Leukemia Research 84 (2019) 106176

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Clinical implications of cytogenetic heterogeneity in Philadelphia chromosome positive (Ph+) adult B cell acute lymphoblastic leukemia following tyrosine kinase inhibitors and chemotherapy regimens

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ARTICLE INFO

Keywords:
Philadelphia chromosome (Ph)
BCR-ABL1
Variant t(9, 22)
Complex t(9, 22)
B-ALL
B cell acute lymphoblastic leukemia
Cytogenetic heterogeneity
additional chromosomal abnormalities (ACAs)
Tyrosine kinase inhibitor (TKI)
Overall survival (OS)
Relapse-free survival (RFS)

ABSTRACT

We retrospectively studied a cohort of 144 adults with Philadelphia chromosome/*BCR*-ABL*1* positive B acute lymphoblastic leukemia (Ph + B-ALL) to assess the clinical implications of cytogenetic heterogeneity in this disease. The study group included 85 men and 59 women that were sorted into 6 subgroups based on karyotypic findings in the stemline as follows: 32 patients with t(9;22) as a sole aberration, 23 with t(9;22) plus 1 additional chromosomal abnormality (ACA), 26 with t(9;22) as part of a complex karyotype, 18 showing a variant-/complex- t(9;22), 30 with t(9;22) as the stemline with ACAs in the sideline(s), and 15 patients who had the t(9;22) and hyperdiploidy. In 89 patients 1 clone was identified; 41 had 2 clones and 14 had ≥ 3 clone(s). The median overall survival (OS) was 25.6 months and the median relapse-free survival (RFS) was 20.6 months. Patients with variant-/complex- t(9;22) had poorer OS and RFS when compared with all other subgroups combined ($P = 0.0018$ and $P = 0.0049$, respectively). In addition, patients with ≥ 2 clones had worse OS and RFS than patients with 1 clone ($P = 0.0179$ and $P = 0.0429$, respectively). Multivariate analysis confirmed that variant-/complex-t(9;22) and clone number are independent risk factors. We suggest that conventional chromosomal analysis is of clinical importance for risk stratification of B-ALL patients.

1. Introduction

The t(9;22)(q34;q11.2), also termed the Philadelphia chromosome (Ph) resulting in *BCR-ABL1* fusion, is the most common recurrent cytogenetic abnormality in adult B acute lymphoblastic leukemia (B-ALL), accounting for about 25% of all cases [1–4]. The Ph chromosome in B-ALL patients is known to be associated with a poorer prognosis. Cytogenetic heterogeneity in Ph positive B-ALL (Ph + B-ALL), in the form of additional chromosomal abnormalities (ACAs), has been reported frequently in chronic myeloid leukemia (CML) [5] and childhood or adult Ph + B-ALL [6,7], including gain of an additional Ph or the "+der(22)t(9;22)", gain of the X chromosome, trisomy 8 or 21, high hyperdiploidy (> 50 chromosomes), monosomy 7 or deletions of chromosomes 7p and 9p, among others. In addition, variant-t(9;22), often involving a 3-/4-way translocation that includes t(9;22), has been reported in 5-10% of CML [8] and less frequently in B-ALL cases [9,10]. However, complex-t(9;22) translocations, often accompanied by additional structural aberrations involving the translocated der(9)t(9;22) or der(22)t(9;22), have not been well documented.

Earlier studies, performed before the introduction of tyrosine kinase inhibitor (TKI) therapy, showed that genomic variations in Ph + B-ALL have a negative impact on clinical outcome [6,7]. However, the incorporation of TKIs into various treatment regimens, as well as risk-adapted therapy including early allogeneic hematopoietic stem cell transplantation (allo-HSCT), has improved overall outcomes dramatically [11–18]. In the TKI therapy era, ACAs have been reported to show a variable impact on overall survival (OS) or relapse-free survival (RFS) in CML [19,20]. In B-ALL, the impact of ACAs on prognosis, either at

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https://doi.org/10.1016/j.leukres.2019.106176
Received 11 March 2019; Received in revised form 24 June 2019; Accepted 25 June 2019
Available online 27 June 2019

the time of initial diagnosis or after therapy, in the setting of minimal residual disease (MRD), is more controversial [21–23]. A recent study reported that some ACAs have an adverse impact on outcome in adult Ph + B-ALL patients treated with TKI and HSCT [21].

Many studies have shown that cytogenetic response to treatment is an important prognostic factor in patients with CML [24,25], myelodysplastic syndromes [26], and acute myeloid leukemia [27]. By contrast, only one study has shown a correlation between the persistence of cytogenetic abnormalities at the time of morphologic remission and poorer prognosis in B-ALL patients [22]. A report from our institution showed that "+der(22)t(9;22)" and "-9/9p" ACAs in patients with Ph + B-ALL treated with TKIs or chemotherapy plus a TKI are associated with poorer outcomes [28]. Otherwise, there are limited data available regarding a systematic assessment of the clinical implications of clonal heterogeneity in Ph + B-ALL [6,7,21,23,28] and only rare reports on the clinical impact of variant-/complex- t(9;22) translocations or cytogenetic clone number in adult Ph + B-ALL.

In this study, we retrospectively reviewed the cytogenetic findings in patients with Ph + B-ALL to systematically investigate the clinical implications of ACAs and cytogenetic heterogeneity. Our results suggest that some additional karyotypic findings in Ph + B-ALL have prognostic impact and are useful for risk stratification.

2. Methods

2.1. Patient involvement

We retrospectively reviewed the cytogenetic data of adult B-ALL patients diagnosed and/or treated at The University of Texas MD Anderson Cancer Center from January 1, 2004 through December 31, 2014. All patients with t(9;22)(q34.1;q11.2) and variants identified by conventional cytogenetic analysis and *BCR-ABL1* fusion identified by fluorescence *in situ* hybridization (FISH) were included. B-ALL patients with a cryptic *BCR-ABL1* fusion identified by FISH or PCR only and patients with a history of CML were excluded for the study. Clinical, pathologic, and laboratory data were reviewed. The study was approved by the Institutional Review Board.

2.2. Immunophenotypic, conventional cytogenetics and FISH methods

Eight-color flow cytometric immunophenotypic analysis was performed according to standard procedures and the antibodies assessed included reagents specific for CD2, CD3, CD4, CD5, CD7, CD9, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD25, CD33, CD34, CD38, CD45, CD52, CD56, CD58, CD66c, CD79a (cytoplasmic), CD117, CD123, HLA-DR, myeloperoxidase, IgM (cytoplasmic), kappa and lambda light chains (Becton-Dickinson Biosciences, San Jose, CA), and TdT (Supertechs Inc, Bethesda, MD). The laboratory established cut-off for minimal residual disease (MRD) is 0.01%.

