Immunogenotype Changes Prevail in Relapses of Young Children with TEL-AML1-Positive Acute Lymphoblastic Leukemia and Derive Mainly from Clonal Selection

E. Renate Panzer-Grümayer,1,2 Giovanni Cazzaniga,3 Vincent H.J. van der Velden,4 Laura del Giudice,5 Martina Peham,1,2 Georg Mann,2 Conny Eckert,6 Andre Schrauder,7 Giuseppe Germano,5 Jochen Harbott,8 Giuseppe Basso,5 Andrea Biondi,3 Jacques J.M. van Dongen,4 Helmut Gadner,1,2 and Oskar A. Haas1,2

Abstract

Purpose: Variations of the immunogenotype and TEL deletions in children with TEL-AML1 + acute lymphoblastic leukemia support the hypothesis that relapses derive from a persistent TEL-AML1 + preleukemic/leukemic clone rather than a resistant leukemia. We aimed at elucidating the relationship between the immunogenotype patterns at diagnosis and relapse as well as their clinical and biological relevance.

Patients and Methods: Immunoglobulin and T-cell receptor gene rearrangements were analyzed in 41 children with a TEL-AML1 + acute lymphoblastic leukemia and an early (up to 30 months after diagnosis; n = 12) or late (at 30 months or later; n = 29) disease recurrence by a standardized PCR approach.

Results: In 68% of the patients (group I), we identified differences in the immunogenotype patterns, whereas no changes were observed in the remaining 32% (group II). The divergence resulted more often from clonal selection than clonal evolution and consisted predominantly of losses (0-6, median 5) and/or gains (0-4, median 1) of rearrangements. The frequency and number of clonal immunoglobulin/T-cell receptor rearrangements in group I was higher at diagnosis (2-13, median 5) than at relapse (2-7, median 4), whereas it was the lowest in group II (1-5, median 3). Although group I children were younger at diagnosis, there was no correlation between particular immunogenotype patterns and remission duration.

Conclusion: These findings imply that the clonal heterogeneity in younger children most likely reflects an ongoing high recombinatorial activity in the preleukemic/leukemic cells, whereas the more uniform repertoire observed in older children mirrors end-stage rearrangement patterns of selected cell clones that evolved during the prolonged latency period.

With a frequency of ~25%, the translocation t(12;21) (p13;q22) and its molecular genetic counterpart, the TEL-AML1 gene fusion, is the most common specific genetic rearrangement in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The initial view that this rearrangement concurs with an extraordinarily favorable prognosis already during fetal development but it is considered insufficient to cause clinically overt leukemia by itself (2). Further events are required but seem to be rare because the incidence of therapy studies the incidence of TEL-AML1 + cases at diagnosis and relapse was found to be similar (reviewed in ref. 1).

The TEL-AML1 gene fusion is an early, or perhaps even the first, event in leukemia development. It commonly occurs already during fetal development but it is considered insufficient to cause clinically overt leukemia by itself (2). Further events are required but seem to be rare because the incidence of

Authors’ Affiliations: 1Children’s Cancer Research Institute; 2St. Anna Kinderspital, Vienna, Austria; 3M. Tettamanti Research Center, Pediatric Clinic, San Gerardo Hospital, University of Milan Bicocca, Monza, Italy; 4Department of Immunology, Erasmus MC Rotterdam, Rotterdam, the Netherlands; 5Department of Pediatrics, University of Padova, Padua, Italy; 6Department of Pediatrics, Charité Medical Center, Berlin, Germany; 7Department of Pediatrics, Hannover Medical School, Hannover, Germany; and 8Department of Pediatrics, Justus Liebig University, Giessen, Germany.

Gadner,1,2 and Oskar A. Haas1,2

Immunogenotype Changes Prevail in Relapses of Young Children with TEL-AML1-Positive Acute Lymphoblastic Leukemia and Derive Mainly from Clonal Selection

E. Renate Panzer-Grümayer,1,2 Giovanni Cazzaniga,3 Vincent H.J. van der Velden,4 Laura del Giudice,5 Martina Peham,1,2 Georg Mann,2 Conny Eckert,6 Andre Schrauder,7 Giuseppe Germano,5 Jochen Harbott,8 Giuseppe Basso,5 Andrea Biondi,3 Jacques J.M. van Dongen,4 Helmut Gadner,1,2

Received 6/8/05; revised 7/20/05; accepted 8/9/05.

