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Clinical implications of clonal chromosomal abnormalities in Philadelphia negative cells in CML patients after treated with tyrosine kinase inhibitors

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ABSTRACT

Emergence of clonal chromosomal abnormalities in Philadelphia chromosome-negative (CCA/Ph-) cells in chronic myeloid leukemia (CML) patients during the treatment with tyrosine kinase inhibitors (TKIs) is an interesting phenomenon. Although previous studies revealed some potential impact of CCA/Ph- on CML patients' outcome, clinical significance of CCA/Ph- in CML patients remains to be further elucidated. We retrospectively reviewed the patients with CML evaluated at Genoptix Medical Laboratory in Carlsbad, California from 2005 to 2015. Twenty-four CML patients with CCA/Ph- cells were identified. These include 18 patients with single chromosomal abnormality, 4 patients with double chromosomal abnormalities, and two patients with complex cytogenetic abnormalities. In addition to trisomy 8 and monosomy 7, we identified that 20q- was also a common abnormality in CCA/Ph- cells. Most of the patients with CCA/Ph- cells demonstrated no significant dysplasia or increased blasts with two exceptions: one patient with persistent 7q- exhibiting mild dysmegakaryopoiesis, suggestive of an early evolving myelodysplastic syndrome, and another patient with complex cytogenetic abnormalities who developed acute myeloid leukemia after gained *MLL* amplification. One patient with complex cytogenetic abnormalities showed optimal response to TKI treatment, no overt dysplasia, and no disease progression during almost 4-years of follow-up. More interestingly, FISH tests could identify more cases with double chromosomal abnormalities and these cases showed suboptimal responses to TKI treatments. Our observation indicates that 20q- was also a common abnormality in CCA/Ph- cells, further FISH tests revealed additional CCA/Ph-, and the majority of CML patients with two or more chromosomal abnormalities in Ph- cells showed inferior response to TKI treatments. The results of our study suggest that CML cases with CCA/Ph- may represent a group of patients with heterogeneous genetic alterations.

Introduction

Chronic myelogenous leukemia (CML) is a clonal hematologic disorder characterized by the presence of a fusion oncogene, *BCR-ABL*, which leads to uncontrolled proliferation of myeloid cells. The fusion gene is the results of reciprocal translocation (9;22) (q34; q11) known as Philadelphia (Ph) chromosome [1]. The successful use of tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL oncoprotein has significantly improved the prognosis of this disease, so that the survival of CML patients is nearly identical to that of the general population [2,3]. During treatments with TKIs it has been shown that a small percentage of CML patients developed other chromosomal abnormalities in the Ph+ and Ph- cells [4,5]. The development of additional clonal chromosomal abnormalities (CCA) in Ph+ cells is considered as cytogenetic clonal evolution, suggesting disease progression [4]. However, the clinical significance of emerging clonal chromosomal abnormalities

Abbreviations: CML, Chronic myelogenous leukemia; Ph, Philadelphia chromosome; TKI, Tyrosine kinase inhibitor; CCA, Clonal chromosomal abnormalities; PCR, Polymerase chain reaction; FISH, Fluorescence in situ hybridization; MDS, Myelodysplastic syndrome; AML, Acute myeloid leukemia; CCyR, Complete cytogenetic response; MMR, Major molecular response; BM, Bone marrow.

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in Ph- cells remains uncertain [5]. The reported common clonal chromosomal abnormalities in CCA/Ph- cells include trisomy 8, monosomy 7 and loss of Y chromosome [6]. The incidence of CCA/Ph- cells in various treatment groups is estimated to be 2–17% [5,7–9]. In the present study, we report 24 patients with CCA/Ph- cells. In addition to trisomy 8 and monosomy 7, we identified that chromosome 20q- was also a common abnormality in CCA/Ph- cells. Furthermore, we have identified one case with deletion of chromosome 5q, one case with monosomy 8, and two cases with complex cytogenetic abnormalities. The results from this investigation may help to understand the pathogenic and prognostic implications of the clonal chromosomal abnormalities in CML.

