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Secondary cytogenetic aberrations in childhood Philadelphia chromosome positive acute lymphoblastic leukemia are nonrandom and may be associated with outcome

NA Heerema[1], J Harbott[2], S Galimberti[3], BM Camitta[4], PS Gaynon[5], G Janka-Schaub[6], W Kamps[7], G Basso[8], C-H Pui[9], M Schrappe[10], M-F Auclerc[11], AJ Carroll[12], V Conter[8], CJ Harrison[13], J Pullen[14], SC Raimondi[9], S Richards[15], H Riehm[10], HN Sather[16], JJ Shuster[17], LB Silverman[18], MG Valsecchi[3] and M Aricò[19], for the Acute Lymphoblastic Leukemia Study Groups: ALL-BFM and CoALL (Germany), AIEOP (Italy), DCLSG (Netherlands), FRALLE (France), CCG, DFCI, POG and St Jude (USA), and UKALL (UK)

[1]Department of Pathology, The Ohio State University, Columbus, OH; USA; [2]Oncogenetic Laboratory, Department of Hematology and Oncology, Giessen, Germany; [3]Department of Clinical Medicine, Prevention and Biotechnology, Section of Medical Statistics, University of Milano-Bicocca, Italy; [4]Midwest Children's Cancer Center, Milwaukee, WI, USA; [5]Childrens Center for Cancer & Blood Diseases, Childrens Hospital, Los Angeles, CA, USA; [6]Department of Hematology & Oncology, Children's University Hospital, Hamburg, Germany; [7]Department of Pediatrics, University Hospital Groningen, Groningen, The Netherlands; [8]Laboratorio di Oncoematologia Dipartimento di Pediatria, Universita di Padova, Padua, Italy; [9]St Jude Children's Research Hospital and University of Tennessee Health Sciences Center, Memphis, TN, USA; [10]Pediatric Hematology & Oncology, MHH Children's Hospital, Hannover, Germany; [11]Hopital Saint Louis, Paris, France; [12]University of Alabama at Birmingham, Birmingham, AL, USA; [13]Cytogenetics Group, Cancer Sciences Division, University of Southampton, UK; [14]University of Mississippi Medical Center, Jackson, MS, USA; [15]Clinical Trial Service Unit & Epidemiological Studies Unit, University of Oxford, UK; [16]Children's Oncology Group, Arcadia, CA, USA; [17]Children's Oncology Group Research Data Center and University of Florida, Department of Statistics, Gainesville, FL, USA; [18] Dana-Farber Cancer Institute and Children's Hospital, Boston, MA, USA; [19]Onco-Ematologia Pediatrica, Ospedale dei Bambini, G Di Cristina, Palermo, Italy

Additional chromosomal aberrations occur frequently in Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) of childhood. The treatment outcome of these patients is heterogeneous. This study assessed whether such clinical heterogeneity could be partially explained by the presence and characteristics of additional chromosomal abnormalities. Cytogenetic descriptions were available for 249 of 326 children with Ph+ ALL, diagnosed and treated by 10 different study groups/large single institutions from 1986 to 1996. Secondary aberrations were present in 61% of the cases. Chromosomes 9, 22, 7, 14, and 8 were most frequently abnormal. Most (93%) karyotypes were unbalanced. Three main cytogenetic subgroups were identified: *no secondary aberrations, gain* of a second Ph and/or >50 chromosomes, or *loss* of chromosome 7, 7p, and/or 9p, while other secondary aberrations were grouped as *combinations* of gain and loss or *others*. Of the three main cytogenetic subgroups, the loss group had the worst event-free survival ($P=0.124$) and disease-free survival ($P=0.013$). However, statistical significance was not maintained when adjusted for other prognostic factors and treatment. Karyotypic analysis is valuable in subsets of patients identified by molecular screening, to assess the role of additional chromosomal abnormalities and their correlation with clinical heterogeneity, with possible therapeutic implications.

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Introduction

With current multiagent chemotherapy regimens, childhood acute lymphoblastic leukemia (ALL) has an overall event-free survival rate approaching 80%.[1–12] However, some patients have a much greater risk for treatment failure. Advances in cytogenetics and then in molecular genetics has allowed recognition of 'genetically defined' ALL subgroups.[13–20] Increasing evidence that some recurring cytogenetic alterations are crucial for leukemogenesis induced several leukemia therapists to hypothesize that such genetic subgroups may represent homogeneous subsets of childhood ALL with prognostic and therapeutic implications. However, limited availability of a full set of cytogenetic and clinical information has largely hampered the assessment of this hypothesis in less common subgroups.

The Philadelphia chromosome (Ph), resulting from t(9;22)(q34;q11.2), occurs in 2–5% of children with ALL, and in approximately 25% of adults with ALL.[21–24] Outcome for both children and adults with Ph+ ALL is poor.[21–25] Molecularly, t(9;22)(q34;q11.2) results in fusion of the *ABL* gene on chromosome 9 with the *BCR* gene on chromosome 22. Three fusion protein products occur, a 210-kDa protein, predominantly found in chronic myeloid leukemia (CML) but also in 50% of adult[26] and a few cases of childhood ALL, a 190-kDa protein present in 50% of adult[26] and in 90% of childhood Ph+ ALL,[27,28] and a rare 230-kDa protein described in chronic neutrophilic leukemia.[29] The genetic rearrangements which give rise to these proteins can be detected by polymerase chain reaction (PCR), fluorescence *in situ* hybridization (FISH) or by standard banded karyotypic analysis, although banded cytogenetic analyses and some FISH probes do not discriminate among the three fusion products.