Note: Presented in part at the 46th Annual Meeting of the American Society of Hematology, 2004, San Diego, California. This collaboration was done using the network of the Biology Group within the International Berlin-Frankfurt-Münster Group.

Requests for reprints: E. Renate Panzer-Grümayer, Children’s Cancer Research Institute, Kinderspitalgasse 6, 1090 Vienna, Austria. Phone: 43-1-40170431; Fax: 43-1-4087230; E-mail: renate.panzer@ccri.at.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Clin Cancer Res 2005;11(21) November 1, 2005 7720 www.aacrjournals.org

SNWK 2000-2268 (J.M.M. van Dongen); and Fondazione Città della Speranza, Consiglio Nazionale delle Ricerche, Ministero dell’Università a Ricerca Scientifica e Tecnologica, Associazione Italiana per la Ricerca sul Cancro (G. Basso).

Requests for reprints: E. Renate Panzer-Grümayer, Children’s Cancer Research Institute, Kinderspitalgasse 6, 1090 Vienna, Austria. Phone: 43-1-40170431; Fax: 43-1-4087230; E-mail: renate.panzer@ccri.at.

© 2005 American Association for Cancer Research.
healthy newborns in whom TEL-AML1+ cells can be detected is ~100 times that of the respective leukemias (3). The latency period is variable and has been reported to range up to 10 years (4). Relapses occur predominantly late and are again responsive to chemotherapy (1). These data together with the observation that deletions of the nonrearranged TEL allele, one of the proposed essential secondary events, may differ between diagnosis and relapse, lead to the hypothesis that at least part of the relapses do not derive from the dominant leukemic clone at diagnosis but rather from a potentially therapy-resistant dormant “preleukemic” clone that is still characterized by a TEL-AML1 fusion gene (5). This notion is supported by several recent studies that focus on the analysis of TEL deletions in such cases (6–8).

Immunoglobulin (IG) and T-cell receptor (TCR) gene rearrangements occur during normal lymphoid development and are widely used as clone-specific markers for clonally expanded lymphoid cells (9). Although these rearrangements are not involved in the leukemogenic process, they nevertheless may provide essential information about the affected cell type and the time of their manifestation during the fetal or postnatal life (10, 11). Specific patterns and types of rearrangements have been attributed to particular, genetically defined leukemia subtypes (12). In line with this observation, we also reported distinct age-related changes of the IG/TCR rearrangements in TEL-AML1+ cases (13). Furthermore, clonotypic IG/TCR rearrangements are also used as specific markers for the surveillance of minimal residual disease. This method has recently been implemented in clinical trials for treatment stratification of childhood leukemia (14–17).

Earlier studies of BCP-ALL (18, 19) as well as an initial study of three relapsed TEL-AML1+ cases suggested that the remission duration could be one of the crucial factors that influence the likelihood that clonal patterns change between diagnosis and relapse (5). However, this notion could not be substantiated in a follow-up study with a larger number of TEL-AML1+ cases (6). Moreover, this latter study also revealed that the differences of the immunogenotype patterns at diagnosis and relapse did not concord with the respective TEL deletion patterns in 6 of 12 cases. To explore these issues further, we determined the frequency of IG/TCR gene rearrangements in a large number of TEL-AML1+ cases at diagnosis and relapse and correlated the respective patterns with clinical variables to gain some insight into their potential clinical or biological relevance.

## Results

The 41 relapses occurred between 6 and 60 months (median 34) after initial diagnosis. The age at diagnosis of the respective children was 3.4 years (median, range 1.7–13.4). Twelve of them relapsed before 30 months after diagnosis (early relapse; range 6–29, median 19) and 29 of them 30 months or later after diagnosis (late relapse; range 30–60, median 41).

