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ARTICLE

Secondary Philadelphia chromosome acquired during therapy of acute leukemia and myelodysplastic syndrome

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Abstract

The Philadelphia chromosome resulting from t(9;22)(q34;q11.2) or its variants is a defining event in chronic myeloid leukemia. It is also observed in several types of de novo acute leukemia, commonly in B lymphoblastic leukemia, and rarely in acute myeloid leukemia, acute leukemia of ambiguous lineage, and T lymphoblastic leukemia. Acquisition of the Philadelphia chromosome during therapy of acute leukemia and myelodysplastic syndrome is rare. We reported 19 patients, including 11 men and 8 women with a median age of 53 years at initial diagnosis. The diagnoses at initial presentation were acute myeloid leukemia ($n = 11$), myelodysplastic syndrome ($n = 5$), B lymphoblastic leukemia ($n = 2$), and T lymphoblastic leukemia ($n = 1$); no cases carried the Philadelphia chromosome. The Philadelphia chromosome was detected subsequently at relapse, or at refractory stage of acute leukemia or myelodysplastic syndrome. Of 14 patients evaluated for the *BCR-ABL1* transcript subtype, 12 had the e1a2 transcript. In 11 of 14 patients, the diseases before and after emergence of the Philadelphia chromosome were clonally related by karyotype or shared gene mutations. Of 15 patients with treatment information available, 7 received chemotherapy alone, 5 received chemotherapy plus tyrosine kinase inhibitors, 2 received tyrosine kinase inhibitors only, and 1 patient was not treated. Twelve patients had follow-up after acquisition of the Philadelphia chromosome; all had persistent/refractory acute leukemia. Thirteen of 15 patients died a median of 3 months after the emergence of the Philadelphia chromosome. In summary, secondary Philadelphia chromosome acquired during therapy is rare, and is associated with the e1a2 transcript subtype, terminal disease stage, and poor outcome.

Introduction

The Philadelphia chromosome resulting from t(9;22)(q34;q11.2) or its variants is the hallmark of chronic myeloid leukemia [1]. It is also observed in several types of de novo acute leukemia, commonly in B lymphoblastic leukemia [2], and rarely in acute myeloid leukemia [3–5], acute leukemia of ambiguous lineage [6, 7], and T lymphoblastic leukemia [8, 9]. Different break point sites within the *BCR* gene result in different *BCR-ABL1* fusion transcript subtypes that may affect the disease phenotype. The typical b2a2/b3a2 fusion transcripts, which encode P210, are found in over 95% of patients with chronic myeloid leukemia. The e1a2 fusion transcript, which encodes P190, is seen in most patients with Philadelphia chromosome-positive B lymphoblastic leukemia, and is associated with monocytosis in chronic myeloid leukemia [10]. Patients with chronic myeloid leukemia and the e1a2 transcript have a higher risk of blastic transformation, poor treatment response, and unfavorable survival compared with patients with the typical b2a2/b3a2 transcripts [11]. Cases of chronic myeloid leukemia that carry the rare e19a2 transcript, which encodes P230, often show prominent neutrophilic maturation or thrombocytosis [12, 13].

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All subtypes of *BCR-ABL1* transcripts encode fusion proteins with constitutive tyrosine kinase activity that is essential for inducing leukemia via increasing tumor cell proliferation and growth, reducing adherence to bone marrow stroma [1], and promoting angiogenesis, metastasis, and a defective apoptotic response [14]. An understanding of the abnormal signaling pathway has led to the development of small molecule tyrosine kinase inhibitors targeting BCR-ABL1. This targeted therapy has dramatically improved the outcome of patients with chronic myeloid leukemia [15], and to a lesser extent, Philadelphia chromosome-positive B lymphoblastic leukemia [16–18]. Improved survival in patients with de novo Philadelphia chromosome-positive acute myeloid leukemia has also been reported anecdotally [19].

Myeloid and lymphoid neoplasms in which the Philadelphia chromosome appears as a primary change at disease onset are well-established entities, and have been extensively studied. However, secondary Philadelphia chromosome acquired during therapy of myeloid or lymphoid neoplasms is extremely rare, and only sporadically described in the form of single-case reports in the literature [20–53]. The biological and clinical features of these cases have not been systematically studied, and the prognostic significance of secondary Philadelphia chromosome is poorly understood. Here, we reported 19 patients who acquired the Philadelphia chromosome during therapy of myeloid or lymphoid neoplasms and provided an overview of cases reported in the literature.

Methods

Patient selection

Patients with myeloid or lymphoid neoplasms diagnosed in our institution from 1998 through 2016 were reviewed for the presence of the Philadelphia chromosome acquired during therapy. Patients with following Philadelphia chromosome-positive diseases were not included: chronic myeloid leukemia, de novo B- or T lymphoblastic leukemia, de novo acute myeloid leukemia, and de novo acute leukemia of ambiguous lineage. Patient demographics, clinical histories, including treatment and follow-up information, and laboratory data were obtained by electronic chart review. This study was approved by the Institutional Review Board at the University of Texas MD Anderson Cancer Center and was conducted in accordance with the Declaration of Helsinki.

Flow cytometric immunophenotyping

Flow cytometric immunophenotypic analysis was performed on bone marrow aspirates using standard multicolor analysis, which evolved substantially during the study interval. The following antibodies were used in various combinations: CD2, CD3 (surface and cytoplasmic), CD4, CD5, CD7, CD13, CD14, CD15, CD19, CD20, CD22, CD25, CD33, CD34, CD36, CD38, CD41, CD45, CD56, CD64, CD117, CD123, HLA-DR, MPO, and TDT (Becton-Dickinson, Biosciences, San Jose, CA, USA). Data were analyzed using FCS Express (De novo Software, Glendale, CA).

Cytogenetic studies

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from unstimulated bone marrow aspirate cultures (24 and 48 h) using standard GTG banding. At least 20 metaphases were analyzed. Results were reported using the 2013 International System for Human Cytogenetic Nomenclature [54]. Fluorescence in situ hybridization (FISH) analysis for *BCR-ABL1* fusion was performed on freshly harvested aspirate smears or cultured cells with LSI-BCR-ABL1 ES fusion probes (Abbott Molecular/Vysis, Des Plaines, IL) using previously described methods [55]. At least 200 interphase nuclei were analyzed.

