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. Haas[1,2]

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 has recently become a matter of discussion because in several 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: [1]Children's Cancer Research Institute; [2]St. Anna Kinderspital, Vienna, Austria; [3]M. Tettamanti Research Center, Pediatric Clinic, San Gerardo Hospital, University of Milan Bicocca, Monza, Italy; [4]Department of Immunology, Erasmus MC Rotterdam, Rotterdam, the Netherlands; [5]Department of Pediatrics, University of Padova, Padua, Italy; [6]Department of Pediatrics, Charite Medical Center, Berlin, Germany; [7]Department of Pediatrics, Hannover Medical School, Hannover, Germany; and [8]Department of Pediatrics, Justus Liebig University, Giessen, Germany

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

Grant support: Austrian Ministry for Education, Science, and Culture (GENAU-CHILD) Österreichische Kinderkrebsforschung and private donations to the Children's Cancer Research Institute (E.R.P-Grümayer); Fondazione Tettamanti, Fondazione Cariplo, Associazione Italiana per la Ricerca sul Cancro, Consiglio Nazionale delle Ricerche/Ministry of Education, University, and Research-Legge 449/97 (A. Biondi); Dutch Cancer Society Koningin Wilhelmina Fonds grant SNWLK 2000-2268 (J.M.M. van Dongen); and Fondazione Città della Speranza, Consiglio Nazionale delle Ricerche, Ministero dell'UniversitA a Ricerca Scientifica a Tecnologica, Associazione Italiana per la Ricerca sul Cancro (G. Basso).

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.

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.

doi:10.1158/1078-0432.CCR-05-1239

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 to10 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.

Patients, Materials, and Methods

Patients and materials. Bone marrow samples from 41 *TEL-AML+* cases were collected at diagnosis and relapse and provided by six collaborating centers (Vienna, Hannover, Monza, Padua, Rotterdam, and Berlin). The vast majority of patients were enrolled sequentially in the ALL-BFM 95, ALL-BFM 2000, the AIEOP-ALL 2000, or the Dutch Childhood Leukemia Study Group ALL-8 or ALL-9 treatment protocol. In addition, we also included eight cases from other studies from which DNA was still available (Giessen). Some of the immunogenotype data were already reported previously (6, 8, 20). The presence of the cryptic t(12;21) was determined either by fluorescence *in situ* hybridization or by reverse transcription-PCR of *TEL-AML1* gene transcripts at diagnosis and relapse. All analyses were done in concordance with the informed consent guidelines of the local or national medical ethics committees.

Identification of PCR targets. DNA extraction, quality control of DNA, and screening PCRs for the subsequent analyses were conducted according to standardized protocols at diagnosis and relapse (21). Screening PCRs were done in all samples and included those for the incomplete and complete *IG heavy chain* (*IGH*) genes, the *IG light chain κ-deleting element* (*IGK-Kde*), as well as the incomplete *TCR δ* (*TCRD*) and the *TCR γ* (*TCRG*) rearrangements (9). In some of the diagnostic samples, additional screening PCRs for the most common Vδ2-Jα (22) and *TCR β* (*TCRB*) rearrangements (9) were done. The clonal nature of the amplified products was assessed by heteroduplex analysis. In case multiple bands were present, sequences were obtained from cloned PCR products. The involved segments of *IGH*, *IGK*, *TCRD*, *Vδ2-Jα*, *TCRG*, and *TCRB* gene rearrangements zzzwere identified through comparison with published germ line sequences using the sequence alignment software (V-QUEST) from IMGT (http://imgt.cines.fr:8104/). VDJ_H gene segments were additionally analyzed in the VBASE directory of human *IG* genes (http://www.vbasemrc-cpe.cam.ac.uk/).

Definition of monoclonal and oligoclonal B-cell precursor acute lymphoblastic leukemia. A sample was assumed to be monoclonal if no more than one *IG/TCR* rearrangement per allele was present (i.e., the number of rearrangements detected by PCR had to be equal to or less than two per *IG/TCR* locus). Oligoclonality, on the other hand, was defined by the occurrence of more than two rearrangements per gene or, in the case of *IGH* rearrangements, also by the detection of related sequences.

Statistical analysis. The different variables, such as the number of *IG/TCR* gene rearrangements, the age at initial diagnosis, the age at relapse, the remission duration, and its correlation with the patterns of *IG/TCR* rearrangements, were analyzed with the Wilcoxon two-sample test and, when children were grouped according to age, with the Fisher's exact test. A difference in the frequencies of *IG/TCR* rearrangements between different occasions was analyzed by McNemar's test. A correlation between age and number of rearrangements at diagnosis was assessed by Kendall's rank correlation.

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 28 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.

