IGH@ translocations co-exist with other primary rearrangements in B-cell precursor acute lymphoblastic leukemia

Sally J. Jeffries,[1] Lisa Jones,[2] Christine J. Harrison,[2] and Lisa J. Russell[2]

[1]West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust; and [2]Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle University, Newcastle-upon-Tyne, UK

ABSTRACT

Primary established genetic abnormalities in B-cell precursor acute lymphoblastic leukemia include high hyper-diploidy (51-65 chromosomes), the translocations t(12;21)(p13;q22)/*ETV6-RUNX1* fusion and t(9;22)(q34;q11)/*BCR-ABL1* fusion, *MLL* rearrangements and intrachromosomal amplification of chromosome 21. These rearrangements are of prognostic and therapeutic relevance and are usually mutually exclusive. We identified 28 patients at diagnosis with both a primary genetic rearrangement and an *immunoglobulin heavy chain locus* translocation using chromosomal analysis and fluorescence *in situ* hybridization. Among these patients, the *immunoglobulin heavy chain locus* translocation partner gene was identified in six (*CRLF2, CEBPA, CEBPB, TRA/D@, IGF2BP1* and *IGK@*). Clonal architecture was investigated in 17 patients using multiple color interphase fluorescence *in situ* hybridization analysis, which showed that the translocation was acquired as a secondary abnormality in ten patients, in four patients the etiology was undetermined and in three patients it was observed in a separate clone from the primary chromosomal rearrangement. These findings demonstrate the co-existence of *immunoglobulin heavy chain locus* translocations with other primary chromosomal rearrangements either in the same or separate clones, which may have prognostic significance in B-cell precursor acute lymphoblastic leukemia. *Clinical trials: UKALLXII: Study ID n. ISRCTN77346223 and ALL2003: Study ID n. ISRCTN07355119*

Introduction

Cytogenetic characterization of acute lymphoblastic leukemia (ALL) indicates prognosis and is paramount for risk stratification for treatment. The main cytogenetic abnormalities used for risk stratification are usually mutually exclusive. They include high hyperdiploidy (51-65 chromosomes with the gain of specific chromosomes) and the *ETV6-RUNX1* gene fusion, which are two subtypes associated with a good outcome, whereas *BCR-ABL1* fusion, *MLL* translocations and intrachromosomal amplification of chromosome 21 (iAMP21) are subtypes associated with a poor outcome.[1]

In a recent study to ascertain the incidence of *immunoglobulin heavy chain locus* translocations (*IGH@*-t) in ALL, we identified the co-existence of *IGH@*-t in association with primary chromosomal rearrangements in 28 patients.[7] To investigate these cases in greater detail, we examined the *IGH@*-t and primary chromosomal rearrangements within the same diagnostic bone marrow cells using multiple color fluorescence *in situ* hybridization (FISH) and found a range of clonal architecture.

Methods

Patients

A total of 28 patients were included in this study (*Online Supplementary Table S1*). Of these, 21 were treated on the childhood treatment trial UKALL2003 [including patients with Down syndrome ALL (DS-ALL)] and six on the adult UKALLXII trial. Institutional review board ethical approval was obtained for all patients at each of the col-laborating centers. Informed consent was given in accordance with the Declaration of Helsinki.

Fluorescence *in situ* *hybridization*

The primary cytogenetic changes were originally identified by cytogenetic analysis; however due to the cryptic nature of the translocation, t(12;21), the presence of the *ETV6-RUNX1* fusion was confirmed by FISH or reverse transcriptase polymerase chain reaction analysis. Initial screening for the presence of *IGH@*-t was carried out using the Vysis LSI IGH Break-Apart Rearrangement Probe (Abbott Molecular, Illinois, USA) (Vysis IGH@ probe). A normal cell with intact *IGH@* shows two closely apposed red (R) and green (G) fusion signals (2F). The presence of an *IGH@*-t is indicated by separation of one pair of red and green signals to give the 1R1G1F pattern. Five control slides made with fixed cells from individuals with no known neoplasia were hybridized with this probe. Both visual and automated scoring was performed to determine the cut-off percentages for false positive results (±3 standard deviations). A patient was classified as *IGH@*-positive if the percentage of separated red and green signals was ≥5%, as determined independently by two analysts. Due to the cryptic nature and relative frequency of *CRLF2* involvement in *IGH@*-t, patients with this translocation and available material were screened for *CRLF2* rearrangements using previously described FISH probes and methods.[2] For patients with an *IGH@*-t visible by cytogenetics, either previously published FISH probes or FISH mapping (if material was available) was used to confirm the partner gene.[2-5]

Multiple color interphase FISH was carried out to assess the presence of both the primary rearrangement and *IGH@*-t in the same cells. The

IGH Breakapart LPH 014 probe (Cytocell, Cambridge, UK) or a home-made *IGH@* single color probe was used for the detection of *IGH@*-t.[5] The FISH probes used to detect aneuploidy in the cohort with high hyperdiploidy were designed to mark those chromosomes most commonly gained (chromosomes 4, 14 and 21) or those rarely gained as a control to exclude tetraploid cells (chromosome 9). The probes used to detect the primary rearrangements are detailed in Table 1.

For scoring by eye (independent *IGH@* and *CRLF2* FISH), results were provided by the regional cytogenetics laboratories or scored using an Olympus BX-61 florescence microscope with a x100 oil objective. A minimum of 100 nuclei were scored for each FISH test by two independent analysts. When combined with probes to mark the co-existing primary rearrangement, automated capture and scoring was carried out using an automated Olympus BX-61 8-bay stage florescence microscope with a x60 oil objective. Images were stored and analyzed using the CytoVision 7.1 SPOT counting system (Leica Microsystems, Gateshead, UK).

Three control slides were set up for all multiple color probe combinations using fixed cells from blood samples of individuals with no known neoplasia (Table 1). For the different probe combinations to mark the *IGH@*-t and aneuploidy, 15% was used as the cut-off percentage.[6] The higher cut-off level is required when analyzing multiple probes in the same cells to allow for interference and obscuring of the many signals.

Results

Patients' details and primary cytogenetic changes

In an earlier study to determine the incidence of *IGH@*-t in B-cell precursor-ALL (BCP-ALL), we identified 28 *IGH@*-t positive patients with co-existing established primary chromosomal rearrangements.[7] The clinical, demographic and diagnostic karyotype data of these 28 patients are presented in *Online Supplementary Table S1*.