Chromosome analysis was performed on non-stimulated overnight cultures of bone marrow or leukemic peripheral blood following standard procedures in the Clinical Cytogenetics Laboratory at the time of diagnosis, and follow up when applicable. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013 and 2016). FISH was performed using the LSI *BCR-ABL1* dual color extra signal or dual color dual fusion probe (Abbott Diagnostics/Vysis, Des Plaines, IL) on bone marrow aspirate smears, cultured bone marrow cells or leukemic peripheral blood at the time of diagnosis, and as a part of follow up studies or during the workup of relapse when possible.

2.3. Cytogenetic subgroups

Due to the cytogenetic heterogeneity, we attempted to categorize all patients into 6 cytogenetic subgroups based on chromosomal abnormalities identified in the stemline of diagnostic specimens. Subgroup I; t(9;22) as a sole abnormality (termed as Sole); Subgroup II, t(9;22) and one clonal additional chromosomal abnormality (ACA) (termed as 1ACA); Subgroup III, t(9;22) as part of a complex karyotype with at least 2 or more additional unrelated aberrations (termed as CK); Subgroup IV, apparent 3-/4-way translocation involving the t(9;22) and resulting in a variant-t(9;22) or cases with additional aberrations involving in the translocated chromosomes, the der(9)t(9;22) or the der(22)t(9;22), resulting in a complex-t(9;22) (termed as Variant-/Complex-); Subgroup V, a sole aberration of t(9;22) in the stemline and sideline(s) that show other ACAs assumed to be evidence of "clonal evolution" (termed as CE); and Subgroup VI, t(9;22) as part of hyperdiploidy (defined as $\geq$ 48 chromosomes in the current study) (termed as HPD). In addition, we assessed the number of clones in Subgroups II - VI.

Cytogenetic status at the time of hematological remission was categorized into three groups: 1) Normal karyotype; 2) "Same/persistent", showing the original diagnostic cytogenetic abnormality and 3) "ACA", showing ACAs with or without diagnostic aberrations including t(9;22).

2.4. Clinical remission and minimal residual disease

Hematologic/morphologic remission and relapse were defined according to established criteria [22,29,30]. Minimal residual disease (MRD) assessment was performed using multi-parameter flow cytometry at the time of complete remission (CR) and hematologic or morphologic remission [31]. Time to CR was calculated from the end of the induction to the time of CR achieved. Time to relapse was calculated from time of CR to the time of relapse. Relapse-free survival (RFS) was calculated from the time of CR until relapse or death or last follow up. Overall survival (OS) was calculated from the time of treatment induction until death or the last follow up.

2.5. Statistical analysis

Continuous variables were described using median and range whereas categorical variables were described using frequencies and percentages. The variability across the various cytogenetic categories was tested using ANOVA and by Chi-square test for the categorical variables. Two-tailed significance was established at the value of $P < 0.05$. All calculations were performed using SAS version 9.2 (SAS Institute, Cary, NC). RFS and OS were calculated using GraphPad Prism 6 software (GraphPad Software, Inc. La Jolla, CA, USA). Kaplan-Meier curves for OS were plotted and the log-rank test was applied with a P value < 0.05 being considered as statistically significant. Multivariate analysis was performed using SPSS version 9.3 (SPSS Institute Inc., Chicago, IL, USA).

3. Results

3.1. Demographic and laboratory data

The study group included 144 patients, 85 (59.1%) men and 59 (40.9%) women with a median age of 54 years (range, 18-83). As summarized in Table 1, there were 32 patients (22.2%) in subgroup I (Sole), 23 (15.9%) in subgroup II (1ACA), 26 (18.1%) in subgroup III (CK), 18 (12.5%) in subgroup IV (Variant-/Complex-), 30 (20.8%) in subgroup V (CE) and 15 (10.4%) in subgroup VI (HPD). When all 6 subgroups were compared, cytogenetic heterogeneity did not correlate with patient age, gender, hemoglobin level, platelet count, blast percentage, or *BCR*-ABL*1* FISH positivity. However, Subgroup V (CE) showed the highest white blood cell (WBC) count and subgroup VI (HPD) had the lowest WBC count ($P = 0.008$).

In subgroup IV (Variant-/Complex-), 8 patients showed an apparent variant-t(9;22) with 3-/4- way translocations, and 10 had a complex-t(9;22) showing additional structural aberrations of the der(9)t(9;22)

Table 1
Characteristics of Ph + B-ALL cytogenetic subgroups at diagnosis.

Parameters		I	II	III	IV	V	VI	*P*-value
Cases (n = 144)	**Number**	32	23	26	18	30	15	NA*
	%	(22.2%)	(15.9%)	(18.1%)	(12.5%)	(20.8%)	(10.4%)	
Age (yrs)	Median	49	59	48	52	58	55	0.270
	Range	(18–81)	(22–83)	(19–77)	(20–80)	(28–78)	(35–77)	
Gender	Males	22	14	13	9	19	8	0.710
	Females	10	9	13	9	11	7	
Hemoglobin	Median	9.2	10	10.6	10.7	10.5	9.8	0.281
(g/dl)	Range	(5.2–13.1)	(7.1–15.9)	(6.3–14)	(7.2–16.4)	(7.1–14.2)	(7.6–12.8)	
WBC Count	Median	13.5	10.8	12.6	10	33.9	9.3	**0.008**
(*10^9/L)	Range	(0.2–173.9)	(0.4–112.2)	(1.8–84.6)	(0.8–210)	(1.2–349.9)	(0.9–98.5)	
Platelet Count	Median	42	77	63	40	75	23	0.594
(*10^9/L)	Range	(1.5–670)	(2–423)	(10–238)	(11–661)	(3–802)	(7–237)	
Blasts %	Median	84	84	80	86	77.5	87	0.368
	Range	(27–98)	(47–98)	(45–95)	(52–98)	(38–98)	(44–97)	
Clone numbers								
One (n = 89)		32 (100%)	20 (87%)	16 (61.5%)	10 (55.6%)	0 (0%)	11 (73.3%)	NA*
Two (n = 41)		0	1	8	7	22	3	
Three or more (n = 14)		0	2	2	1	8	1	
Common ACA[#]		NA	9	15	11	23	13	NA*
+der(22) (n = 31)			2	5	6	10	8	
−7 (n = 18)			6	7	2	3	0	
+8 (n = 18)			1	2	3	7	5	
i(17)(q10)(n = 4)			0	1	0	3	0	
FISH % at diagnosis		90	87.4	87	91	86.5	86.8	0.130
Median (range)		(26–100)	(41–100)	(20–100)	(54–99)	(25–100)	(22–100)	

*NA: Not available; WBC: white blood cell, All 6 subgroups are termed as I-Sole, II-1ACA, III-CK, IV-Variant-/Complex-, V-CE, VI-HPD.