Recently, 10 cooperative groups or large single institutions in Europe and the United States initiated an intergroup effort aimed at investigation of rare subsets of childhood ALL. In the first report from this group, we documented that Ph-positive childhood ALL, although a rare and 'genetically defined' subgroup, encompasses a group of patients with heterogeneous clinical features resulting in different treatment outcomes.[30] In all, 267 of 326 Ph+ ALL children treated with intensive chemotherapy achieved first complete remission (CR). When classified by the modified NCI[31]-Rome[32] criteria, those who presented with good

Correspondence: Dr NA Heerema, Department of Pathology, 167 Hamilton Hall, 1645 Neil Ave., Columbus, OH, 43210, USA; Fax: +1 614 292 7072; E-mail: heerema-1@medctr.osu.edu
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prognostic features (white blood cell (WBC) counts $<50\,000/\mu l$ and age <10 years) had a significantly better outcome, compared to those with intermediate prognostic features who had an intermediate outcome, and those with poor prognostic features (WBC counts $>100\,000/\mu l$ and any age) who had an inferior outcome. In these Ph+ patients, those receiving bone marrow transplantation from an HLA-matched sibling donor had a better outcome than those receiving other forms of transplantation or intensive chemotherapy only, in all age and WBC-defined subgroups.[30]

The majority of patients with CML have a t(9;22)(q34;q11.2) as the sole cytogenetic abnormality at diagnosis. However, most Ph+ ALL patients have secondary cytogenetic aberrations,[21,23,33] which might influence the course of the disease and its response to treatment. In an attempt to clarify whether careful evaluation of secondary chromosomal aberrations might aid interpretation of clinical heterogeneity, we examined the karyotypes of the Ph+ childhood ALL patients in the above-described international collaborative study for the types, frequencies, and prognostic significance of the secondary aberrations present at diagnosis. We describe the different types of secondary cytogenetic aberrations and their prognostic impact in this large cohort of Ph+ childhood ALL patients, using all the available information from the previous report together with an extended follow-up.

Materials and methods

Patients

In total, 326 children with Philadelphia-chromosome positive ALL were identified between 1986 and 1996 by 10 cooperative study groups or large single institutions: AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica, Italy), BFM (Berlin-Frankfurt-Munster ALL Study Group, Germany), CCG (Children's Cancer Group, USA), CoALL (Cooperative ALL Study Group, Germany), DCLSG (Dutch Childhood Leukemia Study Group, Netherlands), DFCI (Dana-Farber Cancer Institute ALL Consortium, USA), FRALLE (French Acute Lymphoblastic Leukemia Study Group, France), POG (Pediatric Oncology Group, USA), SJCRH (St Jude Children's Research Hospital, USA), and UKALL (Medical Research Council Childhood Leukaemia Working Party, UK). All studies were approved by institutional review boards and were performed in accordance with the Declaration of Helsinki. Informed consent was obtained according to local regulatory requirements. Presenting features and treatment outcome were provided by each cooperative group, as described elsewhere;[30] follow-up was extended through 1999, with a median of 7.6 years.

Cytogenetic analyses

The contributing groups provided cytogenetic descriptions of 249 of the patients identified as Ph+ by cytogenetic analysis in the international study. Karyotypic descriptions of the remaining 77 patients were not provided or were too incomplete to be included in these analyses. Karyotypic designations were converted to the International System for Human Cytogenetic Nomenclature (ISCN, 1995)[34] when not provided in that nomenclature. An abnormal clone was defined as at least two cells with the same structural abnormality or chromosome gain or at least three cells missing the same chromosome.[34] Karyotype descriptions were examined for frequencies and types of secondary aberrations present. When more than one abnormal clone was identified, the cumulative cytogenetic abnormalities were used in analyses. Rearrangements were described as balanced when no apparent gain or loss of genetic material was recorded. Partial and whole chromosomal gains and losses within a karyotype were considered as cumulative, resulting in net gains or losses of each chromosome. Thus, for a patient with loss of a chromosome and with a derivative chromosome containing part of that chromosome, only the part of the chromosome not present in the karyotype was recorded as a loss, for example, '-7,der(9)t(7;9)(q11;p22)' was counted as loss of 7pter->7q11 for chromosome 7 and as loss of 9pter->9p22 for chromosome 9. Duplication of an abnormality (eg +14, +14) was counted only once, although gain of a second Ph chromosome (+der(22)t(9;22)(q34;q11.2), designated +Ph) was counted. Each rearrangement involving more than one chromosome was counted for each chromosome involved; thus, a three-way translocation was counted three times, once for each chromosome.

The cases were classified into three primary subgroups for analyses: (1) those with loss of chromosome 7, 7p, and/or 9p, not in combination with +Ph or hyperdiploidy with >50 chromosomes (*loss*); (2) those with +Ph and/or hyperdiploidy with >50 chromosomes, not in combination with the above losses (*gain*); and (3) those without secondary aberrations *(no secondary aberrations)*; and two additional subgroups: (4) patients with combinations of the above gains and losses (*combination*), and (5) all patients who had other secondary aberrations (*others*). This classification reflects the most frequently occurring secondary cytogenetic changes in this group of Ph patients, as well as previous analyses of secondary aberrations in Ph ALL.[33,35] The *loss* group theoretically might have loss of tumor suppressor genes, whereas the *gain* group might have relative overexpression of some genes, a different genetic mechanism from loss. Each of these mechanisms might contribute to the pathogenesis of these tumors, but in different ways.

Statistical analysis

Event-free survival (EFS), disease-free survival (DFS), and survival (SUR) were the main end points in analysis of treatment results. EFS was defined as the time from diagnosis to first event, including death during induction therapy, failure to achieve remission, relapse at any site, death during remission, or the development of a second malignant neoplasm. DFS was defined as the time from achievement of CR to relapse at any site, death during CR, or the development of a second malignant neoplasm. SUR was defined as the time from diagnosis to death from any cause. Observations on patients without events were censored at the date of last contact. Probabilities of EFS, DFS, and SUR were estimated using the Kaplan–Meier method,[36] with standard errors (s.e.) calculated according to Greenwood. Curves were compared by means of the log-rank test.[37] The Cox model was used to assess the prognostic impact of cytogenetic data on outcome, adjusting for the modified NCI criteria, sex, and treatment (with a time-dependent variable for transplant, in order to account for the waiting time).[38] No substantial departures from the proportional hazards assumption emerged from graphical checks on the prognostic factors, while an interaction term of time and treatment was included. In this model, two-tailed *P*-values for differences in the risk of treatment failure were derived from the

likelihood-ratio test. The χ^2 test or the Fisher exact test was used to assess the association between biological and clinical presenting features.