Patients and methods

Patients

We conducted a retrospective investigation of patients diagnosed with CML in our institute (Genoptix Medical Laboratory, Carlsbad, California, USA) between 2005 and 2015. This retrospective case study was approved by Sterling IRB (ID:4728). Twenty-four out of 1412 patients with history of CML were identified carrying CCA/Ph- cells during TKI treatment. The TKI treatment includes Imatinib, Dasatinib, and Nilotinib. Cases with a loss of Y chromosome were excluded. The diagnosis of CML was established according to the criteria proposed by World Health Organization (WHO) [10]. The patients' information was summarized in Table 1.

Karyotype analysis

Conventional cytogenetic analysis was performed on G-banded metaphase cells prepared from unstimulated 24-hr and 48-hr bone marrow aspirates cultured using standard techniques. At least 20 metaphases with good quality banding were evaluated for each case when satisfactory cell cultures were available. A clonal cytogenetic abnormality is defined as the same numerical gain or structural abnormalities in at least 2 metaphases or the same numerical loss in at least 3 metaphases. A complex abnormal karyotype is defined as 3 or more cytogenetic abnormalities. The karyotype was documented according to the International System for Human Cytogenetic Nomenclatures (ISCN 2013) [11]. The presence of CCA was defined as development of one or more cytogenetically abnormal clones in patients who had a previously normal karyotype or who acquired additional clones in addition to previous abnormalities, during clinical follow up. Since controversies exist regarding the clinical significance of -Y and it may be an age-related phenomenon, cases with a loss of Y chromosome were not included in this study [12].

Fluorescence in situ hybridization (FISH) analysis

FISH tests were performed on cell suspension prepared from fresh bone marrow aspirate or peripheral blood pellets using standard FISH techniques. FISH probes and target DNA were co-denatured at 73 °C for 5 min, followed by overnight hybridization at 37 °C. At least 200 nuclei were examined for each probe whenever possible. The FISH analysis included probes specific for chromosome 5q, chromosome 7p/7q, chromosome 8, chromosome 20q and *BCR/ABL*.

PCR for BCR/ABL

BCR-ABL t(9;22) quantitative assay was performed in Genoptix Medical Laboratory in Carlsbad, California, USA. Briefly, patient RNA was isolated and reverse transcribed to complementary DNA (cDNA). The *BCR/ABL* and *ABL* reference gene sequences were amplified in duplicate using multiplexed quantitative real-time PCR. This assay can detect the major, minor, and micro *BCR/ABL* breakpoints and has an analytical sensitivity of better than 0.002%.

Results

Chromosomal abnormalities

Twenty-four out of 1412 patients with history of CML were identified to have CCA/Ph- cells during treatment with TKIs. The observed frequency of CCA/Ph- was approximately 2%. Table 1 summarized the patients' information. The chromosomal abnormalities were identified in patients receiving three different TKIs including Imatinib, Dasatinib, and/or Nilotinib. Seventeen out of 24 patients were male, suggestive of male predominance. Majority of these patients (15 out of 24) were between 50 and 70 years old. The gender and age distribution reflexes the epidemiological features in general patient population with CML.

Among these 24 patients, 18 patients had single chromosomal abnormality, including 9 patients with a trisomy 8, 6 patients with a deletion of 20q, 2 patients with a deletion of 7q, and 1 patient with a monosomy 8. Four patients had double chromosomal abnormalities, including 1 patient with +8 and +mar, 1 patient with +8 and 20q-, 1 patient with 5q- and −7/7q-, and 1 patient with 20q- and −7. Two patients had complex cytogenetic abnormalities.

We further analyzed the persistence of CCA/Ph-. Persistent CCA/Ph- was defined as continuously positive CCA/Ph- results in sequential three or more times of cytogenetic/FISH follow-up. Persistent CCA/Ph- was observed in 7 patients including 3 patients with persistent +8, 1 patient with persistent 7q-, 1 patient with persistent +8 and +mar, and 2 patients with persistent complex karyotypes. 13 patients showed transient CCA/Ph-. The other 4 patients had only one time of abnormal cytogenetic result with no follow-up cytogenetic data available.