Detection of BCR-ABL1 transcripts

Reverse transcription-quantitative polymerase chain reaction (PCR) for detection of *BCR-ABL1* transcripts [e1a2, e13a2 (b2a2), and e14a2 (b3a2)] was performed using RNA extracted from bone marrow or peripheral blood samples with sensitivities of 1 in 10,000 and 1 in 100,000, respectively, according to methods described previously [56, 57]. Quantitative results were expressed as the percent ratio of *BCR-ABL1* to *ABL1*. The sizes of fusion transcripts were determined by capillary electrophoresis.

Mutation analysis

Mutation analysis was performed using DNA extracted from bone marrow aspirate samples in a subset of patients using the following techniques. Next-generation sequencing-based analysis was performed to detect somatic mutations in the entire coding sequences of 28 genes (*ABL1*, *EGFR*, *GATA2*, *IKZF2*, *MDM2*, *NOTCH1*, *RUNX1*, *ASXL1*, *EXH2*, *HRAS*, *JAK2*, *MLL*, *NPM1*, *TET2*, *BRAF*, *FLT3*, *IDH1*, *KIT*, *MPL*, *NRAS*, *TP53*, *DNMT3A*, *GATA1*, *IDH2*, *KRAS*, *MYD88*, *PTPN11*, *WT1*) or in hotspots of 53 genes (*ABL1*, *CSF1R*, *FGFR1*, *HRAS*, *KRAS*, *PIK3CA*, *SRC*, *AKT1*, *CTNNB1*, *FGFR2*, *IDH1*, *MET*, *PTEN*, *STK11*, *ALK*, *DNMT3A*, *FGFR3*, *IDH2*, *MLH1*, *PTPN11*, *TP53*, *APC*, *EGFR*, *FLT3*, *JAK2*, *MPL*, *RB1*, *VHL*,*ATM*, *ERBB2*, *GNA11*, *JAK3*, *NOTCH1*, *RET*, *XPO1*,*BRAF*, *ERBB4*,

Table 1 Clinical characteristics of patients with secondary Philadelphia chromosome

No.	Initial Dx	Sex/age (years)	Dx at Ph emergence	Interval to Ph (months)	BCR-ABL1 transcript	Tx and response before Ph	Tx and response after Ph	OS from Ph (months)	Patient status	Disease status
1	AML	M/22	AML, 1st relapse	9	e1a2	Chemo	Chemo	1	Dead	Refractory
						CR	No response			
2	AML	F/31	AML, 3rd relapse	17	e1a2	Chemo, HSCT	Chemo, sorafenib	3	Dead	Refractory
						CR	No response			
3	AML	F/26	AML, 3rd relapse	26	b3a2 and b2a2	Chemo, HSCT	Chemo, dasatinib, ponatinib	4	Dead	Refractory
						CR	No response			
4	AML	F/57	AML, 3rd relapse	26	e1a2	Chemo, HSCT	Chemo, dasatinib	4	Dead	Refractory
						CR	No response			
5	AML	F/66	AML, 1st relapse	9	NA	Chemo, HSCT	Chemo	1	Dead	Refractory
						CR	No response			
6	AML	M/63	AML, refractory	9	b2a2	Chemo	Chemo, dasatinib	NA	Lost in F/U	NA
						No response	Initial response			
7	AML	M/65	AML, 2nd relapse	15	e1a2	Chemo	Chemo, dasatinib	9	Dead	Refractory
						CR	No response			
8	AML	M/52	AML, refractory	6	NA	Chemo	Chemo	4	Dead	Refractory
						No response	No response			
9	AML	M/28	AML, 1st relapse and progression	18	NA	Chemo	Chemo	NA	Lost in F/U	NA
						No response	NA			
10	AML	M/42	AML, 3rd relapse and progression	14	e1a2	Chemo, HSCT	Chemo	5	Dead	Refractory
						No response	No response			
11	AML	M/39	AML, refractory	11	e1a2	Chemo	Chemo	8	Alive	Refractory
						No response	No response			
12	MDS	F/48	sAML, 2nd relapse	14	e1a2	Chemo	Chemo	2	Dead	Refractory
						CR	No response			
13	MDS	F/58	sAML, progression	72	e1a2	Chemo	NA	NA	Lost in F/U	NA
						Progression				
14	MDS	M/78	sAML, 1st relapse, then persistent MDS	20	NA	Chemo	NA	NA	Lost in F/U	NA
						CR				
15	MDS	M/69	sAML, refractory	38	NA	Chemo	No treatment	1	Dead	Refractory
						Refractory				

Table 1 (continued)

No.	Initial Dx	Sex/age (years)	Dx at Ph emergence	Interval to Ph (months)	BCR-ABL1 transcript	Tx and response before Ph	Tx and response after Ph	OS from Ph (months)	Patient status	Disease status
16	MDS	M/62	sAML, 1st relapse and Refractory	42	e1a2	Chemo Refractory	Imatinib, nilotinib No response	3	Dead	Refractory
17	B-ALL	M/64	B-ALL, 1st relapse	26	e1a2	Chemo CR	Chemo, bosutinib, HSCT CR	6	Alive	CR
18	B-ALL	F/53	B-ALL, 1st relapse and refractory t-AML	40	e1a2	Chemo No response	Imatinib with CR and refractory t-AML	4	Dead	Refractory t-AML
19	T-ALL	F/18	2nd relapse and refractory	19	e1a2	Chemo Refractory	Chemo No response	1	Dead	Refractory

Dx diagnosis, *Tx* treatment, *Ph* Philadelphia chromosome, *Interval* interval time from initial diagnosis to the emergence of Ph, *OS* overall survival, *AML* acute myeloid leukemia, *sAML* secondary AML, *MDS* myelodysplastic syndrome, *B-ALL* B lymphoblastic leukemia, *T-ALL* T lymphoblastic leukemia, *t-AML* therapy-related AML, *M* male, *F* female, *Chemo* chemotherapy, *CR* complete remission, *HSCT* hematopoietic stem cell transplantation, *F/U* follow-up, *NA* not available