Results

Cytogenetic data

Secondary aberrations were present in 153 (61%) of the 249 Ph+ ALL cases with karyotypic descriptions (Figures 1 and 2, Table 1). Most types of aberrations, including partial and whole chromosomal gains and losses and balanced and unbalanced translocations, were identified as secondary aberrations. All chromosomes were involved in secondary aberrations; however, some chromosomes were more frequently abnormal than others, with chromosomes 9, 22, 7, 14, and 8 most frequently abnormal (Figures 1 and 2). The majority of karyotypes with secondary aberrations were unbalanced (143; 93%); only 10 (7%) were balanced. Similarly, most aberrations were unbalanced; only 31 balanced secondary aberrations were observed. Karyotypes often were complex; 60 cases had $\geqslant 3$ independent aberrations in addition to t(9;22)(q34;q11.2), and only eight of these were hyperdiploid with gains of various chromosomes (including +Ph) as the only additional aberrations. In all, 38 cases had more than one abnormal clone. In 36 of these, the simplest clone was Ph+. One case was 45,XY,-7/45,idem,t(9;22)(q34;q11.2), and one was 46,XY,t(9;22)(q34;q11),del(11)(q23)/47,XY,del(11)(q23),+18, suggesting that the Ph was secondary in these cases.

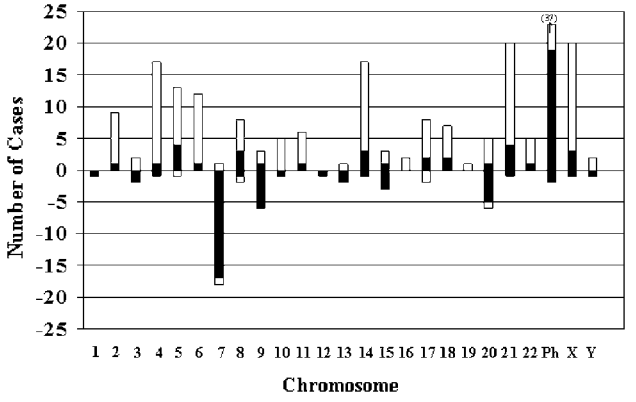


Figure 1 Numerical secondary aberrations in children with Ph+ ALL. Bars above the abscissa indicate gains and bars below the abscissa indicate losses of chromosomes. Black areas represent cases with 50 or fewer chromosomes, and clear areas represent cases with more than 50 chromosomes.

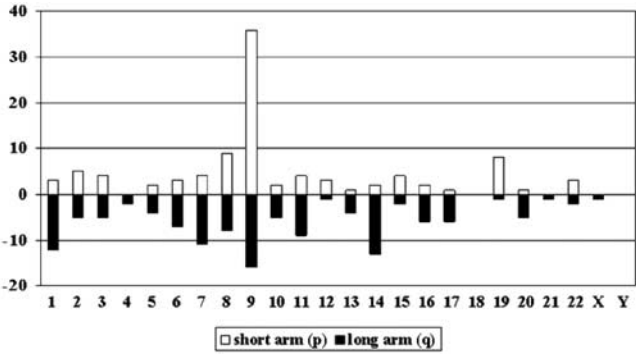


Figure 2 Breakpoints of chromosomes with secondary structural aberrations in children with Ph+ ALL. Bars above the abscissa indicate p-arm breakpoints and bars below the abscissa indicate q-arm breakpoints. Note that breakpoints in a p-arm may result in q-arm losses and *vice versa*.

Table 1 Cytogenetic characteristics of 153 childhood Ph+ ALL patients with secondary aberrations

Ploidy	Number	%
Pseudodiploid	62	41
Hypodiploid	39	26
45 chromosomes	33	22
⩽44 chromosomes	6	4
Hyperdiploid 47–50 chromosomes	30	20
Hyperdiploid 51–64 chromosomes	20	13
Near-tetraploid	2	1
Recurring abnormalities		
Loss 9p	33	22
Loss 7	13	8
Loss 7p	5	3
+Ph	16	10
Abnormal 12p	6	4
t(8;14)(q11;q32)	4	3
der(19)t(1;19)(q23;p13)[a]	2	1
Complex Ph	7	5
dup(1q)[b]	9	6
Loss 3p[c]	5	3
Loss 6q[d]	6	4
Loss 11q[e]	6	4
Loss 17p[f]	6	4
−20	6	4
Loss 20p[g]	8	5
Loss 20q[g]	9	6

Secondary abnormalities present in at least five patients and not depicted in Figures 1 and 2 are listed. t(8;14) and der(19)t(1;19) are included for completeness.
[a]Both were secondary abnormalities.
[b]Smallest region of overlap (SRO) q23–>q44 in eight of the nine patients.
[c]Two with −3, SRO p13->pter.
[d]No SRO identified.
[e]SRO q23–q24.
[f]Two with −17, three with i(17q).
[g]Six with −20, no SRO identified.

Numerical abnormalities were frequent, and all chromosomes were gained and/or lost (Figure 1). Gains were more common than losses with 205 whole chromosome gains, including +Ph. Importantly, 157 of the whole chromosome gains were in the 22 hyperdiploid karyotypes with >50 chromosomes (Figure 1). Whole chromosome losses were less common (52 losses), and only chromosome 7 was lost in a significant number of cases (18 cases; Figure 1).

Structural aberrations were common secondary changes (Figure 2); they usually were unbalanced and all chromosomes except Y and 18 had structural aberrations. Chromosome 9 had the most structural abnormalities, particularly with p-arm breakpoints (55 structural aberrations, 42 resulting in whole or partial p-arm loss). Four chromosome 9 rearrangements were balanced, each with a different breakpoint and involving a different partner chromosome.