Additional chromosomal abnormalities in CCA/Ph- cells detected by FISH tests

Since rare cases with CCA/Ph- were reported to evolve into myelodysplastic syndrome, the majority of CML cases with single chromosomal abnormality in CCA/Ph- cells were further analyzed by using an MDS-associated FISH panel, including probes for +8, 20q-, 5q-, and 7p-/7q-, to observe the possibility of an evolving myelodysplastic syndrome during follow-up in our institute (Table 2). This approach revealed more additional (double) chromosomal abnormalities than karyotyping only (Case 20, 21, 22 in Table 2). The additional chromosomal abnormalities seem from different clones.

Potential association between CCA/Ph- and molecular response to TKI therapy

At the time of detection of CCA/Ph-, all 24 patients were receiving TKI treatments. All 18 patients with single chromosomal abnormality (case 1 to 18) demonstrated negative or less than 0.1% of *BCR/ABL* fusion transcripts, indicating major molecular response (MMR), except case 10 had *BCR/ABL* level higher than 0.1% (0.11%). These cases were treated with only one type of TKIs, either Imitinib or Nilotinib. All cases with double chromosomal abnormalities (cases 19 to 22) showed suboptimal responses to TKI treatments with no achievement of MMR (Table 1). Cases 20 to 22 also had no complete cytogenetic response (CCyR). Case 23 was a 67-year-old male patient with a history of CML. This case was reported in our previous publication[13]. During follow-up this patient showed relapsed CML and persistent complex karyotypes

Table 1
Characteristics of CML patients with CCA/Ph-.

Case	Age/Sex	TKIs	CCA/Ph-	Duration of follow-up	Cytogenetic response	Molecular response (PCR)	Major morphological findings in BM
1	65/F	Imatinib	+8 (persistent)	7 years and 2 months	CCyR	MMR	Normocellular No dyspoiesis
2	45/M	Imatinib	+8 (persistent)	6 years and 10 months	CCyR	MMR	Normocellular No dyspoiesis
3	71/F	Imatinib	+8	6 years and 4 months	CCyR	MMR	Normocellular No dyspoiesis
4	62/M	Imatinib	−8	4 years and 11 months	CCyR	MMR	Mild hypercellular No dyspoiesis
5	54/M	Imatinib	+8	0	CCyR	MMR	Mildly hypocellular No dyspoiesis
6	56/M	Imatinib	+8	1 year and 9 months	CCyR	MMR	Not available
7	40/M	Dasatinib	+8 (persistent)	4 years and 3 months	CCyR	MMR	Normocellular No dyspoiesis
8	61/F	Dasatinib	+8	2 years and 1 month	CCyR	MMR	Normocellular No dyspoiesis
9	37/F	Nilotinib	+8	1 year and 4 months	CCyR	MMR	Not available
10	51/M	Nilotinib	+8	0	CCyR	No MMR (0.11)	Mildly hypocellular No dyspoiesis
11	85/M	Imatinib	20q-	0	CCyR	MMR	Mildly hypercellular No dyspoiesis
12	75/M	Imatinib	20q-	1 year and 5 months	CCyR	MMR	Mildly hypocellular No dyspoiesis
13	57/M	Imatinib	20q-	1 year and 5 months	CCyR	MMR	Normocellular No dyspoiesis
14	68/F	Nilotinib	20q-	1 year and 3 months	CCyR	MMR	Normocellular No dyspoiesis
15	51/F	Dasatinib	20q-	7 months	CCyR	MMR	Mildly Hypocellular No dyspoiesis
16	67/M	Dasatinib	20q-	6 years and 2 months	CCyR	MMR	Normocellular No dyspoiesis
17	43/M	Imatinib	7q- (persistent)	3 years and 7 months	CCyR	MMR	Normocellular Mild dysmegakaryopoiesis
18	39/M	Imatinib	7q-	2 years and 2 months	CCyR	MMR	Mildly hypocellular No dyspoiesis
19	54/M	Imatinib/ Dasatinib/ Nilotinib	+8 and +mar (persistent)	4 years and 3 months	CCyR	No MMR	Normocellular No dyspoiesis
20	67/F	Imatinib/ Dasatinib/ Nilotinib	+8 and 20q-	1 year and 3 months	No CCyR	No MMR	Mildly hypocellular No dyspoiesis
21	54/M	Imatinib/ Dasatinib/ Nilotinib	5q- and −7/7q-	5 years and 1 month	No CCyR	No MMR	Normocellular No dyspoiesis
22	54/M	Imatinib	20q- and −7	2 years and 3 months	No CCyR	No MMR	Mildly hypocellular No dyspoiesis
23	67/M	Imatinib/Dasatinb	Persistent complex karyotype with +6, +8, 11q-, and up to 3 mar (*MLL* amplification)	4 year and 4 months	No CCyR	No MMR	Moderately hypercellular Progress to AML
24	96/M	Imatinib	Persistent complex karyotype with 20q-, +1, 1p-, 1q-, and t(1;7) (p32;q21)	3 years and 10 months	CCyR	MMR	Normocellular No dyspoiesis