GNAQ, *KDR*, *NPM1*, *SMAD4*, *CDH1*, *EZH2*, *GNAS*, *KIT*, *NRAS*, *SMARCB1*, *CDKN2A*, *FBXW7*, *HNF1A*, *KLHL6*, *PDGFRA*, *SMO1)*. The next-generation sequencing assays were performed using the Illumina MiSeq sequencer (Illumina, San Diego, CA) with a sensitivity of 5% as described previously [58, 59]. *FLT3*-ITD, *FLT3* D835, and *NPM1* (exon 12, codons 956–971) mutations were assessed by PCR followed by capillary electrophoresis on a genetic analyzer (Prism ABI 3130, Applied Biosystems, Foster City, CA) as described previously [60]. *NRAS* (codons 12, 13, 61), *KRAS* (codons 12, 13, 61), *IDH1* (exon 4, codons 87–138), *IDH2* (exon 4), *KIT* (exons 8 and 17), and *JAK2* (codon 617) mutations were assessed by PCR followed by Sanger sequencing with a sensitivity of 20% as described previously [58, 61]. PCR-based cDNA sequencing of *BCR-ABL1* was performed to detect mutations in codons 221–500 of the *ABL1* kinase domain by using pyrosequencing PSQ96 HS System (Biotage AB, Uppsala, Sweden) per the manufacturer's instructions. The lower limit of detection of the assay is 5% mutation-bearing cells in the test samples. PCR-based microsatellite polymorphism analysis was performed by using GENESCAN on the pre-transplant, donor, and post-transplant samples.

Results

Timing of emergence of secondary Philadelphia chromosome

A total of 19 patients were identified to have secondary t(9;22)(q34;q11.2)/*BCR-ABL1* detected by chromosomal banding analysis during therapy of acute leukemia or myelodysplastic syndrome. The diagnoses at initial presentation were acute myeloid leukemia ($n = 11$), myelodysplastic syndrome ($n = 5$), B lymphoblastic leukemia ($n = 2$), or T lymphoblastic leukemia ($n = 1$) (Table 1). No variant *BCR-ABL1* rearrangements were identified. There were 11 men and 8 women with a median age of 53 years (range, 18–78 years) at initial diagnosis, and 56 years (range, 19–79 years) at time of emergence of secondary Philadelphia chromosome.

Of the 11 patients with an initial diagnosis of acute myeloid leukemia, 6 patients acquired secondary Philadelphia chromosome at time of first to 3rd relapse, 2 acquired secondary Philadelphia chromosome during progression of acute myeloid leukemia after 1–3 relapses, and 3 acquired secondary Philadelphia chromosome during treatment of refractory disease (patients never achieved remission before the emergence of secondary Philadelphia chromosome) (Table 1). Of the five patients with an initial diagnosis of myelodysplastic syndrome, all developed secondary acute myeloid leukemia, and acquired the Philadelphia

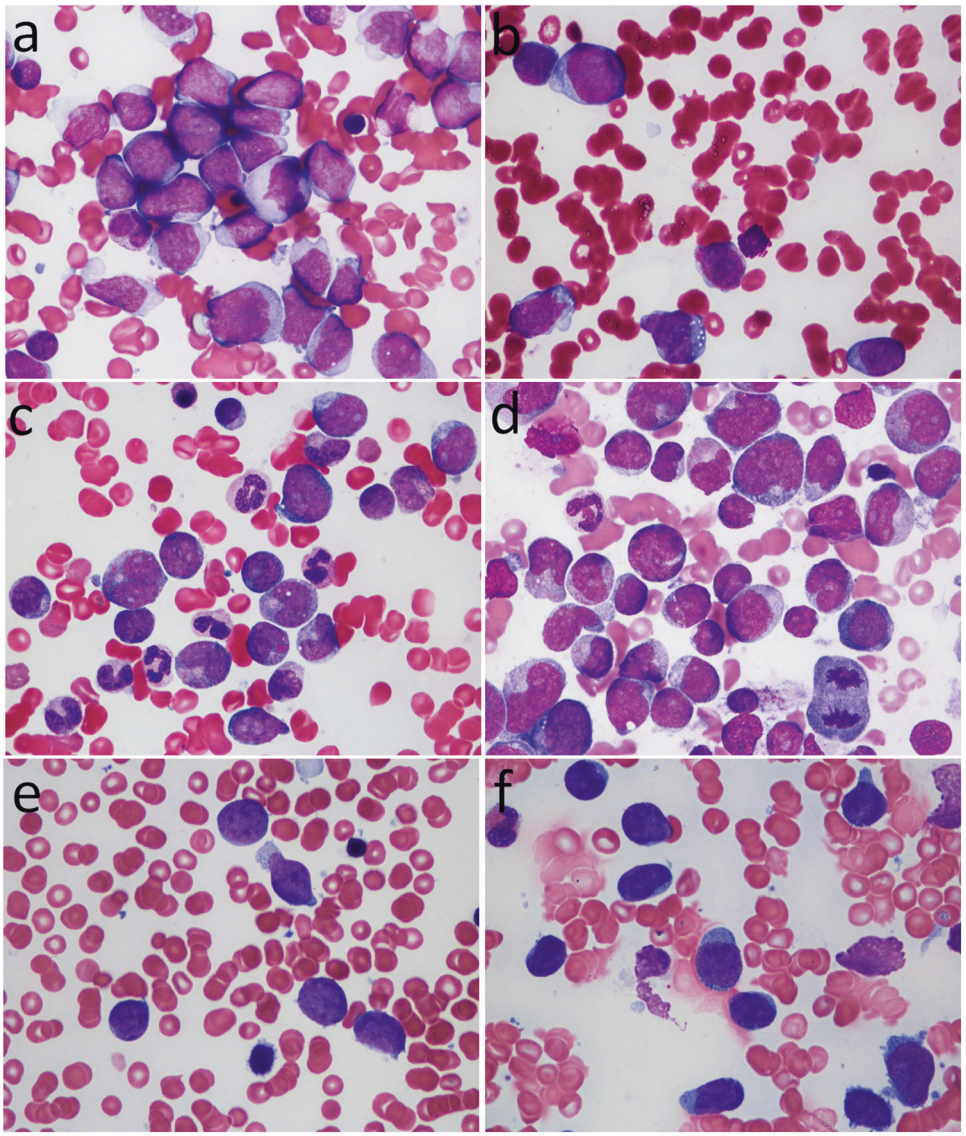

Fig. 1 Morphology of blasts before and after emergence of secondary *BCR-ABL1*. **a**, **b** Blast morphology of patient with acute myeloid leukemia (#8) before (**a**) and after (**b**) the emergence of secondary *BCR-ABL1*. **c**, **d** Blast morphology of patient with myelodysplastic syndrome (#16) before (**c**) and after (**d**) the emergence of secondary *BCR-ABL1*. **e**, **f** Blast morphology of patient with T lymphoblastic leukemia (#19) before (**e**) and after (**f**) the emergence of secondary *BCR-ABL1*. Original magnification: x1000

