**Featured Article**

**TEL Deletion Analysis Supports a Novel View of Relapse in Childhood Acute Lymphoblastic Leukemia**

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**ABSTRACT**

**Purpose:** TEL (ETV6)-AML1 (RUNXI) chimeric gene fusions are frequent genetic abnormalities in childhood acute lymphoblastic leukemia (ALL). They often arise prenatally as early events or initiating events and are complemented by secondary postnatal genetic events of which deletion of the non-rearranged, second TEL allele is the most common. This consistent sequence of molecular pathogenesis facilitates an analysis of the clonal origins of relapse in this leukemia, which has some unusual clinical features.

**Experimental Design:** We compared the boundaries, by microsatellite mapping, of TEL deletions at relapse versus diagnosis in 15 informative patients. Moreover, we compared the relatedness of diagnostic and relapse clones using immunoglobulin and T-cell receptor genes rearrangements and clonotypic TEL-AML1 genomic fusion.

**Results:** Five patients retained the apparent same size TEL deletion, seven had larger deletions, and three had smaller deletions at relapse. In all of the cases evaluated, the clonal relatedness of diagnostic and relapse cells was confirmed by the retention of clonotypic TEL-AML1 genomic sequence and/or at least one identical immunoreceptor gene rearrangement.

**Conclusions:** These data provide further evidence that TEL deletions are secondary to TEL-AML1 fusions in ALL. They are compatible with the novel idea that in at least some cases of childhood ALL, remission occurs with persistence of a preleukemic “fetal” clone, and subsequent relapse reflects the emergence of a new subclone from this reservoir after an independent “second hit,” i.e., independent TEL deletion. To our knowledge, the study is the most extensive and comprehensive analysis of the relationship between diagnostic and relapse clones in childhood ALL presented thus far.

**INTRODUCTION**

TEL-AML1 (ETV6-RUNXI) fusion, via the t(12;21)(p13;q22) chromosomal translocation is a common genetic abnormality in childhood B-cell precursor acute lymphoblastic leukemia (ALL) present in ~20 to 25% of cases (1–5). Studies on identical twins with concordant ALL (6–8) and retrospective scrutiny of neonatal blood spots (Guthrie cards; refs. 8–11) have provided evidence that TEL-AML1 often arises prenatally, possibly as a first or initiating event. A corollary of these data are that a “preleukemic” clone with TEL-AML1 can persist postnatally for extended periods, up to ~14 years (7) and that at least one other postnatal genetic event is required for overt leukemia. The majority of cases of TEL-AML1-positive ALL have deletion of the nontranslocated TEL allele at diagnosis (12–14). TEL deletions vary in size from 10 kb to >10 megabases as judged by microsatellite loss of heterozygosity (LOH) but the minimally deleted regions always affect at least some part of the TEL transcription framework (15, 16). Studies on both singletons (12, 14) and twins (8) with ALL indicate that such TEL deletions are subclonal or secondary to TEL-AML1 and almost certainly postnatal. Candidate preleukemic clones in normal cord blood with TEL-AML1 fusions retain the normal TEL allele (17). Whether other genetic changes in addition to TEL deletion, e.g., kinase mutations (18), are necessary for the clinical development of ALL remains to be established.

These data throw light on the natural history of childhood ALL (19), but they also have clinical implications. One particular issue is whether the putative preleukemic clone with TEL-AML1 fusion but no TEL deletion responds to therapy as does the dominant clone of overt leukemic blasts at diagnosis. One possibility is that they might persist in some patients and, in so doing, provide a reservoir of “at risk” cells for later independent second hits, i.e., TEL deletion and/or other events that would precipitate a de novo ALL masquerading as relapse. The plausibility of this unconventional interpretation of “late” relapse in ALL is supported by the clinical responsiveness of many cases of late or off-treatment ALL relapses (20). In a small pilot study of this issue, we reported that in two patients with off-treatment...
Recurrence of \( g_{\text{db.org}} \). Each PCR reaction contained 8 ng of genomic DNA.

ers are available at The Genome Database (http://gdbwww.gdb.org/). All PCR reactions were performed with nested primers and PCR conditions were published elsewhere (23, 24). To reliably distinguish PCR products from patient DNA, we designed primers for each PCR reaction that yielded a product of 130–300 base-pairs. The presence of a PCR product from a patient DNA sample was then confirmed by sequencing the PCR product.