with +6, +8, 11q-, and up to 3 marker chromosomes in Ph- cells. The marker chromosomes were confirmed as *MLL* amplification. Eventually this patient developed acute myeloid leukemia (AML). Interestingly, case 24 was a 96 year old male patient had a history of CML and persistent complex karyotypes with 20q-, +1, 1p-, 1q-, t(1;7) (p32;q21) after being treated with Imatinib. During almost 4-years of follow-up, this patient had no cytogenetic and molecular evidence of residual or recurrent CML (Fig. 1A and B). PCR studies for *BCR/ABL1* were persistently negative.

Morphological findings of bone marrow biopsies

Morphologic evaluation of bone marrow biopsies from 22 patients with CCA/Ph- demonstrated no evidence of significant dysplasia or increased blasts, with exception of two patients: case 17 (with persistent 7q-) exhibited mild dysmegakaryopoiesis, suggestive of an early evolving myelodysplastic syndrome, and case 23 developed AML after gained *MLL* amplification. Case 6 and 9 showed no overt dyspoiesis on blood smears and had no bone marrow biopsies available for further morphological evaluation. Although case 24 had persistent complex karyotypes with 20q-, +1, 1p-, 1q-, −7, and t(1;7) (p32;q21) during TKI treatment, there was no significant morphological evidence of dyspoiesis and residual disease (Fig. 1C and 1D).

Discussion

In this study, we investigated the occurrence of the CCA/Ph- in patients with CML. Twenty-four out of 1412 patients with history of CML developed chromosomal abnormalities in the Ph- cells during the treatment with TKIs. The observed frequency is approximately 2% which is similar to that reported in the previous

