A Polymerase Chain Reaction Study of the Stability of Ig Heavy-Chain and T-Cell Receptor δ Gene Rearrangements Between Presentation and Relapse of Childhood B-Lineage Acute Lymphoblastic Leukemia


Ig heavy-chain (IgH) and partial V\textsubscript{d}2-D\textsubscript{3} T-cell receptor (TCR) gene rearrangements were investigated, by polymerase chain reaction (PCR) amplification and sequence analysis, in 52 patients at presentation and first relapse and in 14 at both first and second relapse of B-lineage acute lymphoblastic leukemia. In combination, these techniques amplified one or more clonal markers at presentation in 90% of patients (IgH-PCR, 75%; V\textsubscript{d}2-D\textsubscript{3}-PCR, 46%; both, 33%). Changes in the pattern of amplification between presentation and first relapse were seen in 31% of patients positive by IgH-PCR at presentation and in 25% of those positive by TCR\textsubscript{b}-PCR. Only 3 patients showed complete change in their rearrangements, which is suggestive of relapse with a new clone. Furthermore, despite the high reported rates of oligoclonality and clonal evolution at the IgH locus, the results presented show that false-negative minimal residual disease (MRD) detection can be avoided by designing D-N-J probes to all presentation rearrangements. Using a PCR approach for both gene markers, false-negative testing because of clonal evolution would have only occurred in 3 (8%) of the IgH-positive patients, in contrast to 5 (21%) of V\textsubscript{d}2-D\textsubscript{3}-positive patients. Combining these two systems increases the proportion of patients open to study to 90%, allows comparative studies of the sensitivities of the two methods, and reduces the rate of false-negative assessment of MRD caused by clonal evolution to less than 10%. We conclude that large prospective PCR studies of MRD detection should examine gene rearrangements at multiple loci to maximize their applicability and to minimize false-negative relapse prediction.

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Clonal Ig heavy-chain (IgH) and T-cell receptor (TCR) V\textsubscript{d}2-D\textsubscript{3} gene rearrangements can be detected at presentation in 95% and 45% of cases of B-lineage acute lymphoblastic leukemia (ALL), respectively. These provide disease markers that, when amplified by the polymerase chain reaction (PCR) allow highly sensitive tracking of minimal residual disease (MRD). However, if false-negative detection is to be avoided, it is a prerequisite that these rearrangements remain stable throughout the course of the disease.

Southern blot studies show changes in the pattern of rearrangements (clonal evolution) between presentation and first relapse in up to 50% of patients by IgH analysis, but in only 20% of patients by V\textsubscript{d}2-D\textsubscript{3} analysis. In addition, they show more than two IgH rearrangements at presentation ("oligoclonality") in 15% to 45% of patients, a phenomenon seen only infrequently with V\textsubscript{d}2-D\textsubscript{3} rearrangements. This has led to concern regarding the suitability of IgH rearrangements as markers of MRD, although it should be noted that at least one presentation rearrangement is preserved at relapse in most patients. Furthermore, it is unclear how many allele-specific IgH probes would be required to follow disease in oligoclonal patients.

However, recent IgH sequencing studies show that many cases of both oligoclonality and clonal evolution can be explained by secondary gene rearrangement events occurring in subclones of the original disease. These usually disrupt V-N-D sequence but leave D-N-J sequence undisturbed. If probes for tracking MRD are always designed to D-N-J sequence, the effect of these secondary gene rearrangements will be minimized. As a consequence, Southern blot studies may give a false impression of the relative reliability of V\textsubscript{d}2-D\textsubscript{3} and IgH rearrangements when used for MRD analysis.

To assess the true impact of clonal evolution at both loci on prospective trials of MRD analysis, we studied 52 children from presentation to first relapse and 14 from first to second relapse by a combination of IgH- and V\textsubscript{d}2-D\textsubscript{3}-PCR.

MATERIALS AND METHODS

Bone marrow (BM) samples were studied from 55 children (aged 3 months to 16 years) with B-lineage ALL presenting to either the

Royal Hospital for Sick Children (Bristol, UK) or the Hospital for Sick Children (London, UK). A total of 52 children were investigated from presentation to first relapse, and 14 from first to second relapse. All children were diagnosed according to standard morphologic and immunophenotypic criteria. The immunophenotypes of the 55 children were as follows: null ALL (3 cases), common ALL (cALL; 46), pre-B-ALL (3), and B-ALL (1).