chromosome when secondary acute myeloid leukemia developed ($n = 1$), relapsed ($n = 1$), relapsed and became refractory to treatment ($n = 2$), or after relapse with persistent myelodysplastic syndrome (1% blasts in bone marrow) ($n = 1$). The two patients with an initial diagnosis of B lymphoblastic leukemia acquired the Philadelphia chromosome at relapse or at a refractory stage after relapse, respectively. In the patient with T lymphoblastic leukemia, the Philadelphia chromosome was acquired during treatment of refractory disease after two relapses. Among the patients with an initial diagnosis of acute myeloid leukemia or acute lymphoblastic leukemia, the median interval from the initial diagnosis of acute leukemia to the acquisition of secondary Philadelphia chromosome was 16 months (range, 6–40 months). Among the patients with an initial diagnosis of myelodysplastic syndrome, the median interval from the initial diagnosis of myelodysplastic syndrome to the emergence of secondary Philadelphia chromosome was 38 months (range, 14–72 months), and the median interval from the development of secondary acute myeloid leukemia to the emergence of secondary Philadelphia chromosome was 9 months (range, 0–37 months).

Complete blood count data and bone marrow morphology after acquisition of Philadelphia chromosome

At emergence of secondary Philadelphia chromosome, 18 patients had anemia (range, 8.4–11.5 g/dl), 16 had thrombocytopenia (range, 8–104 $\times$ 10^3/μl), and 8 had leukopenia (range, 1.0–3.8 $\times$ 10^3/μl) (Supplemental Table 1). Circulating blasts (range, 2–96%) were seen in 15 patients. In eight patients who had leukocytosis (range, 13.9–89.2 $\times$ 10^3/μl), blasts were the predominant cell population in five

patients. Six patients had absolute monocytosis at the time of emergence of secondary Philadelphia chromosome, and four of them had monocytosis before acquisition of the Philadelphia chromosome. Absolute eosinophilia, basophilia, and left-shifted granulocytes, features of chronic myeloid leukemia, were observed in only one patient (#13).

Bone marrow smears both before and after detection of secondary Philadelphia chromosome were available for retrospective review in nine patients. There was no change in the morphology of blasts before versus after emergence of secondary Philadelphia chromosome (Fig. 1), except in patient #1 whose blasts appeared more monoblastic at detection of secondary Philadelphia chromosome [62]. No immunophenotypic shift in the blasts was detected by flow cytometric analysis in 14 patients with available data (#1, 2, 4, 5, 6, 7, 9, 11, 12, 13, 16, 17, 18, and 19). Given the evolving nature of flow cytometry methods over the course of this study, however, blasts were evaluated with only a limited number of markers in early cases.

Clonal relatedness between diseases before and at emergence of secondary Philadelphia chromosome

Detailed cytogenetic and molecular findings at initial diagnosis, and before and after the emergence of secondary Philadelphia chromosome are provided in Tables 2 and 3.

Karyotypes at initial diagnosis were available in 16 patients (Table 2). Seven patients had a normal karyotype and nine had an abnormal karyotype. In seven of nine patients with an abnormal karyotype at initial diagnosis, the initial chromosomal alterations were present in the secondary Philadelphia chromosome-positive clones, thus the diseases before and at the emergence of the Philadelphia chromosome were clonally related, and the emergence of secondary Philadelphia chromosome may represent clonal evolution.

In five of seven patients with a normal karyotype, tests for molecular mutations with a limited panel were performed in bone marrow samples obtained before and at time of emergence of secondary Philadelphia chromosome. Common molecular mutations were detected before and at emergence of secondary Philadelphia chromosome in four patients (Table 3). *FLT3*-ITD mutation in patients #2 and 10, *NPM1* mutation in patient #4, and *NRAS* mutation in patient #6. Of note, the post-transplant microsatellite polymorphism pattern showed a mixed chimera in three patients tested (#2, 4, and 5), supporting the recipient origin of relapsed disease.

BCR-ABL1 transcript subtype and ABL1 mutation

FISH analyses were performed in 16 patients, and *BCR-ABL1* rearrangement was confirmed positive in all. In 14 patients tested by reverse transcription-quantitative PCR for *BCR-ABL1* transcript subtype, the e1a2 fusion transcripts (P190) were identified in 12 patients (86%). In the other two patients (#3 and 6), b2a2 or simultaneous b2a2 and b3a3 transcripts were identified, respectively. Of five patients analyzed for *ABL1* kinase domain mutations (#2, 3, 7, 16, and 17), only one (#7) showed a mutation (p.G254E) (Table 3).

Evolution of secondary Philadelphia chromosome-positive clones during therapy

Before the emergence of secondary Philadelphia chromosome detectable by chromosomal banding analysis, *BCR-ABL1* rearrangement or fusion transcripts were tested in eight patients by either FISH ($n = 3$) or reverse transcription-quantitative PCR ($n = 4$) or both ($n = 1$). A low level of *BCR-ABL1* fusion transcripts was detected in three patients either at initial presentation (#1, 0.11%; #11, 0.01%) or later during therapy (#18, 0.70%) by reverse transcription-quantitative PCR, but not by FISH. The *BCR-ABL1* transcript subtype was of e1a2 in all three patients. The median time from the detection of low level of *BCR-ABL1* by reverse transcription-quantitative PCR to the emergence of secondary Philadelphia chromosome detected by karyotyping was 9 months (range, 5–11 months).