### Comparison of immunoglobulin/T-cell receptor rearrangement patterns between diagnosis and relapse

Depending on the altered or conserved relapse rearrangement pattern, we defined two groups: group I is composed of 26 cases with a complete or partial change and group II is composed of 13 cases with identical rearrangements (Fig. 1). Thus, the immunogenotype had changed in approximately two thirds of the patients (68%).

### Characteristics of the immunoglobulin/T-cell receptor rearrangements

In group I, the median number of rearrangements per case was 5 (range 2–13) at diagnosis and 4 (range 2–7) at relapse, whereas it was only 3 (range 1–5) in group II. This difference between the two groups at diagnosis is significant (Wilcoxon two-sample test, P = 0.003). Changes at relapse comprised losses (median 5, range 0–6) and/or gains (median 1, range 0–4) of rearrangements. Both gains and losses of rearrangements occurred in 18 cases, only losses in six and only gains in four. The affected genes, their specific types of rearrangements together with their distribution in diagnostic and relapse samples as well as the respective combinations are summarized in Fig. 1 and Table 1.
Immunoglobulin heavy chain locus. IGH rearrangements at diagnosis and/or relapse occurred in altogether 26 of 28 cases in group I and 9 of 13 cases in group II. Patients in group II had only one rearrangement per case, whereas those in group I had up to four IGH rearrangements at diagnosis. At least one IGH rearrangement was conserved at relapse in 20 of 26 cases of group I, but the majority of cases (n = 17) had additional changes that comprised losses (n = 15) and/or gains (n = 9; Fig. 1). Losses of rearrangements may result generally either from clonal selection or evolution. In this cohort of patients, selection was apparent mainly in cases with oligoclonal rearrangements (n = 8), whereas in the remaining cases we could not differentiate between evolution (deletion of IGH rearrangements due to ongoing recombination) and selection processes (n = 4). The distinction between deletion and selection of rearrangements was not possible with our technical approach because it solely detects PCR amplifiable gene configurations but neither deletions nor the germ line configuration. Nevertheless, we have already shown previously that, probably due to the high recombination rate, deletions at the IGH locus are frequent in TEL-AML1 + BCP-ALL (13). Gained rearrangements were either related ones due to VH replacements (n = 4) or they were completely unrelated (n = 4).

Eight leukemias had oligoclonal IGH rearrangements at diagnosis (Table 2). The two or even three related clones in seven of them had evolved exclusively from VH replacements (n = 4) or they were completely unrelated (n = 4).

<table>
<thead>
<tr>
<th>Immunogenotype patterns</th>
<th>IGH</th>
<th>IGK</th>
<th>TCRD</th>
<th>TCRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changed (group I, n = 28)</td>
<td>27/20</td>
<td>21/16</td>
<td>12/12</td>
<td>19/16</td>
</tr>
<tr>
<td>Conserved rearrangements</td>
<td>20/15</td>
<td>7/5</td>
<td>20/14</td>
<td>15/13</td>
</tr>
<tr>
<td>Only at diagnosis</td>
<td>9/9</td>
<td>6/5</td>
<td>2/2</td>
<td>21/16</td>
</tr>
<tr>
<td>Only at relapse</td>
<td>9/9</td>
<td>13/10</td>
<td>6/5</td>
<td>14/10</td>
</tr>
<tr>
<td>Conserved (group II, n = 13)</td>
<td>64</td>
<td>83</td>
<td>47</td>
<td>68</td>
</tr>
<tr>
<td>Conserved rearrangements (%)</td>
<td>64</td>
<td>83</td>
<td>47</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 2. Incidence of oligoclonal IG/TCR rearrangements at diagnosis and relapse

<table>
<thead>
<tr>
<th>Immunogenotype patterns</th>
<th>IG/TCR locus*</th>
<th>Cases†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGH</td>
<td>IGK</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Relapse/relapse in percent</td>
<td>20/7</td>
<td>5/5</td>
</tr>
</tbody>
</table>

*Numbers indicate cases that are oligoclonal at the respective immunoreceptor locus.
†Total number of cases with one or more oligoclonal gene loci.
§One case was oligoclonal at three loci; three of these cases had oligoclonal rearrangements also at relapse.
¶One of these leukemias was oligoclonal at two loci.
||Percentage of all leukemias.

supports the notion that the relapse clone emerged from an oligoclonal population. Only one case (patient 23) was also oligoclonal at relapse. A new IGH rearrangement had developed, which was related to the persistent IGH rearrangement. These data show that subclones with related IGH rearrangements due to V<sub>11</sub> replacement are not only frequently detected at diagnosis but also, albeit to a lower extent, at relapse (Table 1).

