 3q26.2 rearrangement (9/16 or 56.3% vs 24/62 or 38.7%, \( P = 0.21 \)).

**DISCUSSION**

In this study, we investigated the overall landscape and prognostic significance of ACAs as well as treatment response in CML-BP patients treated with TKIs. ACAs were observed in \(~76\%\) of CML-BP cases, \(~80\%\) in MyBP and \(~70\%\) in LyBP. The most common ACAs by the order of decreasing frequency included +8, +Ph, 3q26.2 rearrangement, −7 and i(17q), all with a frequency of over 10%, and +21, −Y and +19, all with a frequency of over 5%. These common ACAs showed lineage specificity. By Pearson correlation analysis, these ACAs were clustered in two different groups with different correlation strength pairwise. CML-BP patients with 3q26.2 rearrangement had a significantly higher rate of ABL1 mutations. Despite the overall worse prognostic impact conferred by ACAs, there was no significant prognostic difference between major-route vs minor-route changes or between simple vs complex ACAs. To our knowledge, this is the largest study of CML-BP in the era of TKI therapy to date.

The distribution of ACAs in CML-BP patients in the era of TKI therapy is substantially different from the pre-TKI era (Table 2). The two high-risk minor-route ACAs in the pre-TKI era, 3q26.2 rearrangement and −7, emerged as the major-route changes in the TKI era. In contrast, +19 became a minor-route change. The frequencies of +8, +Ph and i(17q) were significantly decreased despite retaining the status of major-route changes. When stratified by immunophenotype of BP, a decrease in the frequencies of four traditional major-route changes was observed in both MyBP and LyBP, although the decrease of i(17q) and +19 were more striking in LyBP (Table 3). The increase in the frequency of 3q26.2 rearrangement, a chromosomal alteration typical for acute myeloid leukemia and myelodysplastic syndrome, was solely attributable to its increase in MyBP. Although the higher frequency of −7 in the TKI era was attributable to its increase in both MyBP and LyBP, −7 was significantly more common in LyBP (25.7%) than MyBP (8.9%). When 5% was used as the cutoff value for defining major-route change, however, only 3q26.2 rearrangement emerged as the new member of major-route ACAs, and +17 was downgraded to a minor-route change.

The prognostic significance of major-route changes needs to be reevaluated in the TKI era. Some studies indicated that CML patients with major-route changes had a worse outcome than those with minor-route changes.\(^{2,7,13,14,24,25}\) Although ACAs are considered as an indicator of disease progression, this frequency-based stratification of ACAs may be an oversimplification of their roles in prognosis, which are affected by other concurrent chromosomal changes, disease stage and the nature of ACAs.\(^{22,26}\) Furthermore, some chromosomal changes may simply reflect the genetic instability of CML induced by BCR-ABL1, whereas others can be leukemogenic and cooperate with BCL-ABL1 to induce blast transformation.\(^{26–28}\)

In our recent studies, we found that +8, a major-route ACA, emerging initially or during the treatment, conferred no prognostic impact in the absence of ACAs or other features of AP.\(^{29}\) In contrast, 3q26.2 rearrangement, a traditional minor-route change, conferred a dismal prognosis regardless of the presence or absence of other chromosomal changes.\(^{21}\)

Regarding the prognostic impact of ACAs in BP, most of the studies in the pre-TKI era showed that ACAs conferred a worse prognosis, but others found no prognostic difference between patients with vs without ACAs. In the era of TKI therapy, this study and our previous report show that ACAs indeed confer significant negative prognostic value in BP.\(^{4}\) However, the prognostic impact was not affected by the frequency (major-route vs minor-route), time of emergence (at initial diagnosis vs during therapy), complexity (single vs multiple ACAs) or nature (high risk vs low risk) of ACAs once the disease reaches the stage of BP.\(^{4}\)

In our cohort, about 75% CML-BP patients with ACAs had \(\geq 2\) ACAs. The combinations of ACAs, however, were not random. Pearson correlation analysis revealed two groups of common ACAs: one group included 3q26.2 rearrangement, −7 and i(17q), and the other included +8, +19, +21 and +Ph. The ACAs from the former group tend to be mutually exclusive and independent from ACAs within the latter group. In contrast, the ACAs from the latter group often present with other common ACAs within the group. Despite the lack of prognostic difference between individual ACAs in BP, considering the roles of ACAs in disease progression, the coexistence or mutual exclusivity of various ACAs becomes meaningful. It is reasonable to speculate that +8, +19, +21 and +Ph alone are less powerful and need other concurrent cytogenetic abnormalities to promote progression to BP, whereas 3q26.2 rearrangements, −7 and i(17q), especially 3q26.2 rearrangements and −7, are more potent. This hypothesis regarding the roles of common ACAs in progression and their clustering in BP based on correlation analysis is substantiated.