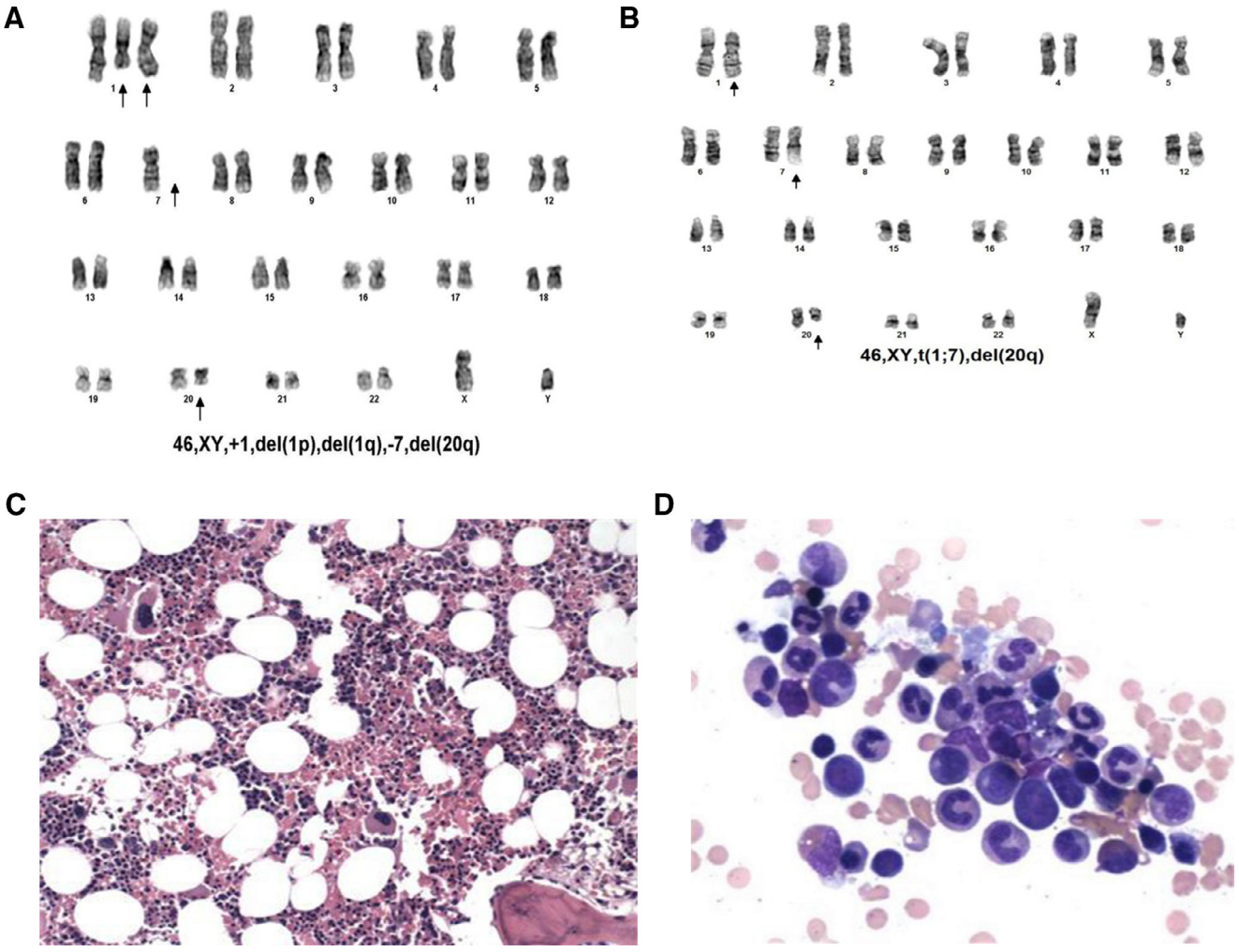

Fig. 1. Representative bone marrow biopsy of case 24. A and B). Karyotypes with complex cytogenetic abnormalities. C. Normocellular bone marrow, core biopsy, H&E. D). Maturing hematopoiesis with no overt dysplasia, bone marrow aspirate smear.

studies by Feldman et al. [9]. The observed frequency is lower than the reports from Bozkurt et al. and Deininger et al. [5,6]. The discrepancy could be due to the fact that the previous studies included -Y in their analyses, which was excluded in the current study. In addition, our study included a larger number of patient samples. Consistent with previous reports, our study demonstrates that trisomy 8 and monosomy 7/7q- are common chromosomal abnormalities in CCA/Ph- cells [6]. Interestingly, our study has identified that 20q- is also a common chromosomal abnormality in Ph- cells, comprising 9/24 cases (37.5%): 6 cases among the cases with single chromosomal abnormality, 2 cases among the cases with double chromosomal abnormalities, and 1 case among the cases with complex cytogenetic abnormalities. In addition, we have detected one case with deletion of chromosome 5q and one case with monosomy 8. Two cases with complex cytogenetic abnormalities have been identified.

Most reports demonstrated that the presence of CCA in the Ph-cells is transient. The persistence of CCA/Ph- was documented in less than 30% of patients [7,15]. Our study also showed that 7 out of 24 patients (~29%) carried persistent chromosomal aberrations as demonstrated by subsequent follow-up cytogenetic analyses. These results suggest that CCA in Ph- cells can be persistent.