Nine *IGH@*-t patients had chromosomal gains indicative of high hyperdiploidy. While all had >50 chromosomes, two DS-ALL patients had constitutional gain of one copy of chromosome 21. One DS-ALL patient (24390) had only four somatic gains, three of which were commonly observed gains in high hyperdiploidy (chromosomes 14, 21 and X). The second patient with DS-ALL (8447) had somatic gains of eight chromosomes and somatic loss of two. However, only three gains (chromosomes X, 8 and 14) were typical of those observed in high hyperdiploidy. Eight patients with *IGH@*-t had the *ETV6-RUNX1* gene fusion, with additional chromosomal abnormalities in four of them. Six *IGH@*-t patients had the *BCR-ABL1* gene fusion, which was cytogenetically cryptic in two of them. Three *IGH@*-t patients had iAMP21 and two had *MLL* translocations, both involving the *AFF1* gene at 4q21.

Conventional cytogenetics and dual color fluorescence in situ *hybridization*

From cytogenetic analysis, eight patients had a visible translocation involving chromosome band 14q32. Interphase and metaphase FISH analysis using the Vysis *IGH@* probe and other home-made probes confirmed that *IGH@*-t was present in all 28 patients and identified the partner genes in six (*CRLF2, CEBPA, CEBPB, TRA/D@, IGF2BP1* and *IGK@*, as shown in *Online Supplementary Table S1*). The partner genes involved in the remaining 22 patients are unknown.

Multiple color fluorescence in situ *hybridization analysis to determine clonal architecture*

To assess the presence of the primary rearrangement and the *IGH@*-t within the same cells, a combination of probes were hybridized simultaneously to the same slide for 17 patients with available material. In all patients a normal population was observed. However, the percentage of nuclei

Table 1. Multicolour FISH probe combinations, probe details and cut-off values used.

Target genes/Chromosomes	Probe combination	Cut-off (%)
IGH@/Chr4/Chr21	IGH Breakapart LPH 014 (Cytocell, Cambridge, UK) Chr4 - RP11-46I19, RP11-71F5, RP11-386I15 Chr21 - RP11-272A03, RP11-396Gll	15[#] (4)
IGH@/Chr9/Chr21	IGH Breakapart LPH 014 (Cytocell) Chr9 - CEP 9 Spectrum Aqua Probe (Abbott Molecular) Chr21 - RP11-272A03, RP11-396Gll	15[#] (6)
IGH@/Chr14/Chr21	IGH Breakapart LPH 014 (Cytocell) Chr14- CTD-3034B12, RP11-676G2 Chr21 - RP11-272A03, RP11-396Gll	4
RUNX1/IGH@	RUNX1 Breakapart (home grown) - RP11-272A03 (SR), RP11-396G11 (SG) IGH@ single colour (home grown* - SGd)	4
BCR/IGH@	BCR FISH DNA Probe, Split Signal (Dako) IGH@ single colour (home grown* - SGd)	5
IGH@/RUNX1	IGH Breakapart LPH 014 (Cytocell) RUNX1 single colour (home grown, SGd) - RP11-272A03, RP11-396G11	5
MLL/IGH@	MLL Breakapart LPH 013 IGH@ single colour (home grown* - SGd)	5
IGH@/BCR	IGH Breakapart LPH 014 (Cytocell) BCR single colour (home grown - SGd) - RP11-42J21, RP11-757F24, RP11-761L13, RP11-869I1	6

*SGd: spectrum gold; SR: spectrum red; SG: spectrum green. *The home-made* IGH@ *probe consisted of the following clones: RP11-150I6, RP11-45K1, RP11-346I20, RP11-676G2, RP11-72N10, RP11-101G24 and RP11-47P23.# Due to the increased number of expected signals in a clone with both high hyperdiploidy and* IGH@-t, we increased the cut-off value for detecting this to 15% to take into consideration the increased likelihood of signal obscurity and/or co-localization of probe signals.*

harboring *IGH@*-t differed according to whether the studies were conducted with the Vysis *IGH@* probe, used individually, or with the Cytocell *IGH@* probe or home-made *IGH@* probe used for the detection of *IGH@*-t in combination with the primary rearrangements in some patients. This is likely a consequence of hybridization of a single probe scored by eye compared to simultaneous multiple color probe combinations scored by an automated procedure.

IGH@-*t* is a secondary event

Thirteen patients (8447, 23110, 22105, 11438, 10281, 22420, 20951, 2618, 10285, 21733, 10859, 11061 and 21940) harbored a population of nuclei with the primary rearrangement in the absence of *IGH@*-t.

Examples of each primary rearrangement with clonal architecture
High hyperdiploidy

In patient 10281 with high hyperdiploidy (Figure 1i-iii), the Vysis *IGH@* probe alone showed a breakpoint within the variable region in 27% of nuclei. By multiple color FISH, 30% of nuclei showed chromosomal gains in the absence of *IGH@*-t. Two abnormal populations without *IGH@*-t had gains of chromosomes 4, 14 and 21 (30%). One of these populations acquired *IGH@*-t on the background of the initial numerical gains (38%), which further evolved with loss of one copy of chromosomes 14 and 21 (11%).

ETV6-RUNX1 fusion

In patient 20951 with *ETV6-RUNX1* (Figure 2), the Vysis

IGH@ probe alone showed a breakpoint within the variable region in 59% of nuclei. By multiple color FISH, *IGH@*-t was detected in 44% of nuclei in association with *ETV6-RUNX1*, as indicated by the *RUNX1* probes. The *IGH@*-t in this patient appeared to have arisen as a secondary event, as 14% of nuclei harbored the *EVT6-RUNX1* translocation and an additional copy of chromosome 21 only. However, as this patient had a variant FISH signal pattern using the Vysis *IGH@* probe, this case was classified as having an undetermined etiology because of the difficulties of interpretation when one signal was absent.

BCR-ABL1 fusion

In patient 21733 with *BCR-ABL1* (Figure 3), the Vysis *IGH@* probe alone showed 9% of interphase nuclei with *IGH@*-t. Using multiple color FISH, 79% of interphase nuclei showed *BCR-ABL1* only with an additional 6% showing both *BCR-ABL1* and *IGH@*-t.

Intrachromosomal amplification of chromosome 21

In patient 11061 with iAMP21, the Vysis *IGH@* probe showed 47% interphase nuclei with *IGH@*-t (Figure 4), while multiple color FISH showed 97% of cells with iAMP21, of which only 21% had *IGH@*-t in addition to iAMP21.