DNA Preparation

BM mononuclear cells were isolated by centrifugation on either a Ficoll/Hypaque (Flow Laboratories, Irvine, CA) or 60% Percoll (Pharmacia, Uppsala, Sweden) gradient, washed in phosphate-buffered saline (PBS), pelleted, and stored at –70°C. DNA preparation was performed either by a guanidinium isothiocyanate-cesium trifluoroacetate technique or by lysis of the mononuclear cells with NP-40 followed by sodium dodecyl sulfate/proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation.

Where stored cells were not available, DNA was extracted from stored BM slides by the following technique. Briefly, an area of BM smear varying from 0.5 to 1 cm² (depending on the thickness of the film) was effaced from each slide in 100 μL of PBS, decanted into a small Eppendorf tube, and centrifuged at 15,000g for 5 minutes. After removal of the supernatant PBS by gentle aspiration, 20 μL of PCR buffer (10 mmol/L Tris HCl, pH 8.3; 50 mmol/L KCl; 3

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mmol/L MgCl₂) containing 0.6 U of PRETAQ (GIBCO-BRL, Gaithersburg, MD), a thermostable protease, was added. The sample was overlaid with 20 μL of light mineral oil (Sigma Chemical Co, St Louis, MO) and heated in an automated thermal cycler at 75°C for 10 minutes followed by 94°C for 50 minutes. After centrifugation at 15,000g for 1 minute, 2-μL aliquots of the supernatant were used for PCR analysis. This method allows preparation of a DNA sample suitable for PCR amplification from a small area of a single slide, regardless of its fixation state or staining.

**PCR Amplification**

All samples were investigated by both TCR β-PCR (using specific V62 and D63 primers) and IgH-PCR (using consensus first framework [FR3] and joining [JH] region primers); these typically produce clonal amplification at presentation in approximately 45% and 75% to 80% of patients, respectively. Patients negative by FR3-JH-PCR were further investigated by PCR using consensus first framework (FR1) and JH region primers. Primer positions and sequences are shown in Fig 1.

**IgH-PCR.** FR3-JH-PCR was performed as described previously⁴; 50-μL PCR mixes contained 2 μL of appropriate sample; 200 μmol/L of dATP, dCTP, dGTP, and dTTP; PCR buffer as above; 1 μmol/L of each primer; and 2.5 U Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT) overlaid with 30 μL light mineral oil.

After initial denaturation at 94°C for 3 minutes, each sample underwent 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. FR1-JH-PCR was performed in identical fashion except that an annealing temperature of 58°C was used.

**Vδ2-Dδ3-PCR.** FR3-JH-PCR reaction conditions were used with the addition of a final 10-minute extension step. For analysis of both reactions, 30-μL aliquots were resolved on an 8% nondenaturing polyacrylamide gel. Specific amplification products were excised from the gel, electrophoresed, and precipitated using Qwik-Precip (Advanced Genetics Technologies Corp, Gaithersburg, MD).

**Sequencing by Linear PCR**

Products were sequenced by a modification of the original PCR reaction, using only one primer, as described previously. Sequenced rearrangements were then analyzed into their component V, D, J, and N region sequences using the DNAsis software package (Pharmacia LKB, Uppsala, Sweden).

**Agarose Southern Blot Analysis**

All patients from the Hospital for Sick Children included in this study had previously been investigated as part of a study of MRD assessment by Southern blotting. In short, IgH analysis was performed by restriction digestion using BamHI/HindIII in combina-
STABILITY OF GENE REARRANGEMENTS IN ALL

<table>
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<tr>
<th></th>
<th>V SEQUENCE</th>
<th>N SEQUENCE</th>
<th>D SEQUENCE (021/0 or DEX1)</th>
<th>N SEQUENCE</th>
<th>J SEQUENCE</th>
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<td>GGCCAGCT</td>
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</table>