The exact mechanisms that lead to the development of clonal chromosomal abnormalities in Ph- cells are unknown. Several hypotheses have been proposed. One hypothesis suggests that since CCA/Ph- abnormalities are not present at the time of initial diagnosis, occurrence of chromosomal abnormalities may be related to the leukemic or treatment process [16–18]. The second hypothesis proposes that the chromosomal abnormalities could be arising from preleukemic clones that became evidence by the successful treatment of CML [19,20]. Recent genomic studies demonstrated that the Ph+ and Ph− clones could be either derived from a common progenitor that predated the acquisition of *BCR/ABL* [21] or arose independently [22]. The clone with t(9;22) acquired a proliferative advantage and therefore masks the underlying clonal diversity. Treatment with TKIs would suppress the proliferation of cells with t(9;22), which allows other abnormal clones to emerge. The third hypothesis suggests that Ph- cells emerging after successful CML treatment may be derived from the cells that could have undergone significant telomere shortening [23]. Telomere shortening could be due to prolonged coexistence with the tumor clone, the proliferative effort associated with bone marrow repopulation following successful clearance of Ph+ cells, and/or direct pharmacological inhibition of telomere preservation capabilities due to TKI treatment [23]. Shortened telomeres may lead to functional exhaustion and genomic instability and chromosomal abnormalities. Recent study using target deep sequencing on samples from CML patients has demonstrated diverse patterns of mutation acquisition. While some patients had evidence of preleukemic mutations, other patients acquired new mutations during TKI treatment [17].

Table 2
Karyotypes and FISH results of CML cases with CCA/Ph-.

Case#	Karyotype	MDS-associated FISH panel (% abnormal cells)
1	47,XX,+8[15]/46,XX[5]	+8 (85%)
2	47,XY,+8[17]/46,XY[3]	+8 (91%)
3	47,XY,+8[4]/46,XY[16]	+8 (23%)
4	45,XY,−8[6]/46,XY[14]	Not performed
5	47,XY,+8[20]	Not performed
6	47,XY,+8[11]/46,XY[9]	+8 (60%)
7	47,XY,+8[15]/46,XY[5]	+8 (82%)
8	47,XX,+8[8]/46,XX[12]	Not performed
9	47,XX,+8[2]/46,XX[18]	Not performed
10	47,XX,+8[2]/46,XX[18]	Not performed
11	46,XY,del(20)(q11.2q13.3)[2]/46,XY[18]	20q- (15%)
12	46,XY,del(20)(q11.2q13.1)[7]/46,XY[11]	20q- (47%)
13	46,XY,del(20)(q11.2q13.1)[3]/46,XY[17]	20q- (20%)
14	46,XX,del(20)(q11.2q13.1)[5]/46,XX[15]	20q- (37%)
15	46,XX,del(20)(q11.2q13.1)[2]/46,XX[29]	Not performed
16	46,XY,del(20)(q11.2q13.3)[5]/46,XY[15]	Not performed
17	46,XY,del(7)(q22)[19]/46,XY[1]	7q- (80%)
18	46,XY,del(7)(q22)[3]/46,XY[17]	7q- (14%)
19	47,XY,+mar[3]/47,+8,XY[5]/46,XY[12]	+8 (35%)
20	46,XY,del(20)(q11.2q13.3)[2]/46,XY[18]	+8 (7%); 20q- (16%)
21	46,XY,del(7)(q22)[3]/45, −7, XY[1]/46,XY[16]	7q- (17%); 5q- (5%)
22	45,XY,−7[2]/46,XY[18]	−7 (10%); 20q- (6%)
23	48–49,XY,+8,del(11)(q21),+1–2mar[cp15]	Not performed
24	46,XY,−1,del(1p),del(1q),−7,del(20q)[5]/46,XY,t(1;7) (p32;q21),del(20q)[4]/46,XY[11]	Not performed

The prognostic significance of CCA/Ph- is not clear. Some studies have shown that a deletion of chromosome 7 is associated with an increased risk of evolving to a myelodysplastic syndrome and acute myeloid leukemia [17]. Most reports have demonstrated that the presence of CCA/Ph- has no impact on the long-term outcomes in CML patients [5,24]. Recent report from Issa et.al. demonstrated that when loss of Y chromosome was excluded, patients with CCA/Ph- were found to be associated with decreased survival and increased risk of development of MDS or AML [14]. In agreement with these reports, our study showed no definite morphologic evidence of dyspoiesis. The patient with persistent 7q- in our current report exhibited mild dysmegakaryopoiesis, which may signify an early evolving myelodysplastic syndrome.