(n = 8) or the der(22)t(9;22) (n = 2). All 3-/4- way rearrangements were confirmed by metaphase FISH. ACAs were found in 11 cases in this group including one with hypodiploidy and two with hyperdiploidy. There were no specific patterns of ACAs in this group. In subgroup V (CE), an additional Ph or + der(22)t(9;22) was most frequent (n = 9) and identified in a sideline. In subgroup VI (HPD) with the t (9;22) and hyperdiploidy (range 48-56 chromosomes), the most common chromosome gains in the stemline were +2, +4, +6, +8, +10, +14, +15, +18, +21, and + der(22)t(9;22). When karyotypic results were assessed by total clone number, there were 89 patients with 1 clone, 41 patients with 2 clones and 14 patients with 3 or more clones (Table 1).

The common recurrent ACAs were + der(22)t(9;22) in 31 patients (including 21 in stemline and 10 in sideline), monosomy 7 in 18 patients and trisomy 8 in 18 patients (Table 1).

3.2. Karyotypic, FISH and molecular characteristics at hematological remission

A total of 125 (86.8%) patients achieved hematologic/morphologic remission and conventional cytogenetic analysis was available in 121 (96.8%) patients (Table 2). There were 102 (84.3%) patients in cytogenetic remission and 16 (13.2%) with persistent abnormalities as were detected in the initial diagnostic samples. Subgroups III (CK), IV (Variant-/Complex-) and VI (HPD) showed a complete cytogenetic remission in all patients, whereas some patients in subgroups I, II and V showed persistence of the original abnormality and 3 patients developed ACAs ($P = 0.017$) (Table 2).

BCR-ABL*1* FISH at remission was available in 83 patients and was negative in 64 (77.1%) patients (Table 2). The apparent difference between subgroup I and subgroup VI (HPD) is significant ($P = 0.023$). Minimal residual disease (MRD) status was assessed by flow cytometry in 116 patients at remission and 61 (52.6%) were negative with no significant differences observed among subgroups (Table 2). MRD results did not correlate with clone number ($P = 0.258$, data not shown).

3.3. Response to treatment and overall survival

All patients received standard care including TKIs [28,32,33] and chemotherapy including hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and high-dose cytarabine (hyper-CVAD regimen); 48 (33.3%) patients also received stem cell transplantation (SCT). The median follow up was 25.8 months (range, 0.4-164.5). The clinical response rate among all subgroups was not statistically significant ($P = 0.677$) (Table 3). There were 39 patients who eventually relapsed and the median time from initial response to relapse was 14.1 months. The frequency of relapse did not show statistical differences among cytogenetic

Table 2
Karyotypic/FISH characteristics among 6 Ph + B-ALL cytogenetic subgroups at the time of hematological remission.

Parameter	I	II	III	IV	V	VI	*P*-value
CG[a]	27	19	22	16	26	11	**0.017**
Normal	17 (63%)	17 (89.5%)	22 (100%)	16 (100%)	19 (73.1%)	11 (100%)	
Same Persists	9 (33.3%)	1 (5.3%)	0	0	6 (23.1%)	0	
ACA[#]	1 (3.7%)	1 (5.3%)	0	0	1 (3.8%)	0	
FISH negativity	10/19 (52.6%)	14/16 (87.5%)	15/16 (93.8%)	8/10 (80%)	10/15 (66.7%)	7/7 100%	**0.023**
MRD negativity	13/27 (48.1%)	11/19 (57.9%)	14/22 (63.6%)	9/15 (60%)	9/22 (40.9%)	5/11 (45.5%)	0.659

[a] CG: cytogenetic analysis, [#]ACA: additional chromosomal abnormality; All 6 subgroups are termed as I-Sole, II-1ACA, III-CK, IV-Variant-/Complex-, V-CE, VI-HPD.

Table 3
Clinical characteristics among cytogenetic subgroups in Ph + B.-ALL.

	I	II	III	IV	V	VI	*P*-value
Cases	32	23	26	18	30	15	
Median Follow Up, months (range)	21 (0.4-82)	42 (1.6-110.8)	33 (1.2-129.4)	23 (2.2-74.4)	24 (0.6-164.5)	35 (0.6-96.9)	**0.041**
Initial response % (n)	87.5% (28)	87% (20)	92.3% (24)	88.9% (16)	86.7% (26)	73.3% (11)	0.677
Refractory to induction % (n)	3.1% (1)	4.3% (1)	7.7% (2)	11.1% (2)	6.7% (2)	1.3% (2)	0.785
SCT % (n)	31.3% (10)	26.1% (6)	50% (13)	33.3% (6)	43.3% (13)	0% (0)	**0.026**
Remission % (n)	71.9% (23)	69.6% (16)	73.1% (19)	77.8% (14)	66.7% (20)	86.7% (13)	0.637
Relapse % (n)	28.1% (9)	30.4% (7)	26.9% (7)	22.2% (4)	33.3% (10)	13.3% (2)	0.792
Death Rate % (n)	40.6% (13)	47.8% (11)	53.8% (14)	77.8% (14)	50% (15)	40% (6)	0.189
Median OS (months)	47.9	56.6	76	23.6	53.9	50	0.051
Median RFS (months)	Not reached	47.3	44	17.1	27	Not reached	0.112

All 6 subgroups are termed as I-Sole, II-1ACA, III-CK, IV-Variant/Complex, V-CE, VI-HPD.