The most frequent aberrations, numerical or structural, in descending order of frequency were loss of distal 9p (Figure 3) with a consensus region of 9p22–p24 (48 cases) including monosomy 9 (six cases) and three cases with indeterminate breakpoints, +Ph (37 cases), complete (18 cases) or partial (10 cases) monosomy 7 with loss of 7p in all cases (Figure 4), and high hyperdiploidy (HH, 51–64 chromosomes; 20 cases) usually with +Ph (17 cases). More than one of these aberrations

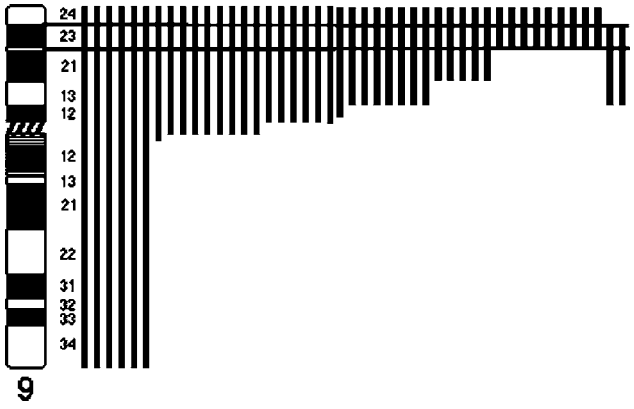


Figure 3 Loss of chromosome arm 9p in childhood ALL Ph+ patients. Each bar represents the deleted region of a single patient. Three cases with indeterminate breakpoints are not represented. A consensus region of loss in all patients is 9p22–p24.

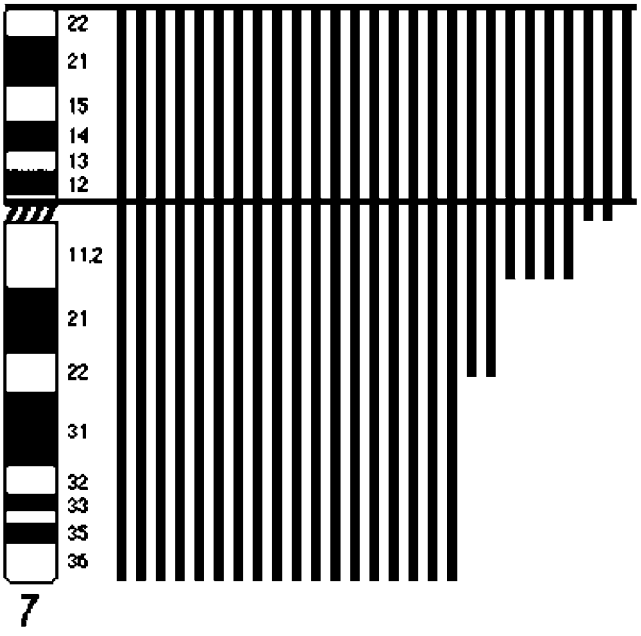


Figure 4 Loss of chromosome 7 in childhood ALL Ph+ patients. Each bar represents the deleted region of a single patient. One case with indeterminate breakpoints is not represented. There are no cases with loss of 7q without loss of 7p. The entire p-arm is lost in all cases.

occurred concurrently in 28 patients, and all combinations were observed (Table 2).

Only two of 37 cases with +Ph had +Ph as the only aberration in addition to t(9;22). Totally, 12 cases had HH and normal chromosomes 7 and 9, three had loss of 9p and 7p, two had HH and loss of 9p, one had HH and monosomy 7, one with 50 chromosomes had monosomy 7 and loss of 9p, one had loss of 9p, and one had monosomy 7 (Table 2). The remaining 14 +Ph cases had various nonrecurring aberrations in addition to t(9;22) and +Ph.

Modal numbers for 19 of the HH cases ranged from 51 to 58, with a mean of 55. One additional case had 64 chromosomes. Two HH cases also had cells with 46 chromosomes with t(9;22) as the sole abnormality, suggesting that the Ph was the primary abnormality. The chromosomes most frequently in excess in decreasing order of frequency in these HH cases were X, Ph, 4, 21, 14, and 6. Both near tetraploid cases were classified as *other*.

In addition to the frequent secondary aberrations listed above, chromosome 14 was abnormal in 34 cases; 17 cases with whole chromosome gains and one with loss (Figure 1). Strikingly, 12 of the chromosome 14 structural aberrations were balanced rearrangements (39% of the balanced rearrangements). These included two t(2;14)(p11;q13), previously unreported, and four t(8;14)(q11;q32), previously reported in Ph+ ALL.[39] The remaining six balanced aberrations of chromosome 14 included a complex Philadelphia chromosome, an inv(14)(q11q32) and translocations with different partner chromosomes (6, 9, 10, and 11) and with different chromosome 14 breakpoints (q11, q13, q24, q32). Two unbalanced rearrangements had chromosome 14 p-arm breakpoints.

Chromosome 8 also was frequently abnormal (27 cases), with trisomy 8 in eight cases (Figure 1). Five balanced rearrangements of chromosome 8 included the four t(8;14)(q11;q32) and one t(8;11)(p10;q10). Four unbalanced rearrangements with chromosome 9 occurred, all with different breakpoints. In all, 10 cases had loss of 8p21 → 8pter, and all 10 of these cases also had loss of 7p and/or 9p.

Presenting features and treatment outcome

The 5-year EFS (s.e.) for the 249 patients was 26.3%. (2.8) and survival was 37.2% (3.1), confirming that this set of patients with a complete cytogenetic evaluation is an unselected sample from the original group of 326 Ph+ patients.[30] When the analysis was restricted to the 206 patients in CR after initial induction

Table 2 Frequency of specific aberrations and their combinations in cytogenetic subgroups of children with Ph+ ALL

Loss		*Gain*		*Combination*	
Aberration	*Number of patients*	*Aberration*	*Number of patients*	*Aberration*	*Number of patients*
−7	13	+Ph	16	del(9p)/HH	1
del(7p)	5	HH	3	−7, +Ph/del(9p)	1
del(9p)	33	+Ph, HH	12	−7, +Ph/HH	1
−7, del(9p)	2			del(9p)/+Ph	1
del(7p), del(9p)	4			del(9p)/+Ph/HH	2
				del(9p)/+Ph/del(7p)	3
				−7/+Ph	1
Total	57		31		10