MLL translocation

In patient 21940 with an *MLL* translocation, the Vysis *IGH@* probe alone showed a rearrangement in 13% of interphase nuclei (Figure 5). Multiple color FISH showed the pres-
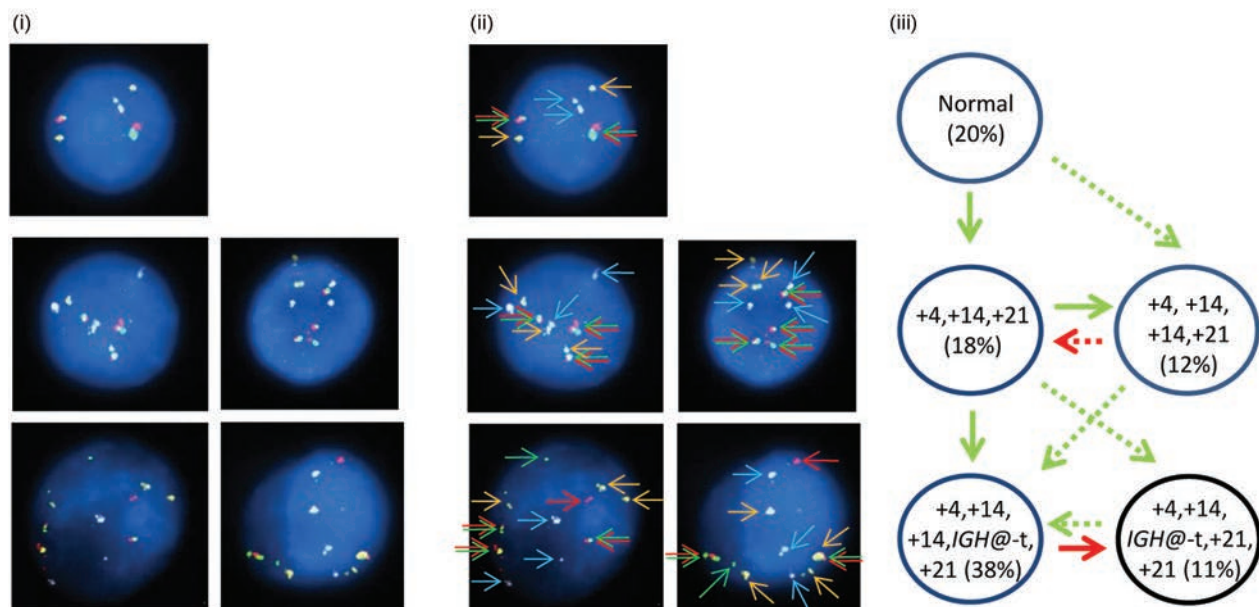

Figure 1. Images depicting the emergence of high hyperdiploidy prior to the *IGH@*-t in patient 10281. (i) Captured interphase nuclei hybridized with FISH probes to track the high hyperdiploidy (chromosome 4; aqua, chromosome 21; gold) and *IGH@*-t/+14 (red/green). (ii) The same captured interphase nuclei, including colored arrows depicting the signals that were scored: blue arrows highlight the aqua probe marking copies of chromosome 4, the yellow arrows highlight the gold probe marking copies of chromosome 21 and the red/green double arrows highlight the *IGH@* breakapart probe that marks both the copy number of chromosome 14 and also detects the presence of a translocation. (iii) Cartoon depicting possible models of evolutionary progression by either gain (green arrows) or loss (red arrows). Solid and dashed lines highlight the possible alternative evolutionary routes taken by the sample. The black circle represents the FISH signal pattern which correlates with the abnormal karyotype observed at metaphase analysis. No cytogenetically visible *IGH@*-t was observed in these cells.

ence of *IGH@*-t in addition to the *MLL* translocation in 8% of cells with 82% having only *MLL* translocation with no evidence of *IGH@*-t.

IGH@-t and primary rearrangements are independent clones

In three patients (24390, 11520 and 3737), independent abnormal clones were identified. In patient 24390, cytogenetics and FISH identified independent high hyperdiploidy and *IGH@*-t abnormal clones. Chromosomal analysis showed a high hyperdiploidy karyotype with 51 chromosomes including constitutional gain of chromosome 21 (+21c), consistent with this patient having DS-ALL. Interphase and metaphase FISH using the Vysis *IGH@* probe showed a normal population with two intact *IGH@* signals (28% interphase nuclei) (Figure 6Ai). Three abnormal populations were also observed: (ii) an additional chromosome 14 with intact *IGH@* signals observed on all three chromosomes 14 in 10/23 metaphases and 29% interphase nuclei; (iii) an *IGH@*-t in 2/23 metaphases and 11% interphase nuclei but with no evidence of gain of chromosome 14; (iv) a rearrangement of both copies of the *IGH@* locus in 9/23 metaphases and 28% interphase nuclei but no evidence of gain of chromosome 14. The quality of the metaphase cells with *IGH@*-t was too poor to establish the number of chromosomes present. The FISH and cytogenetic results indicated two independent abnormal clones with either high hyperdiploidy or a cytogenetically cryptic *IGH@*-t. Further supporting evidence for two independent abnormal clones included a significant difference in the nuclear morphology of the interphase cells. The nuclei with *IGH@* rearrangements (Figure 6Aiii-iv) were significantly smaller than those
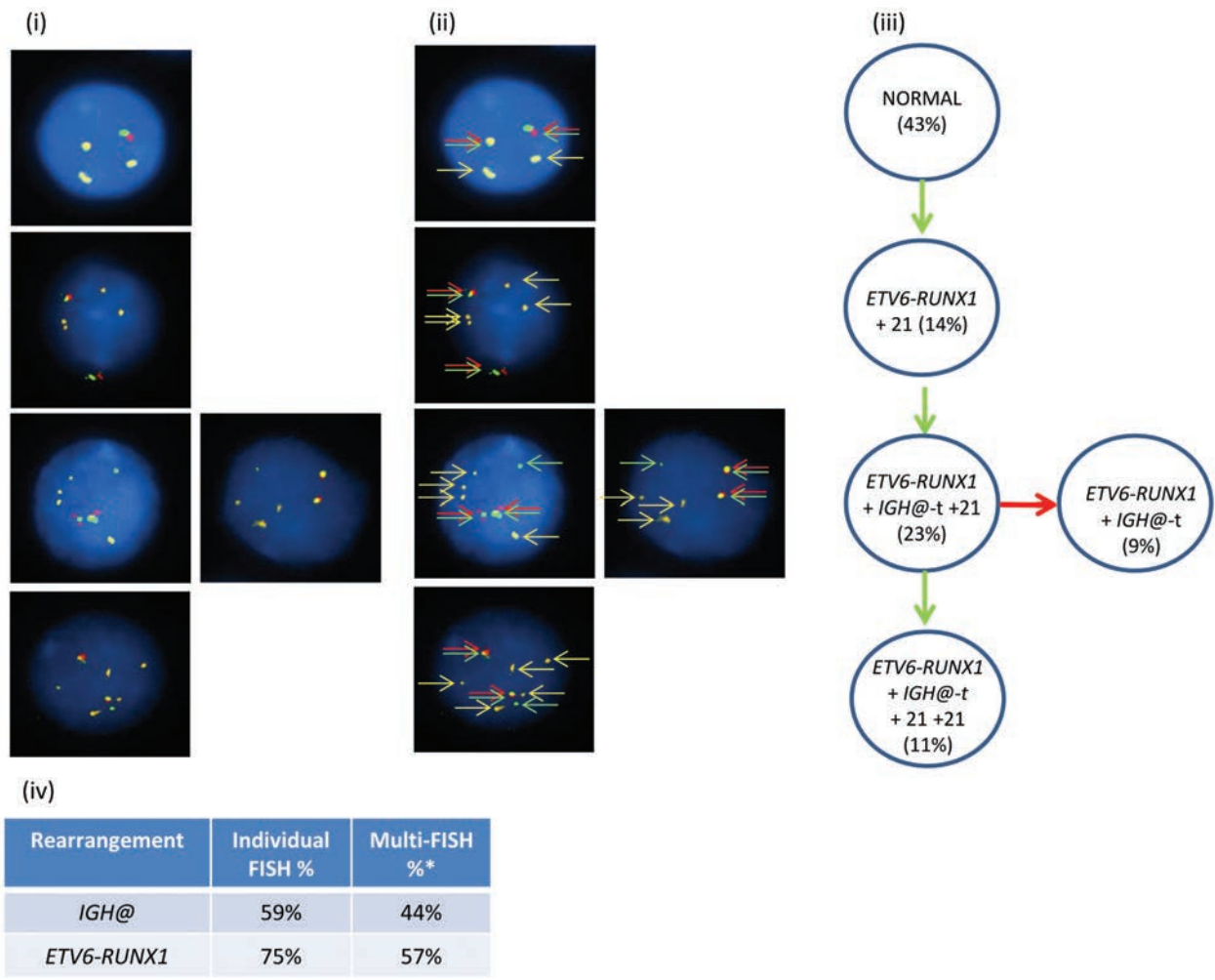