We analyzed bone marrow samples of 17 patients with \( T{\text{-}}L{\text{-AML1}} \)-positive ALL for the presence of \( T{\text{-}}L{\text{-AML1}} \) gene rearrangements using a screening PCR protocol. TEL-AML1 fusion sequences were amplified by PCR using patient DNA as template. PCR products were then purified and sequenced. The presence of a PCR product from a patient DNA sample was then confirmed by sequencing the PCR product.

**Immunoglobulin/TCR Genes Rearrangements.** As a test of clonality, the analysis of immunoglobulin genes rearrangements was performed in 16 patients (UPN 1-16). The data from cases 7 to 13 and 15 to 16 have been published elsewhere. In immunoglobulin rearrangements, the analysis of immunoglobulin genes rearrangements was performed in 16 patients (UPN 1-16). The data from cases 7 to 13 and 15 to 16 have been published elsewhere.

**RESULTS**

**Deletions of the Second TEL Allele.** We analyzed bone marrow samples of 17 patients with \( T{\text{-}}L{\text{-AML1}} \)-positive ALL. Immunoglobulin/TCR Genes Rearrangements. As a test of clonality, the analysis of immunoglobulin genes rearrangements was performed in 16 patients (UPN 1-16). The data from cases 7 to 13 and 15 to 16 have been published elsewhere.

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(27). All of the clonal rearrangements detected and sequenced in relapse samples of patients UPN 1, 3, 6, 7, 9, 10, 12, and 13 were also shown to be present as major rearrangements in the diagnostic samples. Correspondingly, patient UPN 14 had three clonal rearrangements detected at second relapse, all of which were also present in the first-relapse sample. One additional rearrangement in the first-relapse sample was detected in this patient. Patient UPN 5 had two rearrangements maintained between presentation and diagnosis, and another two rearrangements were changed but, nonetheless, seem related between the two time points (one IgH rearrangement with identical (N)-D-J sequence and different V\textsubscript{H} segment and another IgH rearrangement with identical D-J joining and V\textsubscript{H} segment but different V-(N)-D fusion region). Patients UPN 2, 4, 8, 11, and 16 had at least one “new” clonal rearrangement at relapse compared with the screening performed on the presentation sample, but other rearrangements were preserved between presentation and relapse. Patient UPN 15 showed a very different rearrangement pattern between diagnosis and relapse, with four rearrangements detected at presentation and two different rearrangements at relapse. However, in this patient both of the new rearrangements found at relapse were successfully amplified from the presentation DNA using patient-specific primers at level 5 \times 10^{-4} (detailed analysis of immunoglobulin/TCR rearrangements of this patient was published previously; ref. 28).

**TEL-AML1 Intronic Fusion.** In five relapse samples (UPN 3, 4, 15, 18, and 19) we identified the patient-specific TEL-AML1 genomic fusion sequence. In all of these five patients, we amplified an identical PCR product from the presentation material as confirmed by sequencing analysis. The intensity of the diagnostic product on an agarose gel was comparable with that observed in the relapse sample in all cases. These data

### Table 1: Clinical characteristics and TEL status of analysed patients

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age at presentation (yrs)</th>
<th>1st (2nd) remission duration (mo)</th>
<th>TEL deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>38</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>3.3</td>
<td>25</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>46</td>
<td>U</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>31</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>1.9</td>
<td>24</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
<td>36</td>
<td>U</td>
</tr>
<tr>
<td>7</td>
<td>9.5</td>
<td>55</td>
<td>L</td>
</tr>
<tr>
<td>8</td>
<td>3.2</td>
<td>25</td>
<td>L</td>
</tr>
<tr>
<td>9</td>
<td>4.7</td>
<td>52</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>11.8</td>
<td>24</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>3.1</td>
<td>50</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>3.4</td>
<td>32</td>
<td>U</td>
</tr>
<tr>
<td>13</td>
<td>4.9</td>
<td>16</td>
<td>L</td>
</tr>
<tr>
<td>14*</td>
<td>3.4</td>
<td>NA (35)</td>
<td>S</td>
</tr>
<tr>
<td>15</td>
<td>4.3</td>
<td>48</td>
<td>L</td>
</tr>
<tr>
<td>16</td>
<td>6.1</td>
<td>34</td>
<td>L</td>
</tr>
<tr>
<td>17</td>
<td>4.9</td>
<td>120</td>
<td>U</td>
</tr>
<tr>
<td>18</td>
<td>4.6</td>
<td>76</td>
<td>U</td>
</tr>
<tr>
<td>19*</td>
<td>6.7</td>
<td>NA (66)</td>
<td>S</td>
</tr>
</tbody>
</table>

Abbreviations: L, larger deletion at relapse; U, unchanged; S, smaller deletion at relapse; N, noninformative; NA, not applicable.