Previous studies revealed that there was no significant difference in response to TKI treatment between CML with CCA/Ph- and CML with no CCA/Ph- cases [14]. Similarly, most CCA/Ph- CML cases with single chromosomal abnormality in our current study showed good responses to TKI treatment (CCyR and MMR). However, 4 cases with double chromosomal abnormalities had inferior responses to TKI treatment: only 1 case (1/4) achieved CCyR and no cases (0/4) achieved MMR. Three of the four cases received treatment with Imatinib as well as second-generation TKIs including Dasatinib and Nilotinib. The underlying mechanism of this phenomenon is uncertain. CCA/Ph- cases with double chromosomal abnormalities may carry more underlying genomic alterations/mutations which could integrate with *BCR/ABL* pathway or provide alterative pathways, leading to resistance to TKI treatment. Compared with the most recent study [14] in which only one case had double non -Y chromosomal abnormalities, our current study showed much more cases with double chromosomal abnormalities from separate clones (4 out of 24). The disparity is likely due to more nuclei (more than 200) examined by FISH tests in this current study. This finding suggests that additional FISH tests may help to identify CML cases harboring CCA/Ph- with inferior responses to TKI treatment.

The two cases with complex cytogenetic abnormalities are interesting and had totally different prognoses. It is commonly believed that complex cytogenetic abnormalities are associated with poor prognoses in myeloid neoplasms. Case 23 with complex cytogenetic abnormalities including *MLL* amplification showed recurrent CML and eventually developed AML driven from CCA/Ph- clone [13]. Although case 24 had persistent complex cytogenetic abnormalities with 20q-, +1, 1p-, 1q-, t(1;7) (p32;q21), −7 during almost 4-years of follow-up, this patient showed no cytogenetic and molecular evidence of residual or recurrent CML (persistent CCyR and MMR). The prognostic difference in the two cases may imply that the involved driver mutation(s)/alteration(s) such as *MLL* amplification is likely more important than complex cytogenetic abnormalities themselves.

Conclusions

Our studies indicate that 20q- was also a common abnormality in CCA/Ph- cells. Additional FISH tests detected more CML cases with double chromosomal abnormalities. The majority of CML patients with two or more chromosomal abnormalities in Ph- cells showed suboptimal response to TKI treatments. These results imply that CML cases with CCA/Ph- may represent a spectrum of diseases with heterogeneous molecular alterations. Additional studies are needed to define the molecular determinants and long-term implications of this phenomenon. Further analysis of these CML cases carrying CCA/Ph- with suboptimal response to TKI treatments may help to reveal more molecular mechanisms of TKI resistance.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Hongyu Ni were responsible for doing data analysis and drafting the manuscript. Hong Drum, Xianli Sun, Xianfeng Zhao, Bei You, Dongfang Liu, and Chen Liu helped in data analysis and reviewing the manuscript. **Paris Petersen, Yin Xu** and **Derek Lyle** helped in collecting data. **Jie-Gen Jiang** was responsible for study designing, supervision, and drafting the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

The study has been examined and certified by the Ethics Committee of Sterling IRB (ID:4728), in agreement with institutional guidelines.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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No.