Of particular interest in the context of minimal residual disease screening is our observation regarding one patient (patient 19) whose prominent relapse rearrangement had not been detected with screening PCRs, but was identified with a DN<sub>11</sub> clone-specific primer that was used for minimal residual disease analysis, which indicates that a V<sub>11</sub> replacement had occurred.

**Immunoglobulin light chain κ-locus.** Twenty cases in group I had an IG<sub>K</sub>-κ<sub>-locus</sub> rearrangement either at initial diagnosis and/or at relapse compared with 10 cases in group II (Fig. 1). Up to five rearrangements per leukemia were identified at either occasion in group I, whereas in group II only one and two rearrangements were present in seven and three patients, respectively. In group I, 16 of the 18 cases with an IG<sub>K</sub>-κ<sub>-locus</sub> rearrangement at diagnosis had at least one rearrangement conserved at relapse, including five patients with additional losses and/or gains of rearrangements (Fig. 1). In two cases (patients 11 and 21) oligoclonal populations with three and five IG<sub>K</sub>-κ<sub>-locus</sub> rearrangements were present at diagnosis. The latter patient retained three of the five rearrangements at relapse, whereas another patient (patient 18) kept one IG<sub>K</sub> rearrangement, but also gained two additional ones (Fig. 1; Table 2).

**T-cell receptor δ-locus.** Twenty patients from group I had at least one TCRD rearrangement at diagnosis and/or at relapse, whereas only five patients in group II had one (n = 4) or two (n = 1) rearrangements (Fig. 1). In group I, only one rearrangement per leukemia was preserved at relapse (n = 12). No further changes took place in five of them; however, in the others, losses (n = 6) predominated over losses and gains (n = 1). Eight patients did not have a conserved rearrangement at relapse. Table 1 and Fig. 1 provide detailed information about the respective changes. The original oligoclonality with three and four rearrangements in two patients was lost at relapse (Table 2). The ongoing rearrangements from TCRD to one of the J segments of the TCR<sub>D</sub> locus were not analyzed systematically but data from individual cases indicate that, as expected, heterogeneity of these leukemias increases further, so that even some clonally appearing cases eventually became oligoclonal (patients 29 and 31; Fig. 1).

**T-cell receptor γ-locus.** With 27 cases in group I and 10 in group II, the overall frequency of TCRG rearrangements at diagnosis and/or relapse was high in both groups (Fig. 1). Ten of the 26 patients with a TCRG rearrangement at diagnosis in group I did not have a stable rearrangement because of isolated losses of rearrangements (n = 1) or, more frequently, of losses combined with gains (n = 9). Eight of the 16 patients with at least one stable rearrangement had additional losses or gains of rearrangements.

Because TCRG rearrangements are end-stage recombinations, a loss of rearrangements at this locus indicates that another subclone must have evolved to constitute the relapse clone. The deletion of a preexisting rearrangement by upstream V segments and downstream J segments, however, cannot be formally excluded in this study because the sequences were too short to distinguish between J1.1 and J2.1 or J1.3 and J2.3. It seems unlikely, however, that V-J replacements of rearrangements are common because the relapse V segments were more downstream than the one in the initial clone-specific rearrangement. Only one leukemia at diagnosis and two at relapse appeared oligoclonal. In two additional cases, a third very weak rearrangement was present at diagnosis but did not evolve to a relapse clone.