Fig 2. Clonal evolution at the IgH locus by V-V replacement is shown. Patient no. 7 was investigated by IgH-PCR using consensus FR3 and JH primers. BM samples were studied at presentation, at isolated CNS relapse (13 months from diagnosis), at BM harvest before allogeneic BM transplantation (at 19 months), and at second (BM) relapse (28 months). Sequence analysis (A) showed the rearrangement seen at second relapse to be a V-V replaced version of the presentation rearrangement. D-N-J sequence is fully preserved, although the V-N sequence is corrupted (common N sequence is underlined). Therefore, the tracking of residual disease is not affected if a D-N-J probe is used, as shown in (B) where a Southern blotted polyacrylamide gel is probed with a 32P-labeled oligonucleotide designed to presentation D-N-J sequence (position shown in bold). The additional band in the presentation lane probably represents a lower-level subclone, which resolves rapidly following chemotherapy. BM disease persists at the time of "isolated" relapse. This subclone is eradicated (at least to below the maximum sensitivity of this probe, 10⁻⁶) by BM transplantation, and relapse occurs with a new predominant subclone. No hybridization is seen to normal BM mononuclear cell DNA (N).

Polyacrylamide Southern Blot Analysis

One patient (no. 7), whose IgH rearrangement underwent V-V replacement between presentation and second relapse, was further investigated by Southern blotting of polyacrylamide-resolved PCR products (Fig 2). PCR products from amplification of BM mononuclear DNA at presentation and BM relapse were diluted 1 in 1,000. Thirty-microliter aliquots of these diluted products, together with equivalent volumes of undiluted product from patient BM mononuclear cell DNA at both central nervous system (CNS) relapse and harvest and from normal BM mononuclear cell DNA, were electrophoresed through 8% polyacrylamide. This gel was soaked for 5 minutes in 0.4% sodium hydroxide solution and then blotted by a standard Southern technique onto Hybond N⁺ nylon membranes (Amersham International) using the same solution. Oligonucleotide probes were synthesized (a 391 DNA Synthesizer [Applied Biosystems, Foster City, CA]) to presentation D-N-J sequence and end-labeled using 32P-γATP. Hybridization and washing were performed at 1°C below the calculated melting temperature of the probe, and autoradiography was performed for up to 4 hours.

RESULTS

Rearrangements Amplified at Presentation

IgH-PCR using the consensus FR3 primer successfully amplified rearrangements in 37 patients at presentation. In 2 others, bright single products were obtained with a consensus FR1 primer; sequence analysis of these showed deletion of bases from the 3' end of the anticipated FR3 priming site, a phenomenon that has been described previously. Therefore, 39 of 52 (75%) patients were open to investigation by IgH-PCR between presentation and first relapse.

Only 4 of 39 (10%) patients showed more than 2 rearrangements by IgH-PCR. Three rearrangements were amplified in 2 patients; 1 of whom was trisomic for chromosome 14 on cytogenetic analysis. Five rearrangements were amplified in the other 2 patients, but, in each case, there were only 2 unique sequences; all others were the product of
Fig 3A and B.

**A**

- **Size in base pairs**
  - 151
  - 140
  - 118
  - 100
  - 82
  - 66

- **Patient Number**
  - 2
  - 17
  - 43
  - 21

- **Months from Diagnosis**
  - 0
  - 29
  - 37
  - 45
  - 0
  - 34
  - 52
  - 0
  - 26

**B**

- **Size in base pairs**
  - 140
  - 118
  - 100

- **Patient Number**
  - 9
  - 48
  - 51

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secondary gene rearrangement events. V\&2-D\&3-PCR amplified rearrangements in 24 of 52 (46%) patients at presentation; no patient had more than 2 rearrangements amplified. Overall, 47 (90%) of the 52 leukemias studied at presentation showed distinct clonal electrophoretic bands by either IgH- or TCR \&6-PCR; 17 (33%) were amplified by both techniques.

### Stability of Rearrangements Between Presentation and First Relapse

**Stable rearrangements.** Identical rearrangements at presentation and relapse, in both number and sequence, were seen in 27 of 39 patients (69%) by IgH-PCR and in 18 of 24 (75%) by V\&2-D\&3-PCR. Examples of 4 patients who relapsed with identical rearrangements by IgH-PCR are shown in Fig 3A.

**Clonal evolution.** Of the 39 patients, 12 (31%) changed the number or sequence of amplified rearrangements between presentation and relapse by IgH-PCR, compared with 6 (25%) by V\&2-D\&3-PCR. However, 9 of the 12 who changed by IgH-PCR relapsed with related subclones and partially or completely retained rearrangements seen at presentation. The same was true for only 1 of the 6 who changed by V\&2-D\&3-PCR. Details of all patients showing clonal evolution between presentation and first relapse, including suggested mechanisms for the changes seen, are given in Table 1. Three examples are shown in Figs 3B and C. The etiology of band loss at relapse is unclear; in no case did cytogenetic analysis show chromosome 14 deletion.