Abbreviations: HH = high hyperdiploidy, more than 50 chromosomes; del = deletion; +Ph = gain of Philadelphia chromosome.

therapy, the 5 years DFS (s.e.) was 31.2% (3.3). When the patients were classified according to the modified NCI[31]-Rome[32] criteria, the 5-year DFS (s.e.) for the low-risk group (70 patients; <10 years of age, WBC <10 000/μl) was 48.1% (6.0), for the intermediate-risk group (69 patients; all others) 27.0% (4.4), and for the worst group (67 patients; any age, WBC >100 000/μl) 17.9% (4.7) ($P=0.0001$); and the significant role of the modified-NCI criteria, as shown in the former paper,[30] was retained. In this study, the presence of secondary aberrations *per se*, regardless of type, did not influence prognosis, neither EFS nor DFS: for instance, the 5-year DFS (s.e.) of those with and those without secondary aberrations was 31.1% (4.1) and 31.4% (5.4), respectively (log-rank $P=0.81$; Figure 5).

When outcome was analyzed according to the most frequently recurring secondary aberrations, patients with losses of 7, 7p, or 9p had the worst outcome and those with a +Ph had a better outcome (Table 3). Analysis of the prognostic significance of a +Ph in relation to other secondary aberrations showed that patients with a +Ph, irrespective of additional secondary aberrations, had an outcome similar to the rest of the patients (Figures 5 and 6, curve labeled 'Overall'). However, when the frequently recurring additional secondary aberrations were considered, those with +Ph and HH appeared to have a better outcome and those with +Ph and other aberrations a worse outcome (Figure 6).

The patients also were classified, using the most common recurring secondary aberrations, by '*loss*', '*gain*', or both gain and loss of genetic material, '*combination*' (Tables 2 and 4).

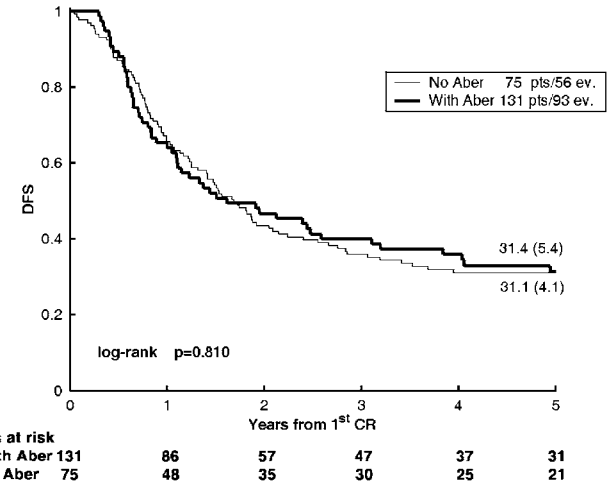


Figure 5 Disease-free survival in childhood Ph+ ALL patients with (*With Aber*) and without (*No Aber*) secondary chromosome aberrations. Abbreviations: Aber = aberrations; pts = patients; ev = events; DFS = diesease-free survival; CR = complete remission.

According to this classification, 96 patients had no secondary aberrations (*no secondary aberration*), 57 patients had losses of 7, 7p and/or 9p, not in combination with gains of +Ph or with >50 chromosomes (*loss*), 31 patients had gains of a +Ph and/or >50 chromosomes not in combination with loss of 7, 7p and/or 9p (*gain*), 10 patients had combinations of loss of 7, 7p and/or 9p and gain of a +Ph and/or HH (*combination*), and 55 patients had other secondary aberrations (*other* group; Table 4).

When the impact of cytogenetics was analyzed by the three primary cytogenetic subgroups (ie *loss, gain, no secondary aberrations*), a difference in outcome was suggested in EFS (log-rank $P=0.124$) and was statistically significant in DFS (log-rank $P=0.013$). The main feature was that the *loss* subgroup had the worst prognosis, with a 5-year EFS (s.e.) and DFS (s.e.) of 14.0% (4.6) and 15.1% (4.9), respectively (Figure 7a and b, which for descriptive purposes also include the subgroup *others*).

Leukemia relapse was the most common cause of treatment failure (Table 4). Patients with *losses* showed the highest proportion of relapses either in the marrow or in the CNS. Also of interest, patients with no additional aberrations had a higher rate of resistance to initial induction therapy compared to patients either with *losses* or *gains*.

There appeared to be no major differences among the cytogenetic subgroups with regard to the distribution of clinical

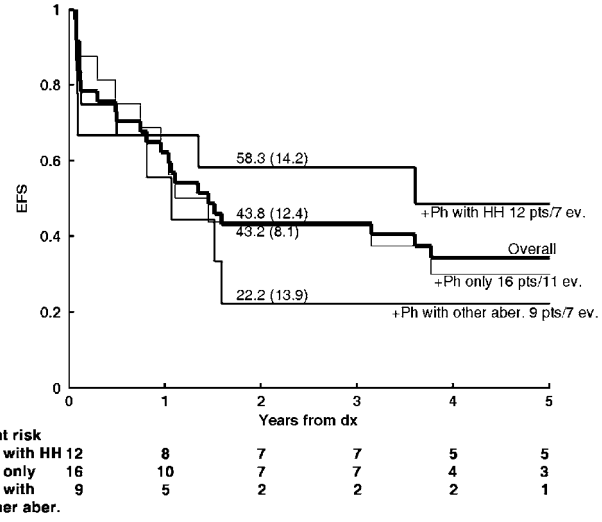


Figure 6 Event-free survival in childhood Ph+ ALL patients with duplication of the Ph chromosome (+Ph). The *Overall* curve overlaps with the curves shown in Figure 5. Abbreviations: EFS = event-free survival; +Ph with HH = high hyperdiploidy and a second Ph; Overall = all patients with +Ph; +Ph only = +Ph with no additional secondary aberrations; +Ph with other aber = +Ph with any other secondary aberration; Pts = patients; ev = events.