After the emergence of secondary Philadelphia chromosome detected by karyotyping, the Philadelphia chromosome-positive subclones were the major one in 11 patients, simultaneously with the emergence of secondary Philadelphia chromosome in eight patients (#1, 6, 7, 9, 13, 16, 17, and 19) and shortly after the emergence of secondary Philadelphia chromosome in three patients (#4, 12, and 18). The Philadelphia chromosome-positive subclones were a minor clone in the remaining eight patients. Twelve patients had follow-up information on the evolution of Philadelphia chromosome-positive subclones after initial detection. The Philadelphia chromosome-positive subclones were persistent by FISH analysis in six patients (#2, 6, 8, 11, 12, and 16) despite chemotherapy with or without tyrosine kinase inhibitors. In the remaining six patients (#3, 4, 7, 10, 17, and 18), the Philadelphia chromosome-positive subclones were undetectable by FISH analysis, including three patients (#4, 10, and 17) with undetectable *BCR-ABL1* transcripts by reverse transcription-quantitative PCR. Of those six patients with undetectable Philadelphia chromosome-positive subclones, five were treated with tyrosine kinase inhibitors and one received conventional chemotherapy. Interestingly, all 12 patients had persistent or refractory acute myeloid leukemia or acute lymphoblastic leukemia regardless of the presence or absence of the Philadelphia chromosome-positive subclones, including one patient (#18) with refractory therapy-related acute myeloid

Table 2 Secondary *BCR-ABL1* detected by karyotyping, FISH, and molecular analyses

No.	At initial Dx[a]	Immediately before emergence of Ph	At and after emergence of Ph
1	46,XY,t(3;13)(q27;q14) [1]/45,XY,t(3;13)(q27;q14),−7 [19] FISH(−); Q-PCR(+): 0.1%	46,XY [20]	Initial: 45,XY,t(3;13)(q27;q14),-7 [3]/45,XY,t(3;13)(q27;q14),-7,t(9;22)(q34;q11.2) [14]/46,XY [3] FISH(+): 50%
2	46,XX [20]; Q-PCR(−) at 1st relapse	46,XX [1]/46,XY [19][b]	1. Initial: 46,XX,t(9;22)(q34;q11.2) [7]/46,XX [2]/46,XY [11]; FISH(+): 9% 2. Refractory AML, 6 wk after Ph, before sorafenib: FISH(+): 68%; Q-PCR(+): 55.33%
3	NA	46,XX,t(2;17)(p21;p11.2),t(14;17)(q24;q25) [16]/46,XX [4][b]	1. Initial: 46,XX,del(2)(p23),add(14)(q11.2),−17,+mar [8]/46,idem,t(9;22)(q34;q11.2) [2]/46,idem[cp4]/ 46,XX,del(1)(q42),del(11)(q13q23) [4]/46,XX [2]; FISH(+): 6.5%; Q-PCR(+), 9.28% 2. Persistent AML, 11 wk after Ph, 7 wk after ponatinib; FISH(−)
4	46,XX [20] FISH(−) at 2nd relapse	46,XY [20][b]	1. Initial: 46,XX,t(9;22)(q34.1;q11.2) [5]//46,XY [15] FISH(+): 6.5% 2. Persistent AML, 4 wk after Ph: Q-PCR(+): 80.92% 3. Persistent AML, 10 wk after Ph/7 wk after dasatinib: Q-PCR(−)
5	48,XX,+6,+8 [18]/46,XX [2]	46,XX [19][b]	Initial: 46,XX,t(15;17)(q15;q11) [8]/46,XX,t(2;7)(p21;q36),t(9;22)(q34;q11.2),add(12)(p11.2), t(15;17)(q15;q11) [4]/46,X,t(X;15;17)(p22;q15;q11) [8]
6	46,XY [20]; Q-PCR(-) 2 months later	46,XY [20]	1. Initial: 46,XY,t(9;22)(q34;q11.2) [15]/46,XY [5]; FISH(+): 39%; Q-PCR(+) 2. Persistent AML, 2 wk after Ph, before dasatinib: FISH(+): 48%
7	46,XY,inv(16)(p13q22) [20]	46,XY,?del(16)(q22) [3]/46,XY [17]	1. Initial: 46,XY,inv(16)(p13q22) [2]/48,idem,t(9;22)(q34;q11.2),+13,+22 [16]/47,XY,inv(16)(p13q22),+13 [1]/48,XY,inv(16)(p13q22),+13,+22 [1] FISH(+): 4.5% Q-PCR(+): 8.81% 2. Persistent AML, 8 wk after Ph, 4 wk after dasatinib: Q-PCR(+):<0.01%
8	45,XY,inv(3)(q21q26.2),−7 [10]/46,XY [10]	45,XY,inv(3)(q21q26.2),−7 [20]; FISH(−)	Initial: 45,XY,inv(3)(q21q26.2),−7 [19]/44,XY,inv(3)(q21q26.2),−7,t(9;22)(q34;q11.2),−18 [1]; FISH(+): 2.5%
9	46, XY	46,XY [19]	Initial: 46,XY,t(9;22)(q34;q11.2) [14]/46–47,XY,del(3)(p13),t(9;22)(q34;q11.2), + mar[cp4]/46,XY [2]
10	46,XY	46,XY[b]	1. Initial: 46,XY,del(20)(q11.2q13.3) [3]/46,XY,t(9;22)(q34;q11.2) [1]/46,XY,t(1;16)(p33;q24) [1]/ 46,XY [15]; FISH(+): 0.4% 2. Persistent AML, 6 wk after Ph; Q-PCR(−)
11	45,XY,inv(3)(q21q26.2),−7 [19]/45,idem,t(5;12)(q31;p13) [1] Q-PCR(+): 0.01%	45,XY,inv(3)(q21q26.2),−7 [19]/ 45,idem,t(11;17)(p11.2;p11.2) [1]	1. Initial: 8/10/2016 45,XY,inv(3)(q21q26.2),−7 [14]/45,idem,t(9;22)(q34;q11.2) [5]/ 45,idem,t(11;17)(q11.2;q11.2) [1] 2. Refractory AML, 6 wk after Ph: 45,XY,inv(3)(q21q26.2),−7 [19]/46,idem,+21 [1]; FISH(+): 3%
12	NA	45,XX,del(5)(q13q33),inv(6)(p11.2q21), add(7)(p15),−13 [10]//46,XX [10]	1. Initial: 45,XX,del(5)(q13q33),inv(6)(p11.2q21),add(7)(p15),−13 [3]/45,idem,t(9;22)(q34;q11.2) [6]/ 45,idem,17,mar [1]/46,XX [10]; FISH(+): 21%