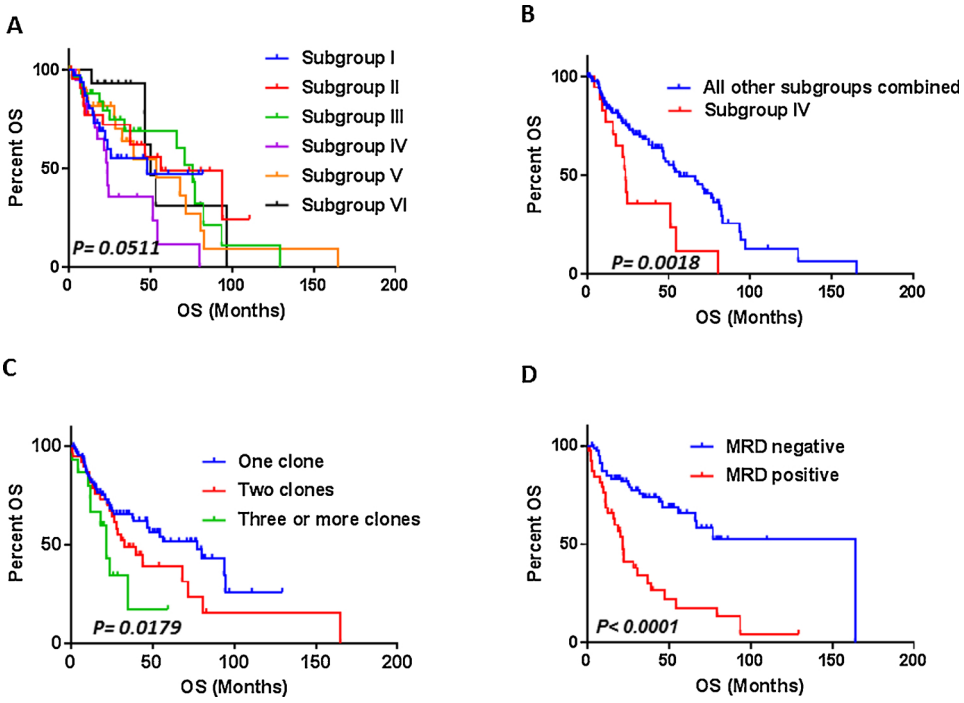

Fig. 1. A, Overall survival among 6 subgroups of Ph + B-ALL. **B,** Overall survival comparison between patients of subgroup IV and all other subgroups combined. **C,** Overall survival among cases with different clone numbers. **D,** Overall survival comparison between patients with MRD positive and MRD negative disease. Subgroups are as follows: I-Sole, II-1ACA, III-CK, IV-Variant-/Complex-, V-CE, VI-HPD.

subgroups (P = 0.792, Table 3) and did not correlated with cytogenetic clone number (P = 0.968, data not shown).

The median OS of the entire cohort was 25.6 months (range, 0.6-165.1 months) and median RFS was 20.6 months (range, 0.4-129.4 months) (Table 3). The median OS among the 6 subgroups did not reach statistical significance (P = 0.051) (Table 3 and Fig. 1A); however, when comparing subgroup IV (Variant-/Complex-) with the other subgroups combined, OS was significantly poorer for patients in subgroup IV (Variant-/Complex-) (23.6 vs 56.6 months, P = 0.0018) (Fig. 1B). In addition, OS was poorest in patients with 3 or more clones, followed by patients with 2 clones when compared with those with only 1 clone (P = 0.0179) (Fig. 1C). When OS was further compared between patients who were MRD positive (n = 55) versus MRD negative (n = 61) after therapy, MRD positive patients had a poorer prognosis (OS 21.9 vs 164 months, P < 0.0001) (Fig. 1D).

RFS was 47.3 months for patients in subgroup II (1ACA), 44 months for subgroup III (CK), 17.1 months for subgroup IV (Variant-/Complex-), 27 months for subgroup V (CE) (P = 0.112), and was not reached for patients in subgroups I and VI (Table 3 and Fig. 2A). Patients in subgroup IV (Variant-/Complex-) showed the poorest RFS compared with patients in all other subgroups combined (Fig. 2B) (17.1 versus 47.3 months, P = 0.0049). In addition, patients with ≥ 2 clones had a shorter RFS than those with only 1 clone (P = 0.0429) (Fig. 2C). MRD positive patients also had poorer RFS when compared with MRD negative patients (P < 0.0001) (Fig. 2D).

There were 48 patients who eventually received SCT. In this subgroup, 26 patients had 1 clone, 15 patients had 2 clones, and 7 patients had 3 clones. The percentage of patients treated with SCT showed no correlation with clone number (P = 0.269). With the exception of patients in subgroup VI, none of whom received SCT (P = 0.026), the percentage of patients who received SCT among subgroups I-V showed no statistical difference (P = 0.39) (Table 3). There was no apparent OS advantage compared to patients who did not undergo SCT in those with 1 clone or 2 clones, respectively. However, SCT did improve the OS in patients who had ≥3 clones (P = 0.0167) (Supplement Fig. 1), although case numbers are limited in this subgroup.

3.4. Multivariate analysis for overall survival

The results of multivariate analysis are summarized in Table 4. We found that variant-/complex-t(9;22) (P = 0.001, HR 2.83, 95% CI: 1.479 - 5.347), clone number (P = 0.003, HR 3.549, 95% CI: 1.541 - 8.172), clinical response at the initial treatment (P = 0.020, HR 2.588, 95% CI: 1.164 - 5.751), and MRD positivity at remission (P < 0.0001, HR 3.252, 95% CI: 1.766 - 5.991) were independent negative risk factors. There were no significant differences observed between patients with or without relapse. In addition, patient age or gender had no clinical impact on OS (Table 4).