Combining the results for both loci, there were only 3 patients (8%), nos. 6, 18, and 49, for whom no relationship existed between presentation and relapse findings. Patient no. 6 was an infant (previously reported in Potter et al\(^\text{20}\)) who presented and relapsed with bands of completely unrelated sequence by both IgH- and V\&2-D\&3-PCR, despite a presentation/relapse interval of only 10 months. Patient no. 18 became PCR-negative by both techniques at relapse. This patient had a normal karyotype at presentation and, although gaining a 5q \(-\) deletion, had no evidence of chromosome 14 loss at relapse. Patient no. 49 showed no relationship between the sequences of the amplified IgH rearrangements at the two events, and V\&2-D\&3-PCR was negative on both occasions. Cytogenetic analysis confirmed that a different clone was present at relapse.

Overall, 2 of 3 patients who showed complete change of IgH rearrangements from presentation to relapse were V\&2-D\&3-PCR-positive; in neither were these TCR\&6 rearrangements stable. All 5 patients with complete change at the V\&2-D\&3 locus were IgH-PCR-positive at presentation. In 3 of these, either partial (D-N-J) or complete IgH rearrangements were retained at relapse.

### Stability of Rearrangements From First to Second Relapse

All 14 patients studied between first and second relapse were positive on IgH-PCR. Of these patients, 12 (86%) relapsed with identical rearrangements in both number and sequence to those seen at first relapse; 1 lost one of two bands, and another (patient no. 7) had a V-V replaced version of the previous rearrangement. The latter patient suffered an isolated CNS relapse 13 months into treatment. Allogeneic BM transplantation was performed 6 months after reinduction, but a further relapse (BM) occurred at 28 months. A single PCR band was seen at diagnosis on polyacrylamide electrophoresis. CSF was not available from first

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### Table: Stability of Gene Rearrangements In ALL

<table>
<thead>
<tr>
<th>Patient</th>
<th>V Sequence</th>
<th>N Sequence</th>
<th>D Sequence</th>
<th>N Sequence</th>
<th>J Sequence</th>
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<td>AGAGAC</td>
<td>GGTTTAC</td>
<td>GAGTTTAC</td>
<td>TGTACC</td>
</tr>
</tbody>
</table>

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Fig 3. PCR analysis of IgH rearrangement at presentation and relapse is shown. (A) Of 39 patients, 27 (69%) presented and relapsed with identical IgH rearrangements. Four examples are shown at presentation (P), first relapse (R1), and in 2 cases also at second relapse (R2). SM, size marker. All bands were cut from the gels and sequenced to confirm their identical nature. The faint stepladder in the background of patient no. 43 (R2) is thought to be caused by amplification of productive rearrangements in normal lymphocytes, whose CDR III sequences vary in length by three base (ie, codon) intervals. (B) Of the 39 patients, 12 (31%) changed their pattern of amplification between presentation and first relapse; 9 presented and relapsed with related rearrangements. Three examples of this are shown. Patient no. 9 relapsed with a single band of identical size to the larger of two presentation bands. Sequence analysis (C) shows perfect presentation of D-N-J sequence between these two rearrangements, excepting the 5 terminal bases of the diversity region DM1. Both rearrangements may have developed independent V to DJ rearrangement in subclones of a precursor cell with a DJ rearrangement only. Patient no. 48 relapsed with a V-V replaced version of the presentation rearrangement, and the respective sequences are shown in (C). Common N sequence at the V-N-D junction is underlined.
relapse, but PCR study of BM mononuclear cell DNA at the
time of second relapse amplified a larger V-V replaced band.
To further investigate the time course of clonal evolution in
this patient, we performed patient-specific oligonucleotide
probing of Southern blotted PCR products from presenta-
tion and from both relapses (see Fig 2).
Of the 14 patients, 8 were V62-D63-PCR-positive at first
relapse; of these 8 patients, 7 had an identical band pattern
at relapse and 1 became negative.