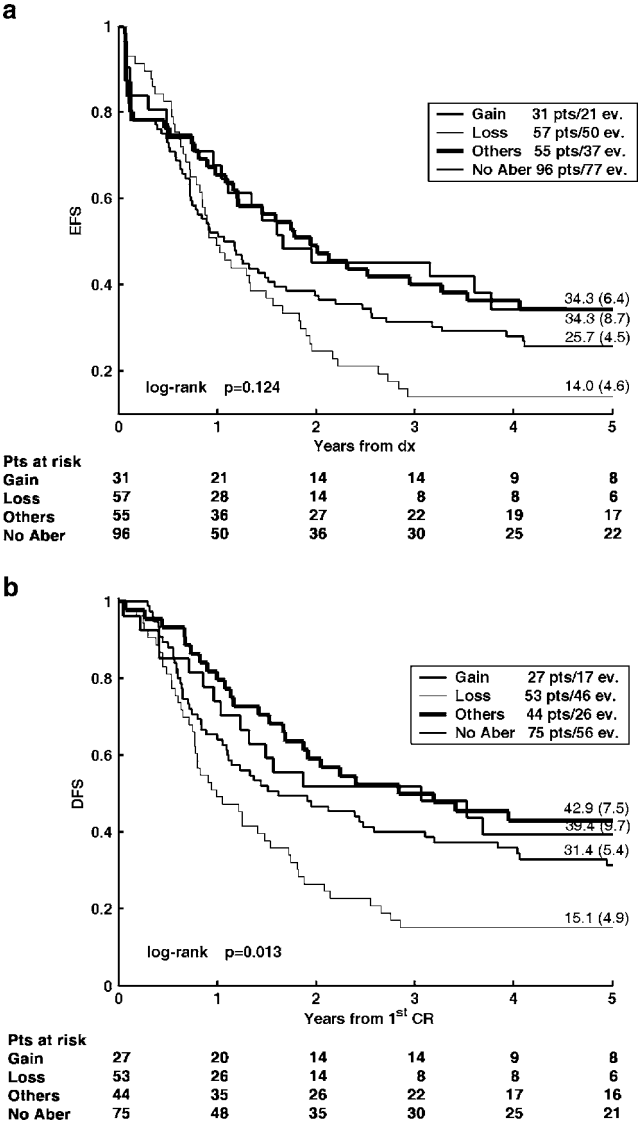
Table 3 Outcome of children with Ph+ ALL according to single cytogenetic gains and losses

	+Ph (n = 16)		*−7 (n = 13)*		*del(9p) (n = 33)*		*HH (n = 3)*		*del(7p) (n = 5)*		*No secondary aberrations (n = 96)*	
	#	%	#	%	#	%	#	%	#	%	#	%
Death in induction	1	6.25	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Resistance	1	6.25	3	23.08	1	3.03	0	0.00	0	0.00	21	21.88
Relapses	8	50.00	8	61.54	28	69.70	1	33.33	4	80.00	42	43.80
Deaths in CCR	1	6.25	1	7.69	4	12.12	2	66.67	0	0.00	10	10.40
SMN	0	0.0	0								4	4.20
CCR	5	31.25	1	15.4	5	15.15	0	0.00	1	20.00	19	19.80
2 year EFS (s.e.)	43.8 (12.4)		15.4 (10)		30.3 (8)						37.5 (4.3)	

Table 4 Pattern of events in 249 children with Ph+ ALL according to cytogenetic subgroups

	No secondary aberrations (n=96)		*Loss (n=57)*		*Gain (n=31)*		*Combination (n=10)*		*Others (n=55)*		*Total (n=249)*	
	#	%	#	%	#	%	#	%	#	%	#	%
Death in induction	0	0.00	0	0.00	1	3.20	0	0.00	1	1.80	2	0.80
Resistance	21	21.88	4	7.00	3	9.70	3	30.00	10	18.20	41	16.50
Relapses	42	43.80	39	68.40	10	32.30	4	40.00	21	38.20	116	46.60
BM	36		32		8		3		17		96	
CNS	4		7		0		1		2		14	
BM + others	2		0		2		0		2		6	
Deaths in CCR	10	10.40	7	12.30	6	19.40	0	0.00	5	9.10	28	11.20
SMN	4	4.20	0	0.00	1	3.20	0	0.00	0	0.00	5	2.00
CCR	19	19.80	7	12.30	10	32.30	3	30.00	18	32.70	57	22.90
2 year EFS (s.e.)	37.5 (4.3)		—		58.3 (14.2)		30 (16.5)		43.1 (6.7)			

In this analysis, patients had only one of the aberrations included in the analysis. Patients with combinations were not included.
Abbreviations: +Ph = gain of a Philadelphia chromosome; del = deletion; HH = high hyperdiploidy with >50 chromosomes; CCR = continuous complete remission; SMN = second malignant neoplasm.
Subgroups: *No secondary aberrations* = patients without secondary aberrations; *Loss* = patients with loss of chromosome 7, 7p, and/or 9p without +Ph and with ⩽50 chromosomes; *Gain* = patients with gain of a Ph chromosome and/or with >50 chromosomes without loss of chromosome 7, 7p, or 9p; *Combination* = patients with combinations of +Ph and/or with >50 chromosomes and with loss of chromosome 7, 7p, and/or 9p; *Others* = patients with other secondary aberrations.



features (Table 5). This also was true when the analyses were limited to the three main cytogenetic subgroups (*no secondary aberrations, gain* and *loss*; *P*-values not shown). Patients with gains of a Ph or with >50 chromosomes tended to be less represented in the class with WBC >100 000/μl (and thus, by definition, in the NCI worst prognostic group).

We used a Cox regression model to assess whether cytogenetics has a role in discriminating outcome independently from other prognostic factors (NCI criteria and sex) and from treatment. This latter covariate was considered in view of the better outcome previously shown to be related to bone marrow transplantation from a matched related donor as compared to chemotherapy alone[30] (Table 6). In the Cox model, based on DFS, the risk of failure in both the *no secondary aberrations* subgroup and in the *gain* subgroup was lower than in the *loss* subgroup, although no longer significantly so (hazard ratio (HR) for no secondary aberrations = 0.75, $P = 0.25$ and HR for gains = 0.56, $P = 0.10$). The modified-NCI criteria and treatment maintained a significant impact on outcome ($P < 0.001$ for both variables).