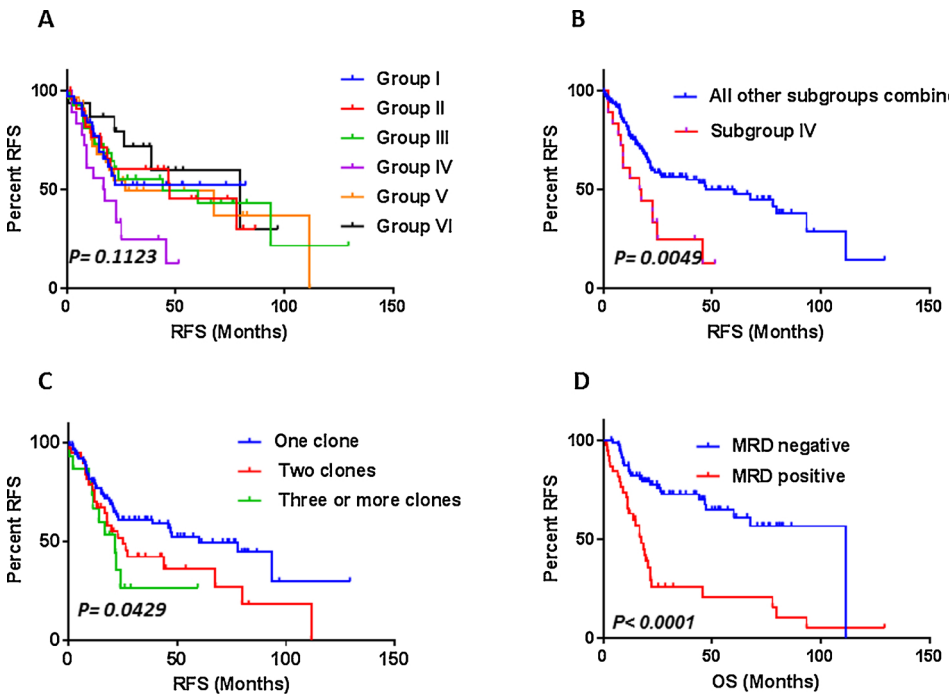

Fig. 2. A, Relapse free survival (RFS) among 6 subgroups of Ph + B-ALL. **B,** Relapse free survival comparison between patients of subgroup IV and all other subgroups combined. **C,** Relapse free survival among cases with different clone numbers. **D,** Relapse free survival comparison between patients with MRD positive and MRD negative disease. Subgroups are as follows: I-Sole, II-1ACA, III-CK, IV-Variant-/Complex, V-CE, VI-HPD.

Table 4
Multivariate analysis of prognostic factors that impact overall survival.

	P-value	HR[a]	95.0 % CI	
			Lower	Upper
Age (≥ 54 vs. < 54)	0.207	1.425	0.822	2.469
Sex (male vs. female)	0.265	1.322	0.809	2.161
Variant-/complex- Ph+ (vs. all others)	**0.001**	2.830	1.497	5.347
Two or more clones (vs. one clone)	**0.003**	3.549	1.541	8.172
No or partial response (vs. complete response)	**0.020**	2.588	1.164	5.751
Minimal residual disease (Positive vs. Negative)	< **0.0001**	3.252	1.766	5.991
Relapse (vs. no relapse)	0.089	1.605	0.931	2.766
SCT[#] (vs. non-SCT)	0.777	1.085	0.616	1.913

[a] HR: hazard ratio, #SCT: stem cell transplantation.

4. Discussion

Although a study from our institution had shown that + der(22)t(9;22) and -9/9p ACAs in patients with Ph + ALL treated with TKI and chemotherapy are associated with poorer outcomes [28], data are limited and studies that have systemically assessed the clinical implications of clonal heterogeneity in Ph + B-ALL patients are few. In this study, we systemically evaluated the cytogenetic characteristics of 144 adults with Ph + B-ALL and correlated these data with clinical outcomes. Our data reveal two potential high-risk cytogenetic subgroups in adult Ph + B-ALL: 1) patients who had a variant-/complex-t(9;22); and 2) patients who had 2 or more clones.

Variants of t(9;22) have been reported in about 3% of Ph + B-ALL patients [9]. Most cases show atypical *BCR-ABL1* fusions that are often confirmed by metaphase FISH [8,9]. On the other hand, complex-t(9;22) has been rarely investigated. Such variant-/complex-t(9;22) abnormalities likely represent a two-step event, as previously proposed [8,34,35], with the first step creating the typical t(9;22) translocation and followed by a secondary event such as a rearrangement involving a third and/or fourth chromosome or, the der(9)t(9;22) or the der(22)t(9;22). In CML, early studies showed that patients with variant-t(9;22) had higher frequency of accelerated phase compared to patients with classic t(9;22), however, there is no impact on patient response rate or OS [8,36] in the TKI therapy era. In B-ALL, the clinical implications of variant-/complex-t(9;22) have not been fully investigated. In this study, we found that patients with either a variant- (n = 8, 5.6%) or a complex- t(9;22) (n = 10, 6.9%) have poorer OS and RFS compared with all other subgroups combined. Although the mechanisms are not fully understood, increased genomic complexity, likely involving breakpoints of t(9;22) or other chromosome loci, is likely associated with refractoriness to induction therapy and fatality (Table 3).

About one-third of Ph + B-ALL patients in this study showed 2 or more cytogenetic clones and this frequency is higher than has been reported in pediatric patients [7]. Patients with ≥ 2 clones more often have a higher WBC count, lower CR rate and higher frequency of disease relapse [34]. One potential explanation could be that second or third Ph + clones that result from clonal evolution could result in increased expression of BCRABL1 chimeric protein thereby affecting drug interactions targeting kinase activity [19]. Of 48 patients (26 with one clone, 15 with two clones and 7 with three clones) who eventually received SCT, our data show that SCT could potentially improve the OS of patients with ≥ 3 clones; however, the case number in this subgroup is limited (Supplement Fig. 1). To date, clone numbers have been studied rarely in B-ALL and reporting these data may prove to be of clinical value if confirmed in additional studies.

Other recurrent ACAs such as monosomy 7, trisomy 8 and i(17)(q10) did not impact OS in this study. For example, monosomy 7 as a sole secondary aberration has been reported frequently in Ph + B-ALL patients and has been associated with an inferior outcome and lower CR rate [6]. However, in the current study there were 18 patients with monosomy 7 and in only 5 patients, the monosomy 7 was a sole secondary abnormality. Although the CR rate in this small subgroup of patients was low (25%, data not shown), their median OS (44.1 months) was comparable to the 47.9 months observed in patients without monosomy chromosome 7. In addition, due to the limitations of a retrospective study, lack of leftover materials from the diagnostic samples precluded high resolution genomic microarray analysis to

assess chromosome 7p deletions, such as *IKZF1* deletions at the exon level, which have been associated with a poorer outcome in Ph + B-ALL patients [37]. The presence of an additional Ph or + der(22)t(9;22) was the most frequent ACA in this study and, in an earlier study from our institution showed a negative impact on prognosis in non-hyperdiploid Ph + B-ALL patients [28]. We did not repeat the same analysis in the current study. In addition, although ACA assessment at the time of CR in adult Ph + B-ALL has been studied [22], we found that only 2.5% of patients had ACAs (Table 2), indicating that ACAs are rare in remission patients with Ph + B-ALL and therefore of limited clinical impact. Although not the focus of this study, assessment for MRD by flow cytometry [38] could predict clinical outcome. As shown by others, MRD detected at remission is associated with a poorer prognosis (Table 4) [39,40]. There were 39 patients who had relapsed disease in this study, however, relapse rate did not correlate with any specific cytogenetic subgroups or clones numbers ($P = 0.792$ and $P = 0.968$, respectively). In addition, 35 (90%) patients with relapsed disease did not have a variant Ph+, a presumed high risk factor. Furthermore, 24 (61.5%) patients with relapsed disease had only one clone, which is associated with lower risk than two or more clones. This result could explain why multivariate analysis did not show relapsed disease as an independent risk factor in this study however, more data are needed to confirm our findings.