Comparison of IgH Southern Blot and PCR Findings

Twelve of the patients studied by IgH-PCR as above had
previously been fully studied by Southern blotting using JH
probes.9 Agreement between the findings of the two types of
study was generally good. At presentation PCR amplified
identical numbers of bands to the number of nongermline
bands seen on Southern blot in 9 patients. Where there was
discrepancy, Southern blot showed the larger number of re-
arrangements; one more than PCR showed in 2 patients,
and two more in 1 patient.

 Differences in the number of rearranged bands seen on
Southern blot between presentation and relapse were ob-
served in 4 patients, of whom 3 gained an extra nongermline
band at relapse. In 1 of these patients, patient no. 7 as
described above, this may be explained by the emergence of a
V-V replaced version of the presentation sequence at relapse
as shown by PCR. In the other, 2, PCR analysis was identical
between presentation and relapse, and the identity of the
new bands is unclear. Another patient (no. 9) dropped from
to three to one nongermline band on Southern blot at relapse,
and, significantly, this was 1 of the patients who had two
bands at presentation but only one at relapse by PCR. The
only major discrepancy was 1 patient (no. 18) who was pos-
tive by both PCR techniques at presentation and became
PCR-negative at relapse but remained identical on Southern
blot.

DISCUSSION

PCR analysis of gene rearrangements has shown that
many cases of oligoclonality at the IgH locus and clonal ev-
olution at both IgH and TCRδ loci can be explained by sec-
ondary gene rearrangements occurring in subclones. Al-
though such changes most profoundly affect the IgH locus
in Southern blot studies, it is not clear at which locus PCR
studies of MRD will be most compromised. We addressed
this question by studying a large group of children by both
techniques at presentation and relapse.

At the IgH locus, three mechanisms of secondary re-
arrangement are described: V-V replacement,15,20,31 inden-
tent V-DJ rearrangement,13 and an open-and-shut
mechanism.21 In all 3 cases, the V-N-D sequence will differ
between subclones, but D-N-J sequence will be completely
preserved. Therefore, D-N-J sequence is the preferred site
when designing oligonucleotide probes for use in monitor-
ing MRD.20,32 This approach will avoid false-negative re-
relapse prediction when relapse occurs with a related sub-
clone. Furthermore, it will minimize the number of probes
required when multiple related rearrangements are present
in subclones.

Oligoclonal rearrangements have been reported in
15% to 45% of B-lineage ALL by Southern blot analysis,
yet we only observed these in 10% of patients in this
study and 16% in a previous one.28 There is no doubt that
direct visualization of products in the manner described
will miss low-level subclones detectable by cloning tech-
niques. Furthermore, incomplete DJ rearrangements will
be detected by Southern blotting but not amplified using a V
region primer. However, this does not appear to adversely
affect the potential for relapse prediction. Most patients relapse with identical or related re-
arrangements to their one or two predominant presenta-
tion rearrangements as amplified by PCR.

In this study 27 of 39 (69%) patients in whom IgH re-
arrangements were amplified at presentation relapsed with
identical rearrangements. Clonal evolution was seen in 12
patients (31%), a rate similar to that reported in most South-
ern blotting studies. Of these 12 patients, 9 relapsed with
subclones closely related to their presentation disease. They
showed varying combinations of loss of presentation re-
arrangements and new rearrangements derived from V-V
replacement or independent V-DJ rearrangement events
(see Table 1). However, in all 9 cases, the use of D-N-J oli-
gonucleotide probes designed to all unique presentation re-
arrangements would have allowed early detection of the
evolving subclones. Expanded to the whole study popula-
tion, this would have required the use of less than 1.5 probes
per patient who was IgH-positive at presentation (56 probes
for 39 patients).

It has been suggested that the instability of IgH re-
arrangements increases as a function of time. In favor of this
idea, Wasserman et al18 identified new rearrangements at
relapse in only 1 of 7 patients relapsing within 3 years from
diagnosis but in 4 of 5 relapsing beyond that point. Although
this could explain why we found less clonal evolution be-
 tween first and second relapse (average interval, 17 months)
than between presentation and first relapse (average in-
terval, 29.9 months), it is perhaps more likely that this re-
ffects inadequate suppression of disease after first relapse.