* Patients 14 and 19 were analyzed only at first and second relapse.

![Fig. 1](image-url) Microsatellite analysis results. Illustrative examples of patients from each TEL deletion group are labeled as follows: L, larger deletion at relapse; U, unchanged; S, smaller deletion at relapse; N, noninformative; \(\bullet\) heterozygous; \(\circ\) deleted (LOH); \(\Box\) noninformative. (PRES, presentation; REM, remission; REL, relapse; SCDR, shortest commonly deleted region; cent, centromere.)
Recurrence of \textit{TEL-AML1}\textsuperscript{+} ALL: Relapse or a New Disease?

Discerning clonal relatedness in relapsed \textit{versus} diagnostic \textit{TEL-AML1} ALL was designed to confirm that the same or related clones are involved and to assess the consistency with which \textit{TEL} deletions alter in relapse compared with diagnosis (21). We previously argued that the appearance of smaller \textit{TEL} deletions in late relapses (\textit{i.e.}, off treatment) of \textit{TEL-AML1} might reflect a new ALL generated from a persistent preleukemic clone rather than a conventional relapse (21). One attraction of this idea was that it might rationalize the otherwise surprising clinical observation that late relapses of \textit{B}-cell precursor/common ALL are often as therapeutically sensitive as \textit{TEL} deletions, as common but secondary events, should be different in their genomic boundaries at diagnosis and relapse. In practice, the only result that is unequivocal in this respect is relapse with a \textit{smaller} \textit{TEL} deletion (three cases described here). Relapse cases with a larger deletion would also be anticipated to occur, and the seven cases described here are, therefore, compatible with this view; however, we cannot exclude the possibility that larger deletions arise by attrition of boundaries of prior smaller deletions. Cases with identical \textit{TEL} deletions at diagnosis and relapse may contradict the hypothesis and one third (5 of 15) of our informative patients had this feature. This could indicate that the dominant clone at diagnosis reappeared in relapse. A caveat here is, however, that the genomic boundaries are defined only with respect to the available microsatellite markers for which the patient is heterozygous. Boundary differences of a modest size (\textit{i.e.}, <0.5 cM) would be undetected. In a recent study, Takeuchi \textit{et al.} (29) found that LOH at loci other than 12p/TEL were also divergent between diagnosis and relapse of childhood ALL, and, in some cases, a deletion present at diagnosis was not evident in relapse cells. The authors suggested that this might indicate the emergence of relapse from a small subpopulation of cells present at diagnosis.

The conclusion from these studies is therefore that late relapse in childhood ALL with \textit{TEL-AML1} does represent, as anticipated, emergence of a related subclone, but in some cases, it is a different dominant clone from that seen at initial diagnosis. Clearly, this result indicates persistence of a subclone in complete remission possibly at levels undetectable in PCR-based minimal residual disease screening. One possibility is that the “different” subclone in relapse is present as a very minor subclone at diagnosis, evades elimination by therapy, and reemerges over time, particularly after cessation of therapy. Evidence for this is now available from immunoglobulin/TCR analysis, which indicates that dominant \textit{TEL-AML1}-positive clones in relapse may be present at low levels (10\textsuperscript{-8} to 5 \times 10^{-3} total) at diagnosis and are poorly or only slowly responsive to induction chemotherapy (27, 28). This is compatible with the view that these cells represent the reservoir of relatively drug-resistant preleukemic cells (28). We assume that these cells may remain in a dormant state during hematologic remission, may be gradually lost, or, if suffering a second hit (\textit{i.e.}, \textit{TEL} deletion or other), will amplify as an overt ALL cell population in later “relapse” (see Fig. 2). This interpretation is difficult to prove unequivocally but accords best with the clinical observations and parallels similar views on the persistence of \textit{AML1-ETO}-positive preleukemic clones in remission of acute myeloid leukemia (30–32). The results of the study are influential for the understanding of pathogenesis and natural history of childhood ALL (33). If correct, they change our understanding of the biology of remission and relapse and may have possible implications for the detection of minimal residual disease. Further-