In the modern era, molecular testing i.e. high-throughput methods such as next generation sequencing, have been shown to have clinical utility in the analysis of acute leukemias including B-ALL. Although chromosome analysis is known to have limited analytic resolution, it nevertheless provides a global view of the genome of leukemic cells. As shown in this study, we conclude that assessing cytogenetic heterogeneity, particularly in identifying variant-/complex-t(9;22) and clonal evolution, by integrated cytogenetic and FISH, has clinical value for accurate risk stratification in adults with Ph + B-ALL.

Contributorship statement

XL designed the study. PJ, JG, LF, CHL, ZT, HYB and XL collected and reviewed cytogenetic and clinical data and LF, XL and HY performed statistic data analysis. RKS and KP performed molecular data review. LJM and PL reviewed pathology data. PJ, JG, LJM and XL wrote the manuscript. All authors approved the manuscript.

Funding

None.

Acknowledgements

The authors wish to thank all the technologists in the Clinical Cytogenetics and Molecular Diagnostics Laboratories for their contributions to the study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.106176.

References

[1] A.V. Moorman, C.J. Harrison, G.A. Buck, S.M. Richards, L.M. Secker-Walker, M. Martineau, G.H. Vance, A.M. Cherry, R.R. Higgins, A.K. Fielding, L. Foroni, E. Paietta, M.S. Tallman, M.R. Litzow, P.H. Wiernik, J.M. Rowe, A.H. Goldstone, G.W. Dewald, Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial, Blood 109 (8) (2007) 3189–3197.

[2] S. Faderl, H.M. Kantarjian, D.A. Thomas, J. Cortes, F. Giles, S. Pierce, M. Albitar, Z. Estrov, Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia, Leuk. Lymphoma 36 (3-4) (2000) 263–273.

[3] J. Gaynor, D. Chapman, C. Little, S. McKenzie, W. Miller, M. Andreeff, Z. Arlin, E. Berman, S. Kempin, T. Gee, et al., A cause-specific hazard rate analysis of prognostic factors among 199 adults with acute lymphoblastic leukemia: the Memorial Hospital experience since 1969, J. Clin. Oncol. 6 (6) (1988) 1014–1030.

[4] B. Gleissner, N. Gokbuget, C.R. Bartram, B. Janssen, H. Rieder, J.W. Janssen, C. Fonatsch, A. Heyll, D. Voliotis, J. Beck, T. Lipp, G. Munzert, J. Maurer, D. Hoelzer, E. Thiel, Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis, Blood 99 (5) (2002) 1536–1543.

[5] A. Fabarius, A. Leitner, A. Hochhaus, M.C. Muller, B. Hanfstein, C. Haferlach, G. Gohring, B. Schlegelberger, M. Jotterand, A. Reiter, S. Jung-Munkwitz, U. Proetel, J. Schwaab, W.K. Hofmann, J. Schubert, H. Einsele, A.D. Ho, C. Falge, L. Kanz, A. Neubauer, M. Kneba, F. Stegelmann, M. Pfreundschuh, C.F. Waller, K. Spiekermann, G.M. Baerlocher, M. Lauseker, M. Pfirrmann, J. Hasford, S. Saussele, R. Hehlmann, K. Schweizerische, Arbeitsgemeinschaft fur Klinische, C.M.L.S.G. the German, Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV, Blood 118 (26) (2011) 6760–6768.

[6] M. Wetzler, R.K. Dodge, K. Mrozek, C.C. Stewart, A.J. Carroll, R. Tantravahi, J.W. Vardiman, R.A. Larson, C.D. Bloomfield, Additional cytogenetic abnormalities in adults with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a study of the Cancer and Leukaemia Group B, Br. J. Haematol. 124 (3) (2004) 275–288.

[7] N.A. Heerema, J. Harbott, S. Galimberti, B.M. Camitta, P.S. Gaynon, G. Janka-Schaub, W. Kamps, G. Basso, C.H. Pui, M. Schrappe, M.F. Auclerc, A.J. Carroll, V. Conter, C.J. Harrison, J. Pullen, S.C. Raimondi, S. Richards, H. Riehm, H.N. Sather, J.J. Shuster, L.B. Silverman, M.G. Valsecchi, M. Arico, Secondary cytogenetic aberrations in childhood Philadelphia chromosome positive acute lymphoblastic leukemia are nonrandom and may be associated with outcome, Leukemia 18 (4) (2004) 693–702.

[8] G. Marzocchi, F. Castagnetti, S. Luatti, C. Baldazzi, M. Stacchini, G. Gugliotta, M. Amabile, G. Specchia, M. Sessarego, U. Giussani, L. Valori, G. Discepoli, A. Montaldi, A. Santoro, L. Bonaldi, G. Giudici, A.M. Cianciulli, F. Giacobbi, F. Palandri, F. Pane, G. Saglio, G. Martinelli, M. Baccarani, G. Rosti, N. Testoni, E.d.A.W.P.o.C.M.L. Gruppo Italiano Malattie, Variant Philadelphia translocations: molecular-cytogenetic characterization and prognostic influence on frontline imatinib therapy, a GIMEMA Working Party on CML analysis, Blood 117 (25) (2011) 6793–6800.

[9] H. Rieder, W.D. Ludwig, W. Gassmann, J. Maurer, J.W. Janssen, N. Gokbuget, S. Schwartz, E. Thiel, H. Loffler, C.R. Bartram, D. Hoelzer, C. Fonatsch, Prognostic significance of additional chromosome abnormalities in adult patients with Philadelphia chromosome positive acute lymphoblastic leukaemia, Br. J. Haematol. 95 (4) (1996) 678–691.

[10] S.Y. Cho, S.Y. Kim, Y.L. Jeon, S.H. Oh, E.H. Cho, W.I. Lee, K.S. Cho, T.S. Park, A novel three-way Ph variant t(8;9;22) in adult acute lymphoblastic leukemia, Ann. Clin. Lab. Sci. 41 (1) (2011) 71–78.