Table 2 (continued)

No.	At initial Dx[a]	Immediately before emergence of Ph	At and after emergence of Ph
			2. Refractory AML, 8 wk after Ph, before dasatinib:45,XX,del(5)(q13q33),inv(6)(p11.2q21), add(7)(p15),t(9;22)(q34;q22.1),−13 [20]; FISH(+): 94%; Q-PCR(+): > 100%
13	46,XX,t(1;12)(p13;q15) [20]	46,XX,t(1;12)(p13;q15) [20]	Initial: 46,XX,t(1;12)(p13;q15)?c [3]/46,XX,t(1;12)(p13;q15)?c,t(9;22)(q34;q11.2) [17]
			FISH(+): 66%; Q-PCR(+): 77.93%
14	47,XY,+8	NA	Initial: 46,XY,t(9;22)(q34;q11.2) [3]/47,XY,+8 [7]/46,XY [10]
15	47,XY,+21	47,XY,+21 [12]/46,XY [8]	Initial: 47,XY,inv(17)(q11.2q23),+21 [2]/47,idem,t(9;22)(q34;q11.2) [2]; FISH(+): 4.5%
16	NA	46,XY,t(3;21)(p21;q22) [8]/46,XY [12]	1. Initial: 46,XY,t(9;22)(q34;q11.2) [17]/46,XY,der(9)t(9;22)(q34;q11.2)inv(9)(p12q32),der(22)t(9;22) [2]
			2. Refractory AML, 5 wk after Ph, 4 wk after imatinib: 46,XY,t(9;22)(q34;q11.2) [18]/ 46,XY,der(9)t(9;22)(q34;q11.2)inv(9)(p12q32),der(22)t(9;22) [1]; FISH(+): 92.5%; Q-PCR(+): >100%
17	46,XY [20]; FISH(−)	46,XY [20]	1. Initial: 48,XY,+5,t(9;22)(q34;q11.2),+10,−21,+der(22)t(9;22) [3]/49,XY,+5,t(9;22),−21,+der(22)t(9;22),+2mar [1]/46,XY [2]; FISH(+): 22.5%; Q-PCR(+): 61.55%;
			2. CR,10 wk after Ph,9 wk after Bosutinib: 46,XY [20]; FISH(-); Q-PCR(-)
18	46,XX [18]	45,XX,−7 [20]; Q-PCR(+) (relapsed/persistent B-ALL, 6 wk before Ph, low quality RNA)	1. Initial (63% blasts): 45,XX,−7 [4]/45,XX,−7,del(18)(q21) [8]/ 46,XX,del(1)(p13),del(4)(p15.2),del(5)(q13),−9,t(9;22)(q34;q11.2),−18,+2mar [1]/46,XX [2]
			2. Persistent B-ALL, 3 wk after Ph, before imatinib: FISH(+): 65.5%, Q-PCR(+): 67.8%
			3. t-AML, 14 wk after Ph, 9 wk after imatinib: 46,XX [30]; FISH(-)
19	68~87[3n],XXX,+1,+2,+3×2,+3q21,+4,+5,+5q13,+9,+9q10x2,+10,+12,+12p11.2,+13,+14,−16,−17,−18,+19,+19p13.1,+20,+20q13.3×2,+22×2,+6mar [5]/46,XX [15]	74~84<4n>,XXX,add(X)(p21),−1,del(1)(p21p36.1),−2, add(11)(q23),−13,−14,−15,−17, der(18)t(17;18)(q11.2;q11.3)x2,add(19)(p13.3),−22,+6~9mar[cp2]/46,XX [18]	Initial: 78~84<4n>,XXX,add(X)(p21)x2,−1,der(1)t(1;9)(p13;q34),−2,add(2)(p12),−4,−5,−9,add(9)(q22)x2,−10,−10,−11,add(11)(q23),−13,−15,−16,−17,−17,der(18)t(17;18)(q11.2;q11.3)x2, add(19)(p13.3),add(21)(p11.2)x2,der(22)add(22)(p11.2)t(9;22)(q34;q11.2),−22,−22,+ider(?)(?q10)t(9;22)(q34;q11.2)x2,+2mar[cp12]/46,XX [8]; FISH(+): 84.5%; Q-PCR(+): 68.41%

Ph Philadelphia chromosome, *FISH* fluorescence in situ hybridization, *Q-PCR* reverse transcription quantitative polymerase chain reaction, *NA* not available, *wk* weeks, *AML* acute myeloid leukemia, *B-ALL* B lymphoblastic leukemia, *t-AML* therapy-related AML, *PR* partial response, *CR* complete remission

[a]Only karyotypes at initial diagnosis are provided; FISH or Q-PCR may be performed later

[b]Post bone marrow transplantation

Table 3 Mutations detected before and at/after emergence of Philadelphia chromosome

No.	Mutations before emergence of Ph	Mutations at/after emergence of Ph
1	*NRAS*(+), *KRAS*(−) *FLT3*-ITD(−), *FLT3* D835(−)	*FLT3*-ITD(−), *FLT3* D835(−)
2	*FLT3*-ITD(+), *FLT3* D835(−), *CEBPA*(+), *IDH1*(−), *IDH2*(−), *KIT*(−), *RAS*(−) *NPM1*(−)	*FLT3* ITD(+), *FLT3* D835(−), *ABL1*(−)
3	NA	*FLT3* ITD(+), *NRAS*(+)[a], *RUNX1*(+)[a], *WT1*(+)[a], *ABL1*(−)
4	*NPM1*(+), *IDH1*(+), *JAK2*(−), *KIT*(−), *FLT3*(−), *IDH2*(−), *KRAS*(−), *NRAS*(−)	*NPM1*(+), *IDH1*(−), *IDH2*(−), *FLT3*(−), *KIT*(−), *CEBPA*(−)
5	*NRAS*(+)	NA
6	*NRAS*(+)[a] *GATA2*(+)[a]	*NRAS*(+)
7	NA	*ABL1*(+), *NRAS*(−), *KIT*(−), *NPM1*(−), *CEBPA*(−)
8	*FLT3*-ITD(−), *FLT3* D835(−), *KRAS*(−), *NRAS* (−)	NA
9	*NRAS*(−), *KRAS*(−)	NA
10	*FLT3*-ITD (+) *FLT3* D835(−), *NPM1* (+)	*FLT3*-ITD(+), *FLT3* D835(−)
11	Negative[a]	*FLT3*-ITD(−), *FLT3* D835(−)
12	*FLT3*-ITD(−), *FLT3* D835(−), *KIT*(−), *NPM1*(−), *RAS* (−), *CEBPA*(−), *IDH1*(−), *IDH2*(−)	NA
13	*FLT3*-ITD(−), *FLT3* D835(−), *KIT*(−), *NPM1*(−), *RAS* (−)	NA
14	NA	*JAK2*(+)[b], *EZH2*(+)[b], *CEBPA*(+)[b]
15	*FLT3*-ITD(−), *FLT3* D835(−), *RAS*(−)	NA
16	*FLT3*-ITD(−), *FLT3* D835(−), *RAS*(−)	*ABL1*(−)
17	NA	Negative[a], *ABL1*(−)
18	NA	NA
19	NA	*TP53*(+)[a]