**Oligoclonal immunogenotype pattern.** At diagnosis, 11 cases (27%, 95% confidence interval 14-42%) appeared oligoclonal in up to three immunoreceptor loci. The highest frequency was found in the IG<sub>H</sub> gene (20%, 95% confidence interval, 5-35%; Table 2). In keeping with the high continuous recombinatorial potential of these leukemias, oligoclonal rearrangements were also identified at relapse in six patients.

**Frequency of immunoglobulin/T-cell receptor rearrangements.** We further analyzed the frequency of rearrangements for the individual IG/TCR loci at diagnosis and, in those from group I, also at relapse (Table 3). The highest frequency of IG<sub>H</sub> and TCRD rearrangements was found in the initial leukemias of group I. At relapse, IG<sub>H</sub> rearrangements were slightly less common, but the TCRD ones were significantly less common (P = 0.004, McNemar’s test), whereas the incidence of IGK

Table 3. Frequency of IG/TCR rearrangements according to immunogenotype patterns at diagnosis and relapse

<table>
<thead>
<tr>
<th>Immunogenotype patterns</th>
<th>IG/TCR locus</th>
<th>Changed (group I)</th>
<th>Conserved (group II)</th>
<th>Overall frequency at diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGH (%)</td>
<td>IGK (%)</td>
<td>TCRD (%)</td>
<td>TCRG (%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>93</td>
<td>64</td>
<td>71</td>
<td>93</td>
</tr>
<tr>
<td>Relapse</td>
<td>82</td>
<td>68</td>
<td>46</td>
<td>93</td>
</tr>
<tr>
<td>Overall frequency at diagnosis</td>
<td>85</td>
<td>68</td>
<td>61</td>
<td>88</td>
</tr>
</tbody>
</table>
and TCRG remained constantly high at both occasions. In cases with a conserved pattern (group II), however, frequency of rearrangements of all immunoreceptor loci was similar to that of the relapses in group I. This difference in the frequencies of rearrangements at the individual gene loci is similar to the one reported previously for the age-related IG/TCR rearrangement patterns of TEL-AML1+ BCP-ALL at diagnosis (13).

**Immunogenotype patterns in relation to age and remission duration.** Following our initial observation of a relationship between age and the frequency of particular rearrangement patterns, such as a decrease of IGH and TCRD and a reciprocal increase of biallelic IGG and TCRG rearrangements with rising age, we wondered whether such age-related associations might also exist in the context of relapses.

The median age of the 28 children in group I was 3.15 years (range 1.7-13.4) and only four of them (14%) were 6 years or older at initial diagnosis. In contrast, the median age of the 13 cases with a conserved pattern (group II), however, frequency of rearrangements of all immunoreceptor loci was similar to the one reported previously for the age-related IG/TCR rearrangement patterns of TEL-AML1+ BCP-ALL at diagnosis (13).

The median age of the 28 children in group I was 3.15 years (range 1.7-13.4) and six of them (46%) were 6 years or older at initial diagnosis. In contrast, the median age of the 13 patients in group II was 4.9 years (range 1.8-12.0) and six of them (46%) were 6 years or older (Table 4). The ungrouped age difference showed a trend toward a higher age in group II with the conserved pattern ($P = 0.09$, Wilcoxon two-sample test). This difference became statistically significant when the patients were grouped according to age (either younger or equal to and older than 6 years; $P = 0.048$, Fisher’s exact test). The remission duration (group I: range 11-60 months, median 34; group II: range 6-56 months, median 32) did not differ significantly between the two groups ($P = 0.7$, Wilcoxon two-sample test). There was no association between age and number of rearrangements at diagnosis ($P = 0.17$, Kendall’s rank correlation).