(i)

(ii)

(iii)

NORMAL (43%)

ETV6-RUNX1 + 21 (14%)

ETV6-RUNX1 + IGH@-t +21 (23%)

ETV6-RUNX1 + IGH@-t (9%)

ETV6-RUNX1 + IGH@-t + 21 +21 (11%)

(iv)

Rearrangement	Individual FISH %	Multi-FISH %*
IGH@	59%	44%
ETV6-RUNX1	75%	57%

Figure 2. Images of FISH interphase nuclei depicting the apparent emergence of an *ETV6-RUNX1* translocation prior to the *IGH@*-t. **(i)** Captured interphase nuclei from patient 20951 hybridized with FISH probes to track the *IGH@-t* (red/green) and the *ETV6-RUNX1* translocation (homemade *RUNX1* probe; gold). **(ii)** The same captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the *RUNX1* rearrangement and the red/green double arrows highlight the *IGH@* break apart probe that marks both the copy number of chromosome 14 and also detects the presence of a translocation. **(iii)** Cartoon depicting possible models of evolutionary progression either by gain (green arrows) or loss (red arrows). Forty-three percent of nuclei were normal for both probes (one red/green fusion appears yellow, this is marked with arrows for clarity as shown in (ii) with 14% showing an additional copy of chromosome 21 in the presence of the *RUNX1* translocation. On the background of the *RUNX1* translocation and additional chromosome 21, a subclone acquired an *IGH@*-t (23%) which subsequently either lost (9%) or gained (11%) a copy of chromosome 21. **(iv)** Table showing the percentage of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell.

with chromosomal gains (Figure 6Ai-ii). In this patient, CRLF2 FISH indicated that this *IGH@*-t was *IGH@-CRLF2*.

Multiple color FISH for *IGH@* and chromosomes 14 and 21 provided additional evidence that the translocation and the copy number abnormalities were present in different clones (Figure 6B). Two intact *IGH@* signals with three copies of chromosome 21, indicating constitutional gain (+21c), were observed in 48% of nuclei. This clone evolved into two independent sub-clones. The first acquired an additional copy of chromosome 21 (4%) which further evolved with gain of chromosome 14 (20%). This clone appeared to lose one copy of chromosome 21 in a further 8% of nuclei. There is a possibility that the latter clone may have evolved from the normal clone, however the hypothesis that high

hyperdiploidy arises in one cell division makes loss of chromosomes at segregation more likely.[8] The second sub-clone harbored a single *IGH@*-t in the presence of +21c. Although this rearrangement was observed in only 2% of nuclei, the metaphase and interphase FISH results described above, using only the Vysis *IGH@* probe, confirmed this result. This clone appeared to have evolved further, with a translocation involving the other *IGH@* locus in 18% of nuclei. *CRLF2* FISH identified a rearrangement, suggesting *IGH@-CRLF2*. The partner gene involved in the second *IGH@*-t remains unknown.

Using individual FISH probes, patient 11520 (Figure 7Ai-iii) showed *IGH@*-t in 32% of cells and *ETV6-RUNX1* in 15% of cells (Figure 7Aiii). Patient 3737 (Figure 7Bi-iii) had
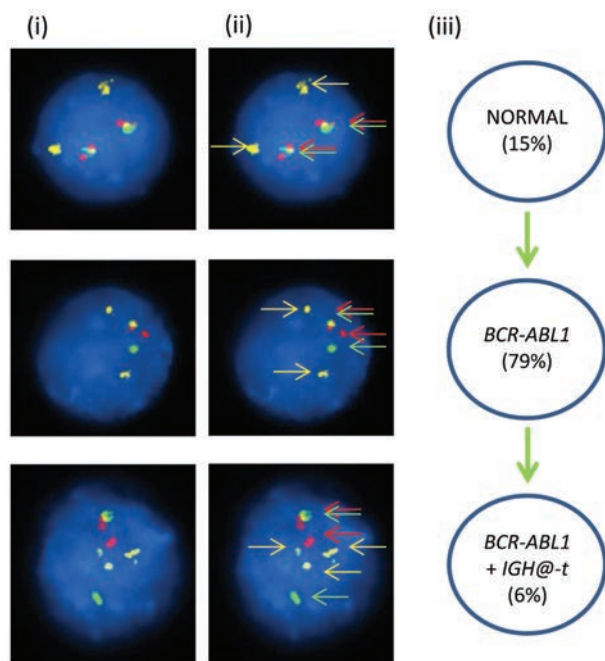
Rearrangement	Individual FISH %	Multi-FISH %*
IGH@	9%	6%
BCR-ABL1	90%	85%