References

[1] Faderl S, Talpaz M, Estrov M, et al. The biology of chronic myeloid leukemia. N Engl J Med 1999;341:164–72.
[2] Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006;355:2408–17.
[3] Gambacorti-Passerini C, Antolini L, Mahon FX, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. J Natl Cancer Inst 2011;103:553–61.
[4] Cortes JE, Talpaz M, Giles F, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood 2003;101:3794–800.
[5] Deininger MW, Cortes J, Paquette R, et al. The prognosis for patients with chronic myeloid leukemia who have clonal cytogenetic abnormalities in philadelphia chromosome-negative cells. Cancer 2007;110:1509–19.
[6] Bozkurt S, Uz B, Buyukasik Y, et al. Prognostic importance of additional cytogenetic anomalies in chronic myeloid leukemia. *Med Oncol* 2013;30:443.
[7] Jabbour E, Kantarjian HM, Abruzzo LV, et al. Chromosomal abnormalities in Philadelphia chromosome negative metaphases appearing during imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Blood 2007;110:2991–5.
[8] Larsson N, Billström R, Lilljebjörn H, et al. Genetic analysis of dasatinib-treated chronic myeloid leukemia rapidly developing into acute myeloid leukemia with monosomy 7 in Philadelphia-negative cells. Cancer Genet Cytogenet 2010;199:89–95.
[9] Feldman E, Najfeldb V, Schuster M, et al. The emergence of Ph-, trisomy-8+ cells in patients with chronic myeloid leukemia treated with imatinib mesylate. Exp Hematol 2003;31:702–7.
[10] Vardiman JW, Melo JV, Baccarani M, et al. Chronic myelogenous leukemia BCR-ABL1 positive. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissue. WHO Press; 2008. p. 32–7.
[11] Simons A, Shaffer LG, Hastings RJ. Cytogenetic Nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res 2013;141:1–6.
[12] Wiktor A, Rybicki BA, Piao ZS, et al. Clinical significance of y chromosome loss in hematologic disease. Genes Chromosomes Cancer 2000;27:11–16.
[13] Jiang JG, Xu Y, Wu Z, et al. Acute myeloid leukemia developed in Ph- cells with *MLL* gene amplification in a patient with chronic myelogenous leukemia. Leuk Lymphoma 2018;23:1–3.
[14] Issa GC, Kantarjian HM, Gonzalez GN, et al. Clonal chromosomal abnormalities appearing in Philadelphia chromosome-negative metaphases during CML treatment. Blood 2017;130:2084–91.
[15] Groves MJ, Sales M, Baker L, et al. Factors influencing a second myeloid malignancy in patients with Philadelphia-negative -7 or del(7q) clones during tyrosine kinase inhibitor therapy for chronic myeloid leukemia. Cancer Genet 2011;204:39–44.
[16] Kharbanda S, Pandey P, Jin S, et al. Functional interaction between DNA-PK and c-Abl in response to DNA damage. Nature (Lond) 1997;386:732–5.
[17] Kim T, Tyndel MS, Kim HJ, et al. Spectrum of somatic mutation dynamics in chronic myeloid leukemia following tyrosine kinase inhibitor therapy. Blood 2017;129:38–47.
[18] Fabarius A, Giehl M, Frank O, et al. Induction of centrosome and chromosome aberrations by imatinib in vitro. Leukemia 2005;19:1573–8.
[19] Zaccaria A, Testoni N, Valenti AM, et al. Chromosome abnormalities additional to the Philadelphia chromosome at the diagnosis of chronic myelogenous leukemia: pathogenetic and prognostic implications. Cancer Genet Cytogenet 2010;199:76–80.
[20] Bumm T, Müller C, Al-Ali HK, et al. Emergence of clonal cytogenetic abnormalities in Ph− cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood 2003;101:1941–9.
[21] Schmidt M, Rinke J, Schäfer V, et al. Molecular-defined clonal evolution in patients with chronic myeloid leukemia independent of the BCR-ABL status. Leukemia 2014;28:2292–9.
[22] Krysiak K, Christopher MJ, Skidmore ZL, et al. A genomic analysis of Philadelphia chromosome-negative AML arising in patients with CML. Blood Cancer J 2016;6:e413.
[23] Lobetti-Bodoni C, Ferrero D, Genuardi E, et al. Telomere loss in Philadelphia-negative hematopoiesis after successful treatment of chronic myeloid leukemia: evidence for premature aging of the myeloid compartment. Mech Ageing Dev 2012;133:479–88.
[24] Wasilewska EM, Panasiuk B, Gniot M, et al. Clonal chromosomal aberrations in Philadelphia negative cells such as monosomy 7 and trisomy 8 may persist for years with no impact on the long term outcome in patients with chronic myeloid leukemia. Cancer Genet 2017;216–217:1–9.