Figure 7 (a) Event-free survival by secondary chromosome aberration subgroups in childhood Ph+ ALL. Subgroups: Gain = patients with gain of a Ph chromosome and/or with >50 chromosomes without loss of chromosome 7, 7p, or 9p; No Aber = patients without secondary aberrations; Loss = patients with loss of chromosome 7, 7p, and/or 9p without +Ph and with ⩽50 chromosomes; Others = patients with other secondary aberrations; Pts = patients; dx = diagnosis. The reported log-rank test refers to the comparison among the first three subgroups (the overall comparison of the four subgroups gives $P = 0.053$). (b) Disease-free survival by secondary chromosome aberration subgroups in childhood Ph+ ALL. Subgroups: Gain = patients with gain of a Ph chromosome and/or with >50 chromosomes without loss of chromosome 7, 7p, or 9p; No Aber = patients without secondary aberrations; Loss = patients with loss of chromosome 7, 7p, and/or 9p without +Ph and with ⩽50 chromosomes; Others = patients with other secondary aberrations; Pts = patients; CR = complete remission. The reported log-rank test refers to the comparison among the first three subgroups (the overall comparison of the four subgroups gives $P = 0.001$).

Table 5 Clinical features of children with Ph+ ALL by cytogenetic subgroup

	No secondary aberrations (n=96)		*Loss (n=57)*		*Gain (n=31)*		*Combination (n=10)*		*Others (n=55)*		*P-value*
	#	%	#	%	#	%	#	%	#	%	
Sex											
Male	60	*62*	40	*70*	19	*61*	7	*70*	37	*67*	*0.854*
Female	36	*38*	17	*30*	12	*39*	3	*30*	18	*33*	
Immunophenotype											
B-lineage	87	*98*	56	*98*	28	*93*	10	*100*	48	*100*	*0.390*
T-lineage	2	*2*	1	*2*	2	*7*	0	*0*	0	*0*	
NK	7		0		1		0		7		
Prednisone response											
Good	20	*77*	7	*100*	7	*88*	3	*60*	11	*73*	*0.500*
Poor	6	*23*	0	*0*	1	*12*	2	*40*	4	*27*	
NK	70		51		23		4		40		
Age (years)											
0–3	11	*11*	4	*7*	5	*16*	0	*0*	5	*9*	*0.653*
3–6	29	*30*	11	*19*	3	*10*	3	*30*	16	*29*	
6–10	20	*21*	18	*32*	7	*23*	4	*40*	14	*25*	
10–15	29	*30*	19	*33*	12	*39*	3	*30*	16	*29*	
>15	7	*7*	5	*9*	4	*13*	0	*0*	4	*7*	
WBC counts/μl											
<25 K	35	*36*	21	*37*	17	*55*	4	*40*	20	*37*	*0.359*
25–50 K	15	*16*	6	*11*	5	*16*	1	*10*	3	*5*	
50–100 K	11	*12*	7	*12*	3	*10*	3	*30*	10	*18*	
>100 K[a]	35	*36*	23	*40*	6	*19*	2	*20*	22	*40*	
Risk group											
Worst[a]	35	*36*	23	*40*	6	*19*	2	*20*	22	*40*	*0.556*
Intermediate	31	*32*	19	*34*	15	*48*	4	*40*	16	*29*	
Best	30	*31*	15	*26*	10	*32*	4	*40*	17	*31*	

Abbreviations: *No secondary aberrations* = patients without secondary aberrations; *Loss* = patients with loss of chromosome 7, 7p, and/or 9p without +Ph and with ⩽50 chromosomes; *Gain* = patients with gain of a Ph chromosome and/or with >50 chromosomes without loss of chromosome 7, 7p, or 9p; *Combination* = patients with combinations of +Ph and/or with >50 chromosomes and with loss of chromosome 7, 7p, and/or 9p; *Others* = patients with other secondary aberrations.
[a]By definition of modified NCI criteria, these categories coincide.

Discussion

This study was aimed to assess whether the clinical heterogeneity observed in a large cohort of patients with Ph+ childhood ALL could be explained partially by the presence of secondary chromosomal abnormalities. The first finding of this study is that, although the presence of secondary aberrations *per se* did not influence prognosis, some specific types of aberrations present at diagnosis may be associated with outcome. The cases could be classified into three well-identified subgroups: those with loss of chromosome 7, 7p, and/or 9p, not in combination with +Ph or hyperdiploidy with >50 chromosomes (*loss*); those with gain of a Ph and/or hyperdiploidy with >50 chromosomes, not in combination with the above losses (*gain*); and those without secondary aberrations (*no secondary aberrations)*. Additional subgroups included all patients with a combination of the above gains and losses (*combination)* and those who had a mixture of other types of secondary aberrations (*others*). Patients with *loss* had the worst outcome in terms of DFS ($P=0.013$). The finding was similar, but lost statistical significance, when the comparison was adjusted for heterogeneity in other known prognostic features and treatment in a Cox regression model.

The second main finding in this study is that although the secondary aberrations present in this cohort of Ph+ ALL patients involved all chromosomes, some chromosomal abnormalities were frequent. Chromosome 9 was abnormal most frequently, particularly loss of the p-arm. Patients with this abnormality were among those that had a particularly poor outcome. Chromosome arm 9p abnormalities are also frequent and associated with very short remissions in adult Ph+ ALL.[33] Aberrations of chromosome arm 9p also have been associated with an adverse prognosis in Ph-negative childhood ALL.[40,41] The combination of Ph+ and loss of 9p may contribute to the particularly poor outcome of these patients. A consensus region of loss of 9p22–24 was identified. A tumor suppressor gene that is frequently lost in Ph+ ALL may be located in this region, possibly contributing to the poor outcome of these patients. Loss of the $p15^{INK4B}$, $p16^{INK4A}$, and $p14^{arf}$ genes, all located on 9p21, near the common region of loss in these patients, is recurrent in pediatric ALL, particularly T-lineage ALL.[42–44] It is not known if loss of one or more of these genes contributes to the poor outcome of Ph+ ALL patients with loss of 9p.