Figure 3. Images of FISH interphase nuclei depicting the apparent emergence of a *BCR-ABL1* translocation prior to the *IGH@*-t. (i) Captured interphase nuclei from patient 21733 hybridized with FISH probes to track *BCR-ABL1* (DAKO BCR split signal probe; red/green) and a home-made *IGH@* probe (gold). (ii) The same captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the home-made *IGH@* probe that detects both the copy number of chromosome 14 and also the presence of a translocation. The red/green double arrows highlight the *BCR* split signal probe that marks the *BCR-ABL1* translocation. (iii) Cartoon depicting a possible model of evolutionary progression by gain (green arrows). A normal population of cells was observed (15%) with the emergence of a clone harboring only the *BCR-ABL1* translocation in 79% of nuclei. This clone subsequently acquired an *IGH@*-t in 6%. (iv) Table showing the percentages of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell.
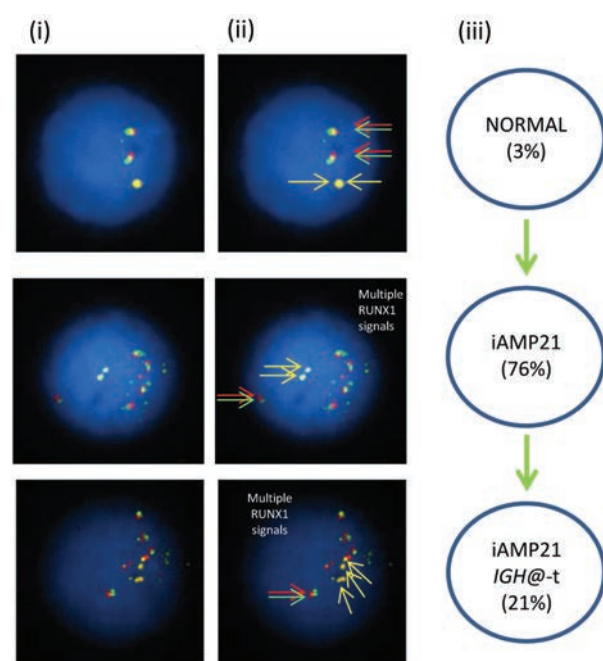
Rearrangement	Individual FISH %	Multi-FISH %*
IGH@	47%	21%
iAMP21	97%	97%

Figure 4. Images of FISH interphase nuclei depicting the apparent emergence of an iAMP21 prior to the *IGH@*-t. (i) Captured interphase nuclei from patient 11061 hybridized with FISH probes to track iAMP21 using a home-made *RUNX1* breakapart probe (red/green) and a home-made *IGH@* single color probe (gold). (ii) The same captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the home-made *IGH@* probe that detects both the copy number of chromosome 14 and also the presence of a translocation. The red/green double arrows highlight the home home-made *RUNX1* breakapart probe that marks the iAMP21 aberration. (iii) Cartoon depicting a possible model of evolutionary progression. Seventy-six percentage of nuclei showed multiple copies of *RUNX1* with 21% of these nuclei also harboring *IGH@*-t. (iv) Table showing the percentages of positive nuclei when samples were hybridised with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell.
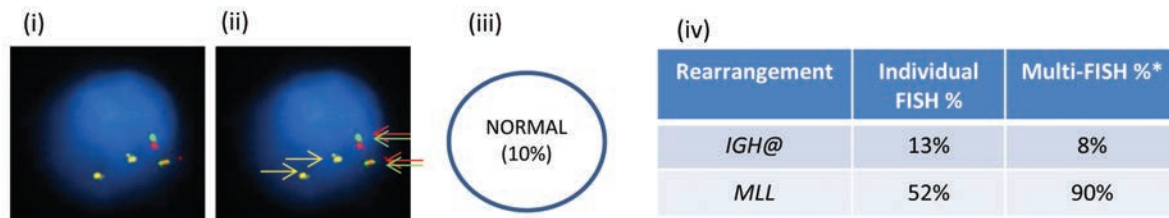
Rearrangement	Individual FISH %	Multi-FISH %*
IGH@	13%	8%
MLL	52%	90%

Figure 5. Images of FISH interphase nuclei depicting the apparent emergence of an *MLL* translocation prior to the *IGH@*-t. (i) Captured interphase nuclei from patient 21940 hybridized with FISH probes to track the *MLL* translocation using a dual color *MLL* breakapart probe (Vysis, Abbott Molecular) (red/green) and home-made *IGH@* probe (gold). (ii) The same captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the home-made *IGH@* probe that detects both the copy number of chromosome 14 and also the presence of a translocation. The red/green double arrows highlight the dual color *MLL* breakapart probe that marks the *MLL* translocation. (iii) Cartoon depicting a possible model of evolutionary progression. An *MLL* rearrangement was observed in 82% of cells and an additional *IGH@*-t in 8% of cells. (iv) Table showing the percentages of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell. *percentages may vary between the FISH results obtained when using an individual probe, and probes in combination, likely reflecting scoring by automation *versus* scoring by eye and increased interference and obscuring of signals when there are many of them.