This study actually provides little support for such an hy-
pothesis. The mean interval from presentation to first re-
relapse for all patients showing clonal evolution at the IgH
locus was 29.7 months compared with 32.1 months for
those with completely stable rearrangements. Furthermore,
new rearrangements at first relapse were seen relatively early
at 10 months (an unrelated sequence) and 24, 24, 25, 26,
and 28 months (all V-V replaced sequences), respectively,
diagnosis. Therefore, we would advocate that the rela-
tive long-term stability of these rearrangements (coupled
with the potential of PCR to study minimal amounts ofslide
material) render them an attractive tool for differentiating
second leukemias from late relapses.

Secondary gene rearrangement at the TCRδ locus is
thought to take the form of further recombination into Ja,
as the first step towards deletion of the δ locus (which
lies nested within the TCRα locus) during TCRα re-
arrangement.25 This will invariably delete the site of the 3'
primer used to amplify V62-D63 rearrangements and, thus,
result in false-negative MRD detection. It may account for
loss of PCR bands at relapse in 4 of 23 patients (17%) V62-
Table 1. Patients Showing Clonal Evolution Between Presentation and First Relapse

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Immunophenotype</th>
<th>Presentation/Relapse Interval (mos)</th>
<th>Band Numbers</th>
<th>Mechanism of Change From Presentation to Relapse</th>
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</tbody>
</table>

D63-PCR-positive at presentation in this series. All presentation bands disappeared in 3 of these 4 cases. The exception (patient no. 24) had two rearrangements at presentation but dropped to one at relapse, implying that it may well prove advisable to track all presentation rearrangements at this locus also. This would require only an extra 0.25 probes per patient (30 probes for 24 patients in this study).

Of 47 patients positive by either IgH- or TCRδ-PCR, we found only 3 (6%) who changed their pattern of amplification in a manner suggestive of relapse with entirely unrelated clones. In 2 patients (nos. 6 and 18), complete change occurred at both loci. The third patient (no. 49) only amplified successfully by IgH-PCR. This rate of complete clonal change is in keeping with the findings of large cytogenetic studies of ALL. These have variously reported relapse with a definitively new clone in only 1 of 116, 2 of 51, and 9 of 98 fully karyotyped cases, ie, all in under 10% of patients.

Our analysis suggests that IgH- and TCRδ-PCR have a complementary role. At the TCRδ locus, 5 of 23 patients (22%) TCRδ-positive at presentation lost all presentation sequence at relapse. However, false-negative relapse prediction could have been avoided in 3 of these cases by concurrent use of IgH probes. In addition, TCRδ examination allowed assessment of an additional 15% of patients who were PCR-negative by the simple IgH-PCR systems described.

In conclusion this study has shown that, contrary to the impression gained from Southern blotting studies, IgH rearrangements provide more reliable markers for relapse prediction in B-lineage ALL than those involving Vb2-D63. However, these techniques should be seen as complementary (together with those of other loci, eg, TCRγ) rather than as mutually exclusive. Using a combination of IgH- and Vb2-D63-PCR, MRD assessment is possible in 90% of B-lineage ALL using an average of 1.8 oligonucleotide probes per patient positive by either or both systems (86 probes for 47 patients). Approximately 30% of patients will amplify by both systems, allowing direct comparison of the sensitivity of IgH- and TCRδ-PCR. Most importantly, the rate of false-negative relapse prediction as a consequence of clonal evolution will be less than 10%.

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REFERENCES

2. van Dongen JJM, Breit TM, Adriaansen HJ, Beishuizen A, Hooijkaas H: Detection of minimal residual disease in acute lymphoblastic leukemia by immunoglobulin marker analysis and polymerase chain reaction. Leukemia 6:47, 1992 (suppl 1)
33. Williams DL, Raimondi SC, Rovera G: Most cases of acute lymphoblastic leukemia (ALL) undergo marked karyotypic shift from diagnosis to relapse. Blood 64:208a, 1984 (suppl 1)
34. Abshire TC, Buchanan GR, Jackson JJ, Shuster JJ, Brock B, Head D, Behm F, Crist WM, Link M, Borowitz M, Pullen DJ: Morphologic, immunologic and cytogenetic studies in children with acute lymphoblastic leukemia at diagnosis and relapse: A Paediatric Oncology Group Study. Leukemia 6:357, 1992
A polymerase chain reaction study of the stability of Ig heavy-chain and T-cell receptor delta gene rearrangements between presentation and relapse of childhood B-lineage acute lymphoblastic leukemia

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