[11] R. Bassan, G. Rossi, E.M. Pogliani, E. Di Bona, E. Angelucci, I. Cavattoni, G. Lambertenghi-Deliliers, F. Mannelli, A. Levis, F. Ciceri, D. Mattei, E. Borlenghi, E. Terruzzi, C. Borghero, C. Romani, O. Spinelli, M. Tosi, E. Oldani, T. Intermesoli, A. Rambaldi, Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00, J. Clin. Oncol. 28 (22) (2010) 3644–3652.

[12] A. de Labarthe, P. Rousselot, F. Huguet-Rigal, E. Delabesse, F. Witz, S. Maury, D. Rea, J.M. Cayuela, M.C. Vekemans, O. Reman, A. Buzyn, A. Pigneux, M. Escoffre, Y. Chalandon, E. MacIntyre, V. Lheritier, J.P. Vernant, X. Thomas, N. Ifrah, H. Dombret, Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study, Blood 109 (4) (2007) 1408–1413.

[13] D.Y. Kim, Y.D. Joo, S.N. Lim, S.D. Kim, J.H. Lee, J.H. Lee, D.H. Kim, K. Kim, C.W. Jung, I. Kim, S.S. Yoon, S. Park, J.S. Ahn, D.H. Yang, J.J. Lee, H.S. Lee, Y.S. Kim, Y.C. Mun, H. Kim, J.H. Park, J.H. Moon, S.K. Sohn, S.M. Lee, W.S. Lee, K.H. Kim, J.H. Won, M.S. Hyun, J. Park, J.H. Lee, H.J. Shin, J.S. Chung, H. Lee, H.S. Eom, G.W. Lee, Y.U. Cho, S. Jang, C.J. Park, H.S. Chi, K.H. Lee, Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia, Blood 126 (6) (2015) 746–756.

[14] F. Ravandi, S. O'Brien, D. Thomas, S. Faderl, D. Jones, R. Garris, S. Dara, J. Jorgensen, P. Kebriaei, R. Champlin, G. Borthakur, J. Burger, A. Ferrajoli, G. Garcia-Manero, W. Wierda, J. Cortes, H. Kantarjian, First report of phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia, Blood 116 (12) (2010) 2070–2077.

[15] M. Yanada, J. Takeuchi, I. Sugiura, H. Akiyama, N. Usui, F. Yagasaki, T. Kobayashi, Y. Ueda, M. Takeuchi, S. Miyawaki, A. Maruta, N. Emi, Y. Miyazaki, S. Ohtake, I. Jinnai, K. Matsuo, T. Naoe, R. Ohno, High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group, J. Clin. Oncol. 24 (3) (2006) 460–466.

[16] A.K. Fielding, J.M. Rowe, G. Buck, L. Foroni, G. Gerrard, M.R. Litzow, H. Lazarus, S.M. Luger, D.I. Marks, A.K. McMillan, A.V. Moorman, B. Patel, E. Paietta, M.S. Tallman, A.H. Goldstone, UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia, Blood 123 (6) (2014) 843–850.

[17] P. Kebriaei, R. Saliba, G. Rondon, A. Chiattone, R. Luthra, P. Anderlini, B. Andersson, E. Shpall, U. Popat, R. Jones, L. Worth, F. Ravandi, D. Thomas,

S. O'Brien, H. Kantarjian, M. de Lima, S. Giralt, R. Champlin, Long-term follow-up of allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: impact of tyrosine kinase inhibitors on treatment outcomes, Biol. Blood Marrow Transplant. 18 (4) (2012) 584–592.

[18] S. Mizuta, K. Matsuo, S. Nishiwaki, K. Imai, H. Kanamori, K. Ohashi, T. Fukuda, Y. Onishi, K. Miyamura, S. Takahashi, M. Onizuka, Y. Atsuta, R. Suzuki, Y. Morishima, K. Kato, H. Sakamaki, J. Tanaka, Pretransplant administration of imatinib for allo-HSCT in patients with BCR-ABL-positive acute lymphoblastic leukemia, Blood 123 (15) (2014) 2325–2332.

[19] W. Wang, J.E. Cortes, G. Tang, J.D. Khoury, S. Wang, C.E. Bueso-Ramos, J.A. DiGiuseppe, Z. Chen, H.M. Kantarjian, L.J. Medeiros, S. Hu, Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy, Blood 127 (22) (2016) 2742–2750.

[20] Z. Chen, C. Shao, W. Wang, Z. Zuo, X. Mou, S.J. Hu, J.A. DiGiuseppe, Y. Zu, L.J. Medeiros, S. Hu, Cytogenetic landscape and impact in blast phase of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy, Leukemia 31 (3) (2017) 585–592.

[21] I. Aldoss, T. Stiller, T.M. Cao, J.M. Palmer, S.H. Thomas, S.J. Forman, V. Pullarkat, Impact of Additional Cytogenetic Abnormalities in Adults with Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia Undergoing Allogeneic Hematopoietic Cell Transplantation, Biol. Blood Marrow Transplant. 21 (7) (2015) 1326–1329.

[22] N.J. Short, H.M. Kantarjian, E.J. Jabbour, S.M. O'Brien, S. Faderl, J.A. Burger, R. Garris, W. Qiao, X. Huang, N. Jain, M. Konopleva, T.M. Kadia, N. Daver, G. Borthakur, J.E. Cortes, F. Ravandi, Prognostic impact of persistent cytogenetic abnormalities at complete remission in adult patients with acute lymphoblastic leukemia, Am. J. Hematol. 91 (4) (2016) 385–389.

[23] B.S. Ko, J.L. Tang, F.Y. Lee, M.C. Liu, W. Tsai, Y.C. Chen, C.H. Wang, M.C. Sheng, D.T. Lin, K.H. Lin, H.F. Tien, Additional chromosomal abnormalities and variability of BCR breakpoints in Philadelphia chromosome/BCR-ABL-positive acute lymphoblastic leukemia in Taiwan, Am. J. Hematol. 71 (4) (2002) 291–299.

[24] E. Jabbour, H. Kantarjian, S. O'Brien, J. Shan, A. Quintas-Cardama, S. Faderl, G. Garcia-Manero, F. Ravandi, M.B. Rios, J. Cortes, The achievement of an early complete cytogenetic response is a major determinant for outcome in patients with early chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors, Blood 118 (17) (2011) 4541-6; quiz 4759.

[25] D. Milojkovic, E. Nicholson, J.F. Apperley, T.L. Holyoake, P. Shepherd, M.W. Drummond, R. Szydlo, M. Bua, L. Foroni, A. Reid, J.S. Khorashad, H. de Lavallade, K. Rezvani, C. Paliompeis, J.M. Goldman, D. Marin, Early prediction of success or failure of treatment with second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia, Haematologica 95 (2) (2010) 224–231.