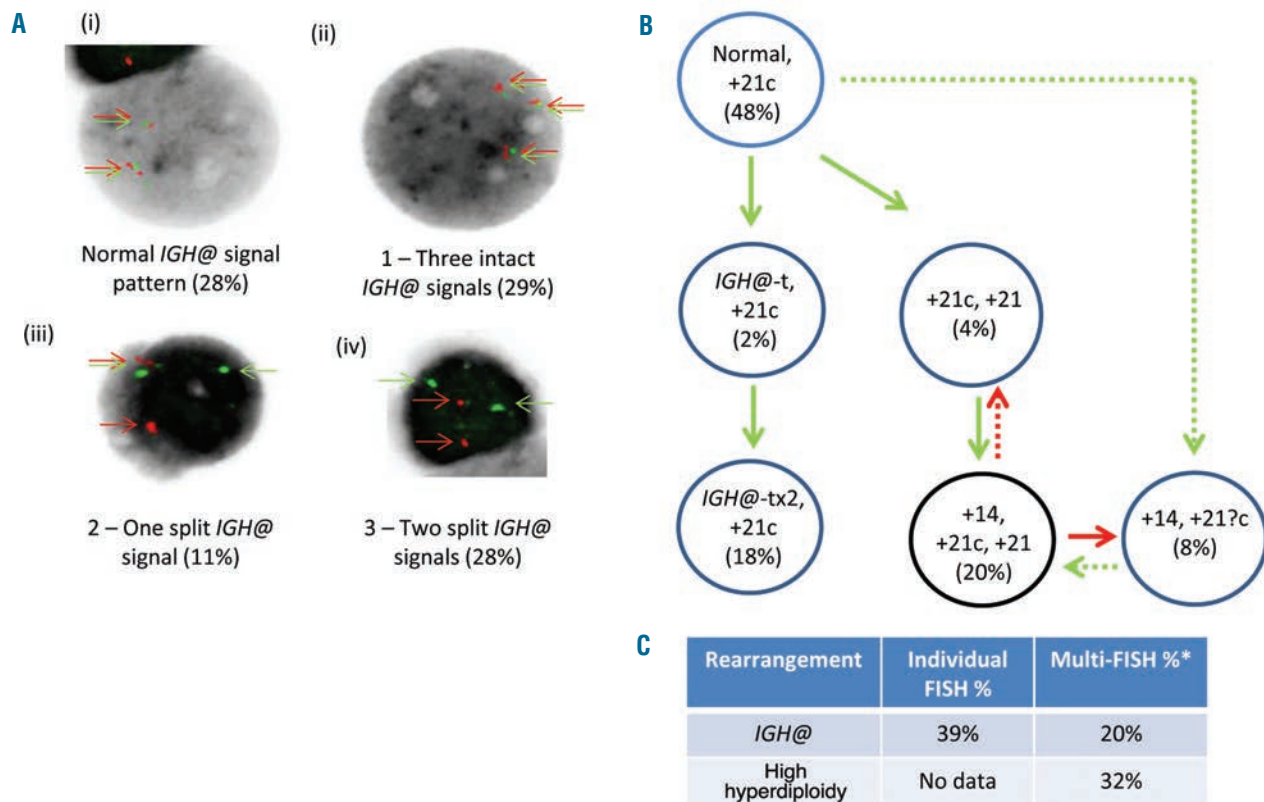| Rearrangement	Individual FISH %	Multi-FISH %*
IGH@	39%	20%
High hyperdiploidy	No data	32%

Figure 6. Dual color and multiple color FISH showing independent aneuploid and *IGH@*-t clones in patient 24390. (A) Dual color *IGH@* FISH showing the presence of a normal clone (i) and three abnormal clones (ii,iii,iv). The red/green colored arrows highlight the signals that were scored. The first abnormal clone (ii) shows an additional copy of the *IGH@* intact fusion, the second abnormal clone (iii) shows the separation of one fusion indicating the presence of a translocation involving one *IGH@* locus and the third abnormal clone (iv) shows the separation of both *IGH@* fusions suggesting the presence of two *IGH@*-t. (B) Cartoon depicting the potential clonal evolution by either gain (green arrows) or loss (red arrows). Solid and dashed lines highlight the possible alternative evolutionary routes taken by the sample. Two intact *IGH@* signals with three copies of chromosome 21 were observed in 48% of nuclei. This clone evolved into two independent sub-clones. The first showed evolution within the high hyperdiploidy clone with additional copies of chromosome 21 (4%), both chromosome 14 and 21 (20%) and loss of one copy of chromosome 21 (8%). The second sub-clone harbored a single *IGH@*-t followed by rearrangement of the second *IGH@* locus (18%). The black circles represent the FISH signal patterns which correlate with the karyotypes observed at metaphase analysis. (C) Table showing the percentages of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell.

IGH@-t in 76% of cells and the *BCR-ABL1* gene fusion in 12% of cells (Figure 7Biii). However, in both patients, multiple color FISH showed that these clones were independent; no cells were observed with either the *ETV6-RUNX1* or *BCR-ABL1* rearrangement in association with *IGH@*-t (Figures 7Aii and 7Bii). The *IGH@*-t partner gene in these patients remains unknown.

Excluding the three patients with independent primary and *IGH@*-t clones, ten patients had *IGH@*-t at the time of presentation of the disease and also at the time when the *IGH@*-t positive clone represented the dominant abnormal clone (largest clone), eight presented when the IGH@-t clone was either at a comparable level to that of the primary rearrangement or a significant minor sub-clone (≥15% of

cells), and seven presented with a minor sub-clone (those close to the false positive level and <15% of cells) (*Online Supplementary Table S1*).