\begin{table}
\centering
\caption{Immunoreceptor gene rearrangements of patients UPN 1 to 6 and 14 at diagnosis and relapse}
\begin{tabular}{lll}
\hline
UPN & Diagnosis & Relapse \\
\hline
Vkl/Kde & Vkl/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg9/Jg1.2 & Vg9/Jg1.2 \\
2 & VH2/JH & VH2/JH \\
Vkl/Kde & Vkl/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
Vg5/Jg1.3–2.3 & Vg5/Jg1.3–2.3 \\
3 & VH4/JH & VH5/JH \\
Vkl/Kde & Vkl/Kde \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
Vg5/Jg1.3–2.3 & Vg5/Jg1.3–2.3 \\
4 & Vkl/Kde & Vkl/Kde \\
RSS/Kde & RSS/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
5 & VH3–33/DH3–10/JH6* & VH3–38/DH3–10/JH6* \\
VH4–31/DH3–10/JH4 & VH5–51/DH3–10/JH6* \\
Vkl/Kde & Vkl/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
Vg5/Jg1.3–2.3 & Vg5/Jg1.3–2.3 \\
6 & VH3–13/DH3–19/JH4 & VH3–13/DH3–19/JH4 \\
Vkl/Kde & Vkl/Kde \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
Vg5/Jg1.3–2.3 & Vg5/Jg1.3–2.3 \\
7 & Vkl/Kde & Vkl/Kde \\
RSS/Kde & RSS/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
14 & Vkl/Kde & Vkl/Kde \\
RSS/Kde & RSS/Kde \\
Vd2/Dd3 & Vd2/Dd3 \\
Vg3/Jg1.3–2.3 & Vg3/Jg1.3–2.3 \\
\hline
\end{tabular}
\end{table}

Abbreviations: V, variable; D, diversity; J, joining regions.
* Four IgH rearrangements with homologous DH-JH sequences.

indicate that the relapse had arisen from the same preleukemic clone present at diagnosis.

\textbf{DISCUSSION}

This molecular scrutiny of clonal relatedness in relapsed \textbf{versus} diagnostic \textit{TEL-AML1} ALL was designed to confirm that the same or related clones are involved and to assess the consistency with which \textit{TEL} deletions alter in relapse compared with diagnosis (21). We previously argued that the appearance of smaller \textit{TEL} deletions in late relapses (\textit{i.e.}, off treatment) of \textit{TEL-AML1} might reflect a new ALL generated from a persistent preleukemic clone rather than a conventional relapse (21). One attraction of this idea was that it might rationalize the otherwise surprising clinical observation that late relapses of \textit{B}-cell precursor/common ALL are often as therapeutically sensitive as \textit{TEL} deletions, as common but secondary events, should be different in their genomic boundaries at diagnosis and relapse. In practice, the only result that is unequivocal in this respect is relapse with a \textit{smaller} \textit{TEL} deletion (three cases described here). Relapse cases with a larger deletion would also be anticipated to occur, and the seven cases described here are, therefore, compatible with this view; however, we cannot exclude the possibility that larger deletions arise by attrition of boundaries of prior smaller deletions. Cases with identical \textit{TEL} deletions at diagnosis and relapse may contradict the hypothesis and one third (5 of 15) of our informative patients had this feature. This could indicate that the dominant clone at diagnosis reappeared in relapse. A caveat here is, however, that the genomic boundaries are defined only with respect to the available microsatellite markers for which the patient is heterozygous. Boundary differences of a modest size (\textit{i.e.}, <0.5 cM) would be undetected. In a recent study, Takeuchi \textit{et al.} (29) found that LOH at loci other than 12p/TEL were also divergent between diagnosis and relapse of childhood ALL, and, in some cases, a deletion present at diagnosis was not evident in relapse cells. The authors suggested that this might indicate the emergence of relapse from a small subpopulation of cells present at diagnosis.

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more, the proposed interpretation might influence the treatment approach to the TEL-AML1-positive relapses.

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