### Table 4. Age at diagnosis and remission duration according to immunogenotype patterns

<table>
<thead>
<tr>
<th>Immunogenotype patterns</th>
<th>Changed</th>
<th>Conserved</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient no.</strong></td>
<td><strong>Age</strong></td>
<td><strong>Remission</strong></td>
</tr>
<tr>
<td>12</td>
<td>1.7</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>1.9</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>52</td>
</tr>
<tr>
<td>22</td>
<td>2.3</td>
<td>34</td>
</tr>
<tr>
<td>24</td>
<td>2.3</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>2.5</td>
<td>44</td>
</tr>
<tr>
<td>21</td>
<td>2.7</td>
<td>42</td>
</tr>
<tr>
<td>26</td>
<td>2.8</td>
<td>50</td>
</tr>
<tr>
<td>27</td>
<td>3.0</td>
<td>34</td>
</tr>
<tr>
<td>11</td>
<td>3.0</td>
<td>47</td>
</tr>
<tr>
<td>9</td>
<td>3.1</td>
<td>50</td>
</tr>
<tr>
<td>16</td>
<td>3.1</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>3.2</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>3.4</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>3.9</td>
<td>30</td>
</tr>
<tr>
<td>18</td>
<td>3.9</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>3.9</td>
<td>48</td>
</tr>
<tr>
<td>13</td>
<td>4.0</td>
<td>34</td>
</tr>
<tr>
<td>1</td>
<td>4.3</td>
<td>48</td>
</tr>
<tr>
<td>17</td>
<td>5.3</td>
<td>30</td>
</tr>
<tr>
<td>28</td>
<td>5.3</td>
<td>30</td>
</tr>
<tr>
<td>23</td>
<td>5.4</td>
<td>35</td>
</tr>
<tr>
<td>15</td>
<td>6.0</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>6.1</td>
<td>34</td>
</tr>
<tr>
<td>25</td>
<td>7.9</td>
<td>35</td>
</tr>
<tr>
<td>19</td>
<td>13.4</td>
<td>25</td>
</tr>
</tbody>
</table>

*Age at diagnosis (years).

1 First remission duration (months).
Nevertheless, it is intriguing to note that the clonal heterogeneity between diagnosis and relapse changes with the age of the patients at diagnosis. Because the vast majority of our patients were consecutively enrolled in the various BFM-based national therapy studies, we can rule out a selection bias. Moreover, the restriction to TEL-AML1+ cases also avoids biases that might have been due to more general age- and genetic subtype–related variations in the IG/TCR gene recombination process (12, 30). The observed age-dependent differences can, therefore, only be attributed to two factors, namely the differential accessibility of the respective IG/TCR loci, which is influenced by the developmental stage of the B precursor cell at the time of transformation and the continuation of the recombination process in these cells during the entire latency period. On the one hand, this process would lead to a continuous formation of new rearrangements at the accessible gene loci (IGK, TCRD, TCRG) and, on the other hand, it would also modify preexisting rearrangements (IGH, TCRD; ref. 23). In contrast to loci that can undergo further recombination, potential end-stage recombinations, such as IGK and TCRG rearrangements (31), are not expected to change with age. Whereas IGH loci may continue to rearrange their genes resulting in V replacements of VDJ rearrangements or in gene deletions, TCRD may further recombine with one of the Jα segments (22, 32). The age-associated decreasing frequency of IGH and TCRD rearrangements at diagnosis and the increase in biallelic end stage IGK and TCRG rearrangements (13), together with the lack of an apparent association between immunogenotype changes at relapse and the length of remissions, support the idea that the vast majority of these rearrangements are already formed in the preleukemic phase of the disease.

The current notion is that the TEL-AML1 gene fusion is one of the first steps in transformation. It is predominantly generated already during fetal development but only additional mutations are supposed to finally set off the development to a clinically apparent leukemia (2). The target cell, in which this gene fusion is formed, is currently unknown. However, recent experiments in mice indicate that the expression of the TEL-AML1 fusion gene inhibits already the earliest stages of B-cell development (33). Our data concord with these observations because the vast majority of leukemias has clonally appearing IGH rearrangements. Furthermore, we can deduce from the type of changes at relapse, namely V_H replacements, that the respective target cell is indeed a B-cell precursor at the pro-B to pre-B transition. In this developmental stage, incomplete to complete VDJ_H rearrangements take place. After the completion of a VDJ_H rearrangement, subclone formation results most frequently or even exclusively in this type of clonal evolution (34). Notably, our data fit also the concept that TEL-AML1 does not transform hematopoietic “stem cells” (35) because relapses with completely unrelated IG/TCR rearrangements were hardly ever detected. Such a pattern would, in analogy with the BCR-ABL+ and the MLL-AF4+ acute leukemias, be expected if a stem cell is targeted (12, 36, 37).