**Table 2.** Changing landscape of common ACAs in CML-BP in TKI vs pre-TKI era

<table>
<thead>
<tr>
<th>Pre-TKI era*</th>
<th>This study</th>
<th>Trend of changes</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+8</td>
<td>39.7%</td>
<td>28.1%</td>
<td>↓</td>
</tr>
<tr>
<td>+Ph</td>
<td>38.3%</td>
<td>26.3%</td>
<td>↓</td>
</tr>
<tr>
<td>i(17q)</td>
<td>20.7%</td>
<td>10.7%</td>
<td>↓</td>
</tr>
<tr>
<td>+19</td>
<td>16.0%</td>
<td>6.3%</td>
<td>↓↓</td>
</tr>
<tr>
<td>+21</td>
<td>8.7%</td>
<td>9.3%</td>
<td></td>
</tr>
<tr>
<td>+17</td>
<td>5.5%</td>
<td>2.2%</td>
<td>↓</td>
</tr>
<tr>
<td>−7</td>
<td>5.0%</td>
<td>13.3%</td>
<td>↑↑</td>
</tr>
<tr>
<td>3q26.2</td>
<td>3.2%</td>
<td>15.2%</td>
<td>↑↑</td>
</tr>
</tbody>
</table>

Abbreviations: ACAs, additional cytogenetic abnormalities; BP, blast phase; CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor. \(^{*}\)Pre-TKI era; data adapted based on the literature review of Johansson et al.\(^{1}\) ↓: decreased; ↓↓: significantly decreased; ↑: increased; ↑↑: significantly increased.
by our recent study on the stratification of ACAs based on the prognostic impact and treatment response in CP, where 3q26.2 rearrangement, −7 and i(17q) form a high-risk group, and +8, +Ph and −Y a low-risk group.22 In separate studies of CML, we found that coexistence of +8 and other ACAs, regardless of the nature of other concurrent ACAs, had a decreased response to TKI treatment and worse survival, whereas +8 alone was associated with a good response to TKI treatment and a good survival.29 On the contrary, although the ACAs from the high-risk group were complex in most cases of BP, the impact of 3q26.2 rearrangement was independent from other concurrent chromosomal abnormalities.23 Whether the negative impact of −7 and i(17q) in CML is similarly independent from other concurrent cytogenetic changes or clinical parameters is unknown. One of the common chromosomal changes in myeloid neoplasms, such as myelodysplastic syndrome and acute myeloid leukemia, is −7.30−32 In myelodysplastic syndrome, −7 is assigned to a high-risk group according to the International Prognostic Scoring System, and myelodysplastic syndrome patients with −7 have high risk of transformation to acute myeloid leukemia. Studies have shown that myeloid neoplasms with i(17q) define a distinctive clinicopathological entity with a high risk of leukemic progression and poor prognosis.33−35

The pattern of ACAs in BP in this study is different from the traditional concept of major-route and minor-route changes in the pre-TKI era. Undoubtedly, TKI treatment is involved in inducing the changes. In this study, about half of the BP patients had ABL1 kinase domain mutations, which are an important factor leading to TKI resistance.34−36 Not of note, 3q26.2 rearrangement has become the most distinctive ‘new member’ of major-route changes in the TKI era. We found that about three-fourth of the BP patients with 3q26.2 rearrangements had ABL1 kinase domain mutations, which was significantly higher than the average incidence of mutations in BP. We propose that TKI resistance has an important role in the changing landscape of ACAs in BP.

ACAs are an important driver of CML progression to BP,38−41 Clonal evolution can lead to a BCR-ABL-independent survival pathway and can help leukemia cells gain an advantage in interclonal competition by escaping TKI-targeted therapy.42,43 Recently, studies have indicated that there are some close and direct connections between the ACAs, clonal evolution, genome instability, TKI resistance and disease progression.44−47 It is reasonable to speculate that ACAs have an important role in developing BP, although the mechanisms that mediate blastic transformation by different ACAs may vary.

In summary, our study revealed a different landscape of ACAs in CML-BP in the era of TKI therapy. The major-route ACAs include +8, +Ph, 3q26.2 rearrangement, −7 and i(17q). In MyBP, +8, 3q26.2 rearrangement and i(17q) were significantly more common, whereas −7 was more common in LyBP. The emergence of 3q26.2 rearrangement as a major-route change correlated with a high frequency of ABL1 mutations in patients bearing this rearrangement. The changes in the distribution of ACAs were reflected differently in MyBP and LyBP. Despite the overall negative prognostic impact conferred by ACAs, the stratification of ACAs into major-route vs minor-route changes provided no prognostic relevance in BP. Although the type of ACAs conferred no prognostic value in BP, the linkage relationship between common ACAs in BP revealed by Pearson correlation analysis may help extrapolate their roles in blastic transformation.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

Table 3. Changing landscape of common ACAs in MyBP and LyBP of CML in TKI vs pre-TKI era

<table>
<thead>
<tr>
<th></th>
<th>MyBP</th>
<th>LyBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-TKI era</td>
<td>This study</td>
</tr>
<tr>
<td>+8</td>
<td>41.3%</td>
<td>33.0%</td>
</tr>
<tr>
<td>+Ph</td>
<td>38.4%</td>
<td>24.6%</td>
</tr>
<tr>
<td>i(17q)</td>
<td>22.9%</td>
<td>14.7%</td>
</tr>
<tr>
<td>+19</td>
<td>16.1%</td>
<td>8.4%</td>
</tr>
<tr>
<td>+21</td>
<td>7.9%</td>
<td>7.9%</td>
</tr>
<tr>
<td>+17</td>
<td>6.1%</td>
<td>2.1%</td>
</tr>
<tr>
<td>−7</td>
<td>3.0%</td>
<td>8.9%</td>
</tr>
<tr>
<td>3q26.2</td>
<td>Not described</td>
<td>22.0%</td>
</tr>
</tbody>
</table>

Abbreviations: ACAs, additional cytogenetic abnormalities; CML, chronic myeloid leukemia; LyBP, lymphoid blast phase; MyBP, myeloid blast phase; NA, not applicable; TKI, tyrosine kinase inhibitor. *Pre-TKI era: data adapted based on the literature review of Johansson et al. †: decreased; ††: significantly decreased; ↑: increased; ↑↑: significantly increased.