Loss of chromosome 7 was also a frequent secondary aberration in this cohort of Ph+ ALL patients, with the entire p-arm the common region of loss. Our results confirm previous reports of smaller numbers of patients, which showed monosomy 7 was an adverse factor in both childhood[35] and adult[33] Ph+ ALL. Additionally, loss of chromosome arm 7p is associated with an adverse outcome in childhood ALL regardless of Ph status (Heerema NA *et al*, Blood 1999; **94**: 503, abstract). The tendency for an adverse prognosis in patients with secondary losses of chromosome 7 or 7p in Ph+ ALL may be the cumulative result of these events. A TSG on chromosome 7 that may contribute to ALL pathogenesis has not been identified.

Hyperdiploidy with >50 chromosomes (HH) in childhood Ph-negative ALL is associated with an excellent outcome.[20,45,46] Ph+ ALL patients with HH have a better outcome than those with loss of chromosome 7, 7p, and/or 9p. Adult Ph+ ALL patients with HH also have a better outcome than those with

Table 6 Treatment outcome in children with Ph+ ALL in complete remission within the three main cytogenetic subgroups, by treatment

	No secondary aberrations (n = 75)						*Loss (n = 53)*						*Gain (n = 27)*					
	Chemo (n = 41)		*MRD-BMT (n = 9)*		*Other BMT (n = 25)*		*Chemo (n = 34)*		*MRD-BMT (n = 5)*		*Other BMT (n = 14)*		*Chemo (n = 13)*		*MRD-BMT (n = 3)*		*Other BMT (n = 11)*	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
No. of events	36		4		16		33		3		10		10		1		6	
Relapses	33	*80.5*	1	*11.1*	8	*32.0*	32	*94.1*	3	*60.0*	4	*28.6*	7	*53.8*	1	*33.3*	2	*18.2*
Deaths in CCR	1	*2.4*	3	*33.3*	6	*24.0*	1	*2.9*	0	*0.0*	6	*42.8*	3	*23.1*	0	*0.0*	3	*27.3*
SMN	2	*4.9*	0	*0.0*	2	*8.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*9.1*
CCR	5	*12.2*	5	*55.6*	9	*36.0*	1	*2.9*	2	*40.0*	4	*28.6*	3	*23.1*	2	*66.7*	5	*45.5*

Abbreviations: *Chemo* = chemotherapy alone; *MRD-BMT* = bone marrow transplant from matched related donor; *Other BMT* = bone marrow transplant from other sources; *CCR* = continuous complete remission; *SMN* = second malignant neoplasm.

abnormalities of 9p or monosomy 7.[33] Interestingly, the pattern of extra chromosomes in these childhood Ph+ ALL patients differs from that in childhood Ph-negative HH patients. Comparison of published data from the CCG[47] for Ph-negative ALL patients with 51–68 chromosomes and these Ph+ HH patients shows that although the frequencies of some trisomies are similar (chromosomes Y, 1, 4, 9, 13, 15, 19, and 22), trisomies of chromosomes 2, 3, 5, 11, and 20 are more than twice as frequent in Ph+ patients, and trisomies of chromosomes 10, 12, 17, and 18 are more than twice as frequent in Ph-negative patients. Trisomies of chromosomes 4, 10, 17, and 18 have been associated with a particularly good outcome in Ph-negative ALL.[47–49] Although trisomy of chromosome 4 was frequent (80% of HH Ph+ patients), trisomy of chromosome 10, 17, or 18 was infrequent in the HH Ph+ patients (25–30% of HH Ph+ patients). The impact of the particular chromosomes present as trisomies on the better outcome of HH Ph+ ALL patients has not been investigated.

CML and Ph+ ALL have markedly different clinical courses although they share the t(9;22)(q34;q11.2). Most CML present with a Ph as the sole cytogenetic abnormality; gain of a second Ph chromosome is associated with accelerated phase or blast crisis and signifies progressive disease.[50] In contrast, the presence of a second Ph at diagnosis of pediatric Ph+ ALL in this study is associated with a more favorable outcome than losses of chromosome 7, 7p, and/or 9p. Notably, although many of the patients with +Ph were also HH, a significant proportion (20; 54%) did not have HH. The Ph chromosome encodes the bcr-abl fusion protein, which is necessary for full expression of Ph+ ALL or CML. In CML it has been hypothesized that a second Ph chromosome increases the amount of this protein, thus accelerating the disease process. However in this study, a second Ph in childhood ALL was not an adverse factor. Recently, it was shown that 10–15% of CML patients have deletions of 9q34 proximal to the ABL gene and/or deletions of 22q11.2 distal to the BCR gene,[51] and patients with 9q34 deletions proximal to ABL have a worse outcome than patients without this deletion,[51] indicating that in CML also, aberrations in addition to t(9;22) at diagnosis may influence the course of the disease.

The secondary aberrations and complex karyotypes at diagnosis of childhood Ph+ ALL nearly always result in unbalanced karyotypes. Unbalanced rearrangements may be associated with progressive disease.[52] Altogether these data suggest that genomic instability producing secondary aberrations may contribute to the aggressive course of Ph+ ALL, which is characterized by a rapidly expanding leukemic cell population with limited growth requirements and thus growth advantage.

Conclusion

This study shows that secondary chromosomal aberrations present at diagnosis of childhood Ph+ ALL are nonrandom, typically are unbalanced, and importantly, may influence treatment outcome. Patients with loss of chromosome 7, 7p, and/or 9p tend to have an inferior outcome compared to patients with gain of a second Ph and/or with >50 chromosomes, those with other secondary aberrations and those without secondary aberrations. The presence of a second Ph did not result in more aggressive clinical behavior, thus suggesting that a dose–effect of the transcript is not in place in this disease. Karyotype analysis remains an important aspect of those ALL subsets which may be readily identified by molecular analysis. Such studies may help

identify the role of additional chromosomal abnormalities and their correlation with clinical heterogeneity, with possible therapeutic implications. Additionally, they identify chromosomal regions for molecular studies, which might unravel the complex leukemogenesis and the mechanism of drug resistance in this disease.

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