IGH@-t etiology is unknown

One patient (7143) showed the presence of both high hyperdiploidy and *IGH@*-t in the same nuclei in all abnormal cells and therefore the point at which *IGH@*-t arose was unknown. In this cases the etiology was classified as undetermined (Figure 8i-iii). Two models of evolution are possible (Figure 8iii). Clonal evolution occurred with either 25% of nuclei gaining a third additional copy of chromosome 21, or 71% of nuclei losing the third additional copy of 21. The *IGH@*-t was cytogenetically visible in this patient and sub-
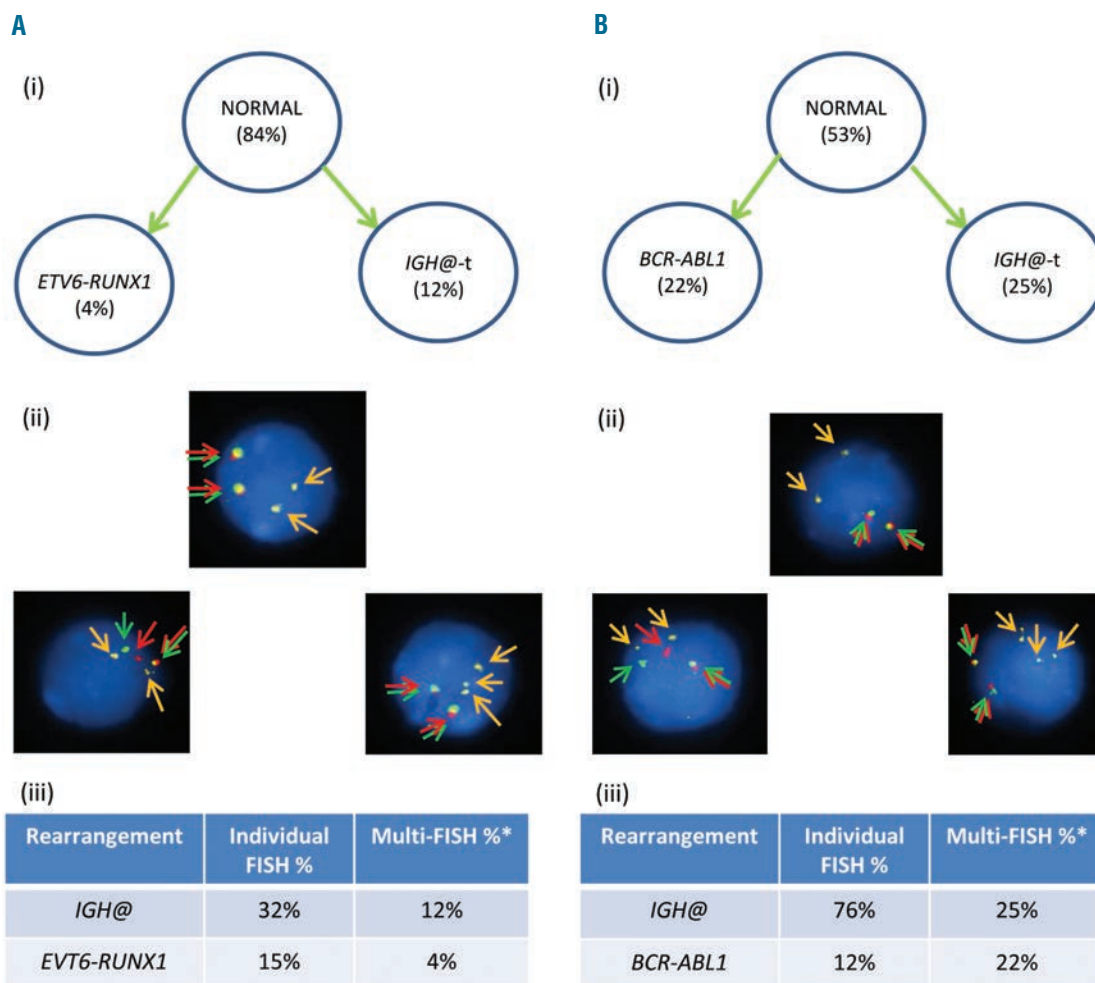

Figure 7. Images of FISH interphase nuclei depicting an independent primary rearrangement and *IGH@*-t clones. (**A**) Patient **11520** with *ETV6-RUNX1* demonstrated using a *RUNX1* breakapart probe (Vysis, Abbott Molecular) (red/green) and home-made *IGH@* probe (gold). (i) Cartoon depicting the possible model of evolutionary progression with both aberrations being observed in separate cells. (ii) Captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the home-made *IGH@* probe that detects both the copy number of chromosome 14 and also the presence of a translocation. The red/green double arrows highlight the *RUNX1* breakapart probe that marks the *ETV6-RUNX1* fusion. (iii) Table showing the percentages of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell. (**B**) Patient **3737** with *BCR-ABL1* demonstrated using the dual color *BCR-ABL1* breakapart probe (Vysis, Abbott Molecular) (red/green) and home-made *IGH@* probe (gold). (i) Cartoon depicting the possible model of evolutionary progression with both aberrations being observed in separate cells. (ii) Captured interphase nuclei, including colored arrows highlighting the signals that were scored: the yellow arrows highlight the gold probe marking the home-made *IGH@* probe that detects both the copy number of chromosome 14 and also the presence of a translocation. The red/green double arrows highlight the *BCR-ABL1* breakapart probe that marks the *BCR-ABL1* fusion. (iii) Table showing the percentages of positive nuclei when samples were hybridized with either the individual probes for each rearrangement (Individual FISH), or when they were investigated together (Multi-FISH) in the same cell. *percentages vary between the FISH results obtained when using an individual probe, or a probe in combination with others.
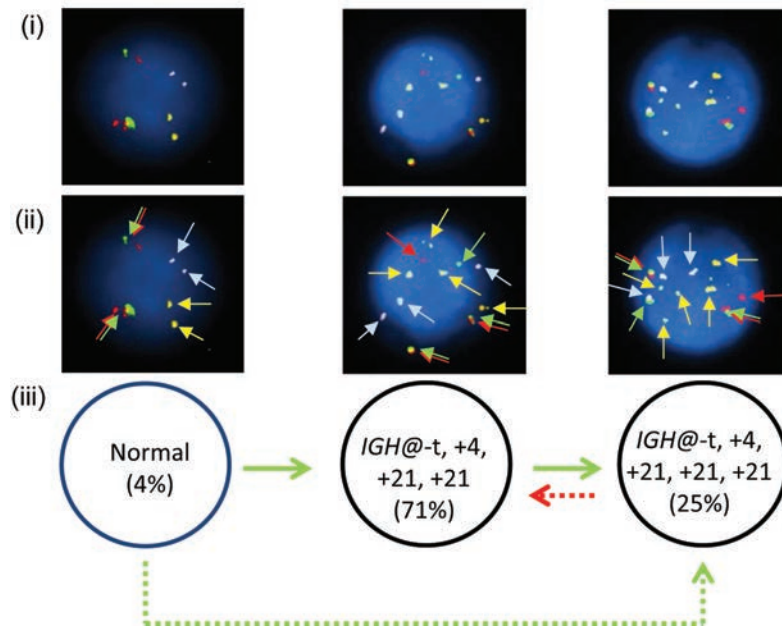
Figure 8. Multiple color FISH for patient 7143 showing co-existing high hyperdiploidy and *IGH@*-t. (i) Captured interphase nuclei from patient 7143 hybridized with FISH probes to track the *IGH@*-t (red/green) (Cytocell), chromosome 4 (aqua) and chromosome 21 (gold). (ii) The same captured interphase nuclei, including colored arrows highlighting the signals that were scored: the red/green double arrows highlight the *IGH@* breakapart probe that marks both the copy number of chromosome 14 and also detects the presence of a translocation, the blue arrows highlight chromosome 4 and the gold arrows chromosome 21. (iii) Cartoon depicting possible models of evolutionary progression by either gain (green arrows) or loss (red arrows). Solid and dashed lines highlight the possible alternative evolutionary routes taken by the sample. The black circle represents the FISH signal pattern which correlates with the abnormal karyotype observed at metaphase analysis.

sequent interphase FISH with a breakapart probe to *CEBPA* confirmed *IGH@-CEBPA*.