Fig. 2. Schematic representation of four distinct modes of disease recurrence that became apparent from the diverse immunogenotype patterns detected at diagnosis and relapse. Circles with the different gray shades, TEL-AML1+ clones with their individual immunogenotype. Their respective location in the gray or white part designates their preleukemic or leukemic nature. Arrows, the critical event, which finally renders a preleukemic cell leukemic nature (diagonal for the initial leukemia and upward pointing for the relapse leukemia). Thin arrows and small empty circles, the propensity for subclone formation at the respective stage of the precursor cell. A. conserved immunogenotype. The immunogenotype changes take place in a preleukemic cell and remain stable and homogeneous when evolving to leukemia as well as relapse. B to D. changed immunogenotype. B. an ongoing rearrangement of the immunogenotype in form of modifications, gains, and/or deletions of rearrangements produces the respective clonal variations, as evident, for instance, in patients 5 and 24. C. the relapse may also result from the clonal selection of a preleukemic subclone that derives from the initial leukemic precursor clone by a V_H replacement as observed, for instance, in patients 3 to 6. The clone with the replaced V_H rearrangement may or may not be detectable at first diagnosis. D. initial leukemia and relapse evolve from two apparently independent TEL-AML1+ subclones with distinct TCRG rearrangements. This pattern is found, for example, in patients 1, 2, 6, and 8.
How do our data now fit into this proposed scheme? If a change of the immunogenotype only depended on the age at initial diagnosis, then also the duration of the first remission should matter, yet we did not find any evidence for that. The important elements in the development of these leukemias and relapses are once again the two Darwinian principles of clonal evolution and selection (38, 39) whereby the various IG/TCR gene rearrangements serve only as very valuable clonal markers that probably do not have any further biological function or prognostic meaning.

In our model, schematically represented in Fig. 2, a precursor B cell with particular VDJH and IGK gene rearrangements expands after the TEL-AML1 gene fusion has taken place. Somatic recombination can then generate a multitude of clones with different TCRD and TCRG gene rearrangements. Those cells, which experience advantageous mutations, continue to expand, rearrange further, and modify their IG/TCR joints accordingly. Ongoing mutation and selection processes during the latency period produce many clones, which are defined, among others, by their individual types of IG/TCR gene rearrangements. One cell of these clones eventually acquires the critical mutation and progresses into a clinically overt leukemia. In addition, a large number of much smaller preleukemic subclones, whose common denominator is the TEL-AML1 fusion gene, may continue to linger around.

The subclones, which escape the therapeutic interventions, will again provide the reservoir for the development of second leukemias in form of relapses.

This model can also explain why, compared with the initial leukemia, the second leukemia has already a significantly reduced number of clonal rearrangements and why the types of IG/TCR gene rearrangements resemble more those of older children at initial disease. In both instances, the cells have already gone through several rounds of expansion and selection and thereby exhausted their potential for recombination. The data presented herein disagree with previous interpretations that the variability of rearrangements directly represents the differentiation stage of the respective affected B cells (6). Instead, we propose now that it indirectly reflects only the differentiation stage of the respective affected B cells (6).

We thank Uli Pötschger for statistical analysis; Maaike de Bie, Marianne Konrad, Uli Monschein, and Susanna Fischer for technical assistance; and Micha Komjáti for preparing Figure 1.

Acknowledgments

We thank Uli Pötschger for statistical analysis; Maaike de Bie, Marianne Konrad, Uli Monschein, and Susanna Fischer for technical assistance; and Micha Komjáti for preparing Figure 1.

References
13. Pui CH, Relling MV, Campana D, Evans WE.
Immunogenotype Changes Prevail in Relapses of Young Children with TEL-AML1-Positive Acute Lymphoblastic Leukemia and Derive Mainly from Clonal Selection


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/21/7720

Cited articles
This article cites 38 articles, 24 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/21/7720.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/11/21/7720.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.