[26] M. Lubbert, P. Wijermans, R. Kunzmann, G. Verhoef, A. Bosly, C. Ravoet, M. Andre, A. Ferrant, Cytogenetic responses in high-risk myelodysplastic syndrome following low-dose treatment with the DNA methylation inhibitor 5-aza-2'-deoxycytidine, Br. J. Haematol. 114 (2) (2001) 349–357.

[27] S. Balleisen, A. Kuendgen, B. Hildebrandt, R. Haas, U. Germing, Prognostic relevance of achieving cytogenetic remission in patients with acute myelogenous leukemia or high-risk myelodysplastic syndrome following induction chemotherapy, Leuk. Res. 33 (9) (2009) 1189–1193.

[28] N.J. Short, H.M. Kantarjian, K. Sasaki, F. Ravandi, H. Ko, C. Cameron Yin, G. Garcia-Manero, J.E. Cortes, R. Garris, S.M. O'Brien, K. Patel, M. Khouri, D. Thomas, N. Jain, T.M. Kadia, N.G. Daver, C.B. Benton, G.C. Issa, M. Konopleva, E. Jabbour, Poor outcomes associated with +der(22)t(9;22) and -9/9p in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia receiving chemotherapy plus a tyrosine kinase inhibitor, Am. J. Hematol. 92 (3) (2017) 238–243.

[29] A.K. Fielding, S.M. Richards, R. Chopra, H.M. Lazarus, M.R. Litzow, G. Buck, I.J. Durrant, S.M. Luger, D.I. Marks, I.M. Franklin, A.K. McMillan, M.S. Tallman, J.M. Rowe, A.H. Goldstone, Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study, Blood 109 (3) (2007) 944–950.

[30] D.A. Thomas, H. Kantarjian, T.L. Smith, C. Koller, J. Cortes, S. O'Brien, F.J. Giles, J. Gajewski, S. Pierce, M.J. Keating, Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy, Cancer 86 (7) (1999) 1216–1230.

[31] J.M. Ribera, A. Oriol, M. Morgades, P. Montesinos, J. Sarra, J. Gonzalez-Campos, S. Brunet, M. Tormo, P. Fernandez-Abellan, R. Guardia, M.T. Bernal, J. Esteve, P. Barba, M.J. Moreno, A. Bermudez, A. Cladera, L. Escoda, R. Garcia-Boyero, E. Del Potro, J. Bergua, M.L. Amigo, C. Grande, M.J. Rabunal, J.M. Hernandez-Rivas, E. Feliu, Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial, J. Clin. Oncol. 32 (15) (2014) 1595–1604.

[32] E. Jabbour, H. Kantarjian, F. Ravandi, D. Thomas, X. Huang, S. Faderl, N. Pemmaraju, N. Daver, G. Garcia-Manero, K. Sasaki, J. Cortes, R. Garris, C.C. Yin, J.D. Khoury, J. Jorgensen, Z. Estrov, Z. Bohannan, M. Konopleva, T. Kadia, N. Jain, C. DiNardo, W. Wierda, V. Jeanis, S. O'Brien, Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study, Lancet Oncol. 16 (15) (2015) 1547–1555.

[33] N. Daver, D. Thomas, F. Ravandi, J. Cortes, R. Garris, E. Jabbour, G. Garcia-Manero, G. Borthakur, T. Kadia, M. Rytting, M. Konopleva, H. Kantarjian, S. O'Brien, Final report of a phase II study of imatinib mesylate with hyper-CVAD for the front-line treatment of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia, Haematologica 100 (5) (2015) 653–661.

[34] A. Hochhaus, S. Kreil, A.S. Corbin, P. La Rosee, M.C. Muller, T. Lahaye, B. Hanfstein, C. Schoch, N.C. Cross, U. Berger, H. Gschaidmeier, B.J. Druker, R. Hehlmann, Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy, Leukemia 16 (11) (2002) 2190–2196.

[35] W. Al-Achkar, A. Aljapawe, S. Almedani, T. Liehr, A. Wafa, A novel cytogenetic abnormality t(7;8)(p11.2:q11.2) and a four-way Philadelphia translocation in an imatinib mesylate-resistant chronic myeloid leukemia patient, Oncol. Lett. 5 (2) (2013) 617–620.

[36] M.M. El-Zimaity, H. Kantarjian, M. Talpaz, S. O'Brien, F. Giles, G. Garcia-Manero, S. Verstovsek, D. Thomas, A. Ferrajoli, K. Hayes, B. Nebiyou Bekele, X. Zhou, M.B. Rios, A.B. Glassman, J.E. Cortes, Results of imatinib mesylate therapy in chronic myelogenous leukaemia with variant Philadelphia chromosome, Br. J. Haematol. 125 (2) (2004) 187–195.

[37] G. Martinelli, I. Iacobucci, C.T. Storlazzi, M. Vignetti, F. Paoloni, D. Cilloni, S. Soverini, A. Vitale, S. Chiaretti, G. Cimino, C. Papayannidis, S. Paolini, L. Elia, P. Fazi, G. Meloni, S. Amadori, G. Saglio, F. Pane, M. Baccarani, R. Foa, IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report, J. Clin. Oncol. 27 (31) (2009) 5202–5207.

[38] G. Gaipa, G. Basso, A. Biondi, D. Campana, Detection of minimal residual disease in pediatric acute lymphoblastic leukemia, Cytometry B Clin. Cytom. 84 (6) (2013) 359–369.

[39] S. Jeha, E. Coustan-Smith, D. Pei, J.T. Sandlund, J.E. Rubnitz, S.C. Howard, H. Inaba, D. Bhojwani, M.L. Metzger, C. Cheng, J.K. Choi, J. Jacobsen, S.A. Shurtleff, S. Raimondi, R.C. Ribeiro, C.H. Pui, D. Campana, Impact of tyrosine kinase inhibitors on minimal residual disease and outcome in childhood Philadelphia chromosome-positive acute lymphoblastic leukemia, Cancer 120 (10) (2014) 1514–1519.

[40] F. Ravandi, J.L. Jorgensen, D.A. Thomas, S. O'Brien, R. Garris, S. Faderl, X. Huang, S. Wen, J.A. Burger, A. Ferrajoli, P. Kebriaei, R.E. Champlin, Z. Estrov, P. Challagundla, S.A. Wang, R. Luthra, J.E. Cortes, H.M. Kantarjian, Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy, Blood 122 (7) (2013) 1214–1221.