Ph Philadelphia chromosome, *NA* not available. Common mutations before and at/after emergence of Ph are underlined

[a]Tested with next-generation sequencing-based analysis with 28 gene panel

[b]Tested with next generation sequencing-based analysis with 53 gene panel

leukemia resulting from treatment of B lymphoblastic leukemia with secondary Philadelphia chromosome.

Treatment and outcome

Before the emergence of secondary Philadelphia chromosome, all 19 patients received chemotherapy (Table 1). In addition, five patients received allogeneic hematopoietic stem cell transplantation. Only nine patients achieved morphologic remission or deeper response, and all nine patients developed relapsed disease.

After the emergence of secondary Philadelphia chromosome, 15 patients had treatment and follow-up information available; the remaining four patients were transferred to other institutions for treatment and were lost to follow-up (Table 1). Seven patients were treated with chemotherapy only, five treated with chemotherapy plus tyrosine kinase inhibitors, two treated with tyrosine kinase inhibitors only, and one not treated due to other comorbid conditions. Thirteen patients died with refractory disease after a median follow-up of 3 months (range, 1–9 months) after the emergence of secondary Philadelphia chromosome. Two patients were living; one (#11) had an initial diagnosis of acute myeloid leukemia, and had refractory disease at last follow-up 8 months after the emergence of secondary Philadelphia chromosome. The other patient (#17) had an initial diagnosis of B lymphoblastic leukemia, and received chemotherapy combined with tyrosine kinase inhibitors, as well as subsequent allogeneic hematopoietic stem cell transplantation after the emergence of second Philadelphia chromosome. The patient was free of residual disease at last follow-up 6 months after the emergence of secondary Philadelphia chromosome. Another patient (#18) with an initial diagnosis of B lymphoblastic leukemia achieved remission with tyrosine kinase inhibitors after the emergence of secondary Philadelphia chromosome. However, this patient developed therapy-related acute myeloid leukemia and died shortly thereafter.

Discussion

We reported 19 patients with Philadelphia chromosome-negative de novo acute myeloid leukemia, myelodysplastic syndrome, B lymphoblastic leukemia, and T lymphoblastic leukemia who secondarily acquired the Philadelphia chromosome during therapy. The emergence of secondary Philadelphia chromosome-positive clones by karyotyping occurred concomitantly with relapsed or refractory disease in de novo acute leukemia or secondary acute myeloid leukemia arising from myelodysplastic syndrome. In most patients, the *BCR-ABL1* transcript subtype was of the e1a2 (encoding P190 BCR-ABLl protein), and the Philadelphia chromosome-positive disease and the original leukemia were clonally related. The Philadelphia chromosome-positive clones were the dominant clones in more than half of the patients when secondary Philadelphia chromosome emerged. Despite the absence of the Philadelphia chromosome-positive clones by karyotyping in 6 patients, 13 of 15 patients with follow-up information died with refractory disease after a median follow-up of 3 months, including one patient with therapy-related acute myeloid leukemia.

Secondary Philadelphia chromosome acquired during therapy of myeloid or lymphoid neoplasms is rare; 36 such cases have been reported in the form of single-case reports in the English literature (Table 4 and Supplemental Table 2) [20–53]. We reviewed these published cases and reclassified them, if necessary or possible, according to the 2016 update of World Health Organization classification [63]. The 36 cases included the following: acute myeloid leukemia ($n = 14$), myelodysplastic syndrome ($n = 10$), B lymphoblastic leukemia ($n = 6$), T lymphoblastic leukemia ($n = 5$), and chronic myelomonocytic leukemia ($n = 1$). There were 20 men and 16 women with a median age of 43 years (range, 3–71 years), approximately 10 years younger than that in our study. Similar to our cases, secondary Philadelphia chromosome was detected at relapse or at a terminal stage of refractory disease in patients with acute leukemia. In patients with myelodysplastic syndrome, the Philadelphia chromosome emerged at time of or after acute transformation in 8 of 10 patients. The median interval from initial diagnosis to secondary acquisition of the Philadelphia chromosome was 15 months (range, 1–120 months). Twenty-one patients had an abnormal karyotype at initial diagnosis or anytime during therapy before secondary Philadelphia chromosome emerged; the abnormalities present before the emergence of secondary Philadelphia chromosome were also observed in the Philadelphia chromosome-positive clones in 19 patients, including one patient with *NRAS* mutation observed before and at emergence of the Philadelphia chromosome. Additionally, one patient with a normal karyotype before secondary acquisition of the Philadelphia chromosome had *NPM1* mutations before and at emergence of the Philadelphia chromosome. The *BCR-ABL1* fusion transcript encoded P190 in 16/24, P210 in 5/24, and both P210 and P190 both in three patients. Of note, in the latter three patients, the Philadelphia chromosome was not detected by karyotyping but P210-encoding transcripts were detected by reverse transcription-quantitative PCR, and P190/e1a2 transcripts emerged later 3, 4, and 14 months before the detection of the Philadelphia chromosome by karyotyping, suggesting that the secondary Philadelphia chromosome encoded P190. *BCR-ABL1* at the onset of the disease was detected in only 2 of 17 patients tested with reverse transcription-quantitative PCR, FISH, or both. Twenty-three patients had treatment information