Discussion

High hyperdiploidy, t(12;21)/*ETV6-RUNX1*, t(9;22)/*BCR-ABL1*, iAMP21 and *MLL* translocations are all established recurrent cytogenetic abnormalities in BCP-ALL.[9] This study has shown that these rearrangements and *IGH@*-t may occur together in both childhood and adult BCP-ALL. *IGH@*-t did not appear to be associated with any particular primary rearrangement, as the frequency observed correlated with the frequency observed in BCP-ALL in general. High hyperdiploidy and *ETV6-RUNX1* are the two most common subtypes of childhood BCP-ALL (~25% each), *BCR-ABL1* is observed in around 9% (3% in children and 15% in adults), whereas iAMP21 and *MLL*-t are two of the relatively rarer subtypes (~2% each). The median age and white cell count at presentation in these patients compared well to those typically observed in BCP-ALL.[9]

This study is the first to look at the existence and evolution of a primary abnormality and a structural rearrangement (*IGH@*-t) in the same cells. Although the number of cases is small, our results support the literature showing that high hyperdiploidy, *ETV6-RUNX1*, iAMP21 and *MLL*-t are primary events.[10,11] *IGH@*-t did not arise as primary events in the presence of these established changes. The models proposed for the four patients described (10281 21733, 11061 and 21940), and confirmed in six others (8447, 22105, 11438, 22420, 2618 and 10859), suggest that in the majority of patients harboring both a primary rearrangement and *IGH@*-t, the *IGH@*-t is a secondary event in leukemia development. In three patients they were apparently independent events and in four the etiology was undetermined.

The partner genes identified in this cohort of patients with both a primary rearrangement and *IGH@*-t were

CRLF2, *CEBPA*, *CEBPB*, *TRA/D@*, *IGF2BP1* and *IGK@*. These genes and others have previously been identified as being deregulated in ALL due to juxtaposition with the *IGH@* enhancer.[2-5,12-14] The translocation, t(14;14)(q11;q32), involving both the *IGH@* and *TRA/D@* loci is typically observed in T-lineage ALL. However, the presence of rearrangements involving *IGH@* and *TRA/D@* in B-lineage neoplasia has been previously reported in the literature, albeit rarely.[7,15] Of the patients with apparently independent clones, the DS-ALL (patient 24390) showed unusual results with different clones having either one or two *IGH@*-t. FISH studies identified one *CRLF2* gene rearrangement, while the other remains unknown. These results suggest the occurrence of one *IGH@*-t with subsequent translocation of the second *IGH@* locus. The apparently unrelated abnormal clones in this patient and two others are likely to have arisen in either a common precursor clone with an unknown rearrangement and/or gene mutation, or both could have arisen as independent leukemias.

Whether these rearrangements represent drivers or passengers may be dependent on which partner gene is aberrantly expressed. Those present as a low level population may be passenger abnormalities with no leukemogenic advantage.

None of the patients in the favorable risk cohort showed any evidence of minimal residual disease after induction therapy. All but two are alive at this time and are in complete remission which has lasted a median of 43 months; the two patients who died had relapsed at 13 and 24 months. It remains to be determined whether *IGH@*-t in the presence of a favorable or intermediate marker alters these risks.

Our study has shown that *IGH@*-t can occur together with primary rearrangements and, when present, *IGH@*-t usually arise as secondary events, likely contributing to the development of the leukemia phenotype. Due to the cryptic nature of some *IGH@*-t we would recommend their exclusion by FISH. Whether *IGH@*-t in the presence of

other primary rearrangements associated with a good or intermediate risk has an impact on outcome has yet to be determined.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 2010;11(5):429-38.

2. Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114(13):2688-98.

3. Chapiro E, Russell L, Radford-Weiss I, Bastard C, Lessard M, Struski S, et al. Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia. Blood. 2006;108(10):3560-3.

4. Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R, et al. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007;109(8):3451-61.

5. Russell LJ, Akasaka T, Majid A, Sugimoto KJ, Loraine KE, Nagel I, et al. t(6;14)(p22;q32): a new recurrent IGH@ translocation involving ID4 in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2008;111(1):387-91.

6. Eastmond DA, Schuler M, Rupa DS. Advantages and limitations of using fluorescence in situ hybridization for the detection of aneuploidy in interphase human cells. Mutat Res Letters. 1995;348(4):153-62.

7. Russell LJ, Enshaei A, Jones L, Erhorn A, Masic D, Bentley H, et al. IGH@ translocations are prevalent in teenagers and young adults with ALL and are associated with a poor outcome. J Clin Oncol. 2014;32(14):1453-62.

8. Paulsson K, Mörse H, Fioretos T, Behrendtz M, Strömbeck B, Johansson B. Evidence for a single-step mechanism in the origin of hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromos Canc. 2005;44(2):113-22.

9. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. Blood Rev. 2012;26(3):123-35.

10. Rand V, Parker H, Russell LJ, Schwab C, Ensor H, Irving J, et al. Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2011;117(25):6848-55.

11. Maia AT, van der Velden VHJ, Harrison CJ, Szczepanski T, Williams MD, Griffiths MJ, et al. Prenatal origin of hyperdiploid acute lymphoblastic leukemia in identical twins. Leukemia. 2003;17(11):2202-6.

12. Russell LJ, De Castro DG, Griffiths M, Telford N, Bernard O, Panzer-Grumayer R, et al. A novel translocation, t(14;19)(q32;p13), involving IGH@ and the cytokine receptor for erythropoietin. Leukemia. 2008;23(3):614-7.

13. Chapiro E, Russell LJ, Struski S, Cave H, Radford-Weiss I, Valle VD, et al. A new recurrent translocation t(11;14)(q24;q32) involving IGH@ and miR-125b-1 in B-cell progenitor acute lymphoblastic leukemia. Leukemia. 2010;24(7):1362-4.

14. Aventín A, Sánchez J, Nomdedéu JF, Estany C, Forcada P, La Starza R, et al. Novel IGH translocations, t(2;14)(q14.3;q32) and t(14;17)(q32;q21), in B-cell precursor acute lymphoblastic leukemia. Canc Genet Cytogen. 2008;185(1):57-9.

15. Wong KF, Kwong YL, Wong TK. Inversion 14q in acute lymphoblastic leukemia of B-lineage. Cancer Genet Cytogen. 1995;80(1):72-4.