Table 4 Summary of clinicopathologic characteristics of patients with secondary Philadelphia chromosome

	Current study	Previous studies [20–53]	Overall
Case number	19	36	55
Age: median; range (yrs)	53 (18–78)	43 (3–71)	48 (3–78)
Interval: median; range (mo)	18 (6–72)	15 (1–120)	16 (1–120)
Disease distribution (*n*)			
AML	11	14	25 (45.5%)
MDS	5	10	15 (27.3%)
B-ALL	2	6	8 (14.5%)
T-ALL	1	5	6 (10.9%)
CMML	0	1	1 (1.8%)
BCR-ABL1 subtype			
P190	12/14	16/24	28/38 (73.7%)
P210	2/14	5/24	7/38 (18.4%)
P190, P210	0	3/24	3/38 (7.9%)
Clonal relatedness[a]			
By karyotype	7/9	19/21	26/30 (86.7%)
By molecular mutation	4/5	2/2[b]	5/6 (83.3%)[b]
Total	11/14	20/22[b]	31/36 (86.1%)
Median OS after Ph (mo)	3.1	4.5	4

Ph Philadelphia chromosome, *Age* age at initial diagnosis, *yrs* years, *Interval* interval time from initial; diagnosis to the emergence of Ph, *mo* months, *n* number

[a]Comparison between diseases at any time before the emergence of Ph and at the emergence of Ph

[b]Including 1 patient with clonally related diseases before and at the emergence of Ph by karyotyping

available, 21 patients received chemotherapy, 8 received tyrosine kinase inhibitors, and 6 received allogeneic hematopoietic stem cell transplantation. Of 33 patients with follow-up information available regarding the disease status, 30 had relapsed or refractory disease and 26 were dead at last follow-up, with a median follow-up of 5 months (range, 1–22 months). For the 16 patients with follow-up information available regarding the evolution of the Philadelphia chromosome-positive clones, the Philadelphia chromosome-positive clones were persistent and/or became the major clone in most patients despite intensive chemotherapy, and only 3 patients achieved cytogenetic remission [32, 52, 53].

The emergence of secondary Philadelphia chromosome can be explained by multiple mechanisms. It may indicate clonal evolution and disease progression. This hypothesis is supported by the findings that the diseases before and after the emergence of secondary Philadelphia chromosome were clonally related by karyotyping or molecular analyses in the vast majority of patients (86%) in our series and in patients reported previously (Table 4 and Supplemental Table 2). This apparently underestimates the clonal relatedness given the limited panel used to detect molecular mutations. Alternatively, in a subset of patients the emergence of secondary Philadelphia chromosome could be due to the expansion of a minute Philadelphia chromosome-positive subclone not detectable initially by cytogenetic methods. The detection of the same transcript subtype of e1a2 before and at time of emergence of the Philadelphia chromosome-positive subclones in three patients (#1, 11, and 18) in this study would substantiate this theory. In all three patients, interestingly, the Philadelphia chromosome-positive subclones clonally related to the previous disease before the emergence of secondary Philadelphia chromosome.

The persistence or refractoriness of disease despite the disappearance of secondary Philadelphia chromosome-positive clones in some patients suggests that *BCR-ABL1* is not essential for the maintenance of the leukemic process. However, the persistence of Philadelphia chromosome-positive clones despite chemotherapy and/or tyrosine kinase inhibitors in some patients and predilection of the e1a2 (P190) subtype rather than the typical b2a2/b3a2 (P210) in most patients support a role of *BCR-ABL1* in disease progression. Both P210 and P190 *BCR-ABL1* fusion transcripts result in new chimeric proteins with increased tyrosine kinase activity. However, P190 was shown to have at least fivefold higher tyrosine kinase activity than P210 in a murine model [64]. Transgenic mice bearing the e1a2 (P190) fusion transcript develop acute leukemia [65], whereas transgenic mice bearing P210 develop chronic myeloid leukemia [66]. Patients with myelodysplastic syndrome with P190 at initial presentation show rapid progression to acute myeloid leukemia [67]. P190 is also associated with transformation of chronic myeloid leukemia to a more aggressive terminal phase disease [68]. In addition, patients with de novo acute myeloid leukemia with P190 have a higher rate of relapse than patients with P210 [19]. Overall, these findings correlate with our finding that P190 is highly associated with disease progression in myeloid and lymphoid neoplasms.

Adding tyrosine kinase inhibitors to the therapeutic regimens has significantly altered the outcome of patients with chronic myeloid leukemia [69, 70] and Philadelphia chromosome-positive acute lymphoblastic leukemia [71, 72], and the efficacy has also been shown in Philadelphia chromosome-positive de novo acute myeloid leukemia [19]. It is worth mentioning that tyrosine kinase inhibitors are effective in rare cases of myelodysplastic syndrome with the Philadelphia chromosome [73] and rare cases with concurrent *BCR-ABL1* and *CBFB* rearrangements [31, 74, 75]. However, the effect of tyrosine kinase inhibitors along with or in place of chemotherapy in patients with late-acquired Philadelphia chromosome in relapsed or refractory disease is not well understood. We reported seven such patients treated with tyrosine kinase inhibitors. In this study, eradication of the Philadelphia chromosome-positive clones detected by FISH analysis was observed in five of six patients with follow-up information although four of them had refractory Philadelphia chromosome-negative disease. Eight such patients reported in the literature received tyrosine kinase inhibitors; seven showed partial or complete response to tyrosine kinase inhibitors although four patients had relapsed or refractory disease at last follow-up. These findings show that incorporating tyrosine kinase inhibitors in the treatment is an effective approach to treat the Philadelphia chromosome-positive clones in most patients. Overall however, prognosis of these patients is still poor due to other factors.

In conclusion, the Philadelphia chromosome, usually seen as a primary change in chronic myeloid leukemia, can also occur secondarily as a late-developing chromosomal abnormality during therapy of myeloid and lymphoid neoplasms. The transcript subtype associated with the late-occurring Philadelphia chromosome is overwhelmingly of the e1a2 (P190), and the disease before and after the emergence of the Philadelphia chromosome are clonally related. Incorporating tyrosine kinase inhibitors into treatment may eradicate the Philadelphia chromosome-positive clones and could potentially change the disease prognosis in a small subset of patients, but most patients do not respond to the treatment and the overall survival is poor.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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