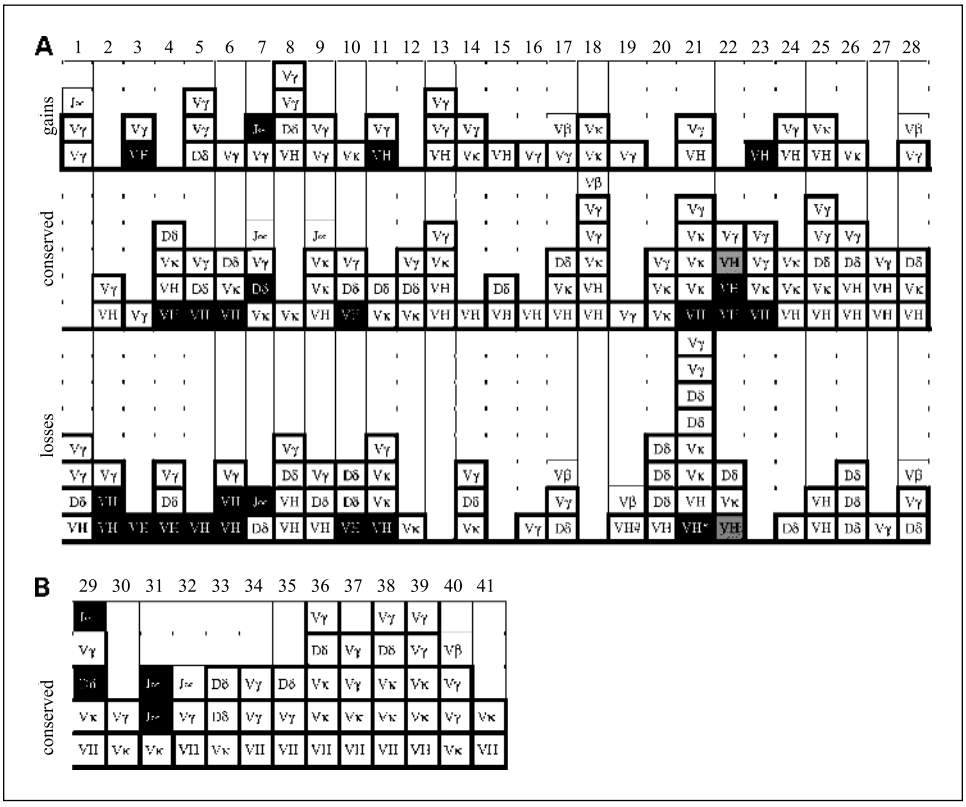

Fig. 1. *IG/TCR* gene rearrangement patterns at diagnosis and relapse in 41 *TEL-AML1* + ALL. *A,* cases with immunogenotype changes between diagnosis and relapse. *B,* cases with a conserved immunogenotype. Rearrangements are subdivided into conserved, gains, and losses, indicating the stable rearrangements, those present only at relapse, and those exclusively present at initial diagnosis, respectively. White squares, unique rearrangements; black squares, related rearrangements; gray squares in patient 22, the other subclones with related *IGH* rearrangements that differ from those related *IGH* rearrangements indicated in black; VH, complete *IGH* V_H-J_H rearrangements; *, D_H-J_H rearrangement; #, *IGH* rearrangement that was detectable at relapse with DJ_H-specific primers but not with the screening PCRs; Vκ, *IGK-Kde* rearrangements; Dδ, incomplete *TCRD* rearrangements; Vγ, *TCRG* rearrangements; Jα, *Vδ2-Jα* rearrangements; Tβ, *TCRB* rearrangements.

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 V_H 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 V_H replacements (Fig. 1). At relapse, one or two of these related rearrangements were lost in all but one patient (patient 22), who retained two related clones. The original clone (i.e., the clone with the most upstream V_H segment) was only preserved in two patients, whereas in the other four cases the clone with the most downstream V_H segment (i.e., the clone with the more "advanced" rearrangement) was preserved. This finding

Table 1. Type, frequency, and stability of *IG/TCR* rearrangements in leukemias according to immunogenotype patterns

Immunogenotype patterns	*IG/TCR* locus			
	IGH	***IGK***	***TCRD***	***TCRG***
	(Σ of rearrangements/rearranged leukemias)			
Changed (group I, n = 28)				
Conserved rearrangements	27/20	21/16	12/12	19/16
Only at diagnosis	20/15	7/5	20/14	15/13
Only at relapse	9/9	6/5	2/2	21/16
Conserved (group II, n = 13)	9/9	13/10	6/5	14/10
Conserved rearrangements (%)	64	83	47	68

Table 2. Incidence of oligoclonal *IG/TCR* rearrangements at diagnosis and relapse

Immunogenotype patterns	*IG/TCR* locus*				
	IGH	*IGK*	*TCRD*	*TCRG*	Cases†
Diagnosis	8	2	2	1	11‡
Relapse	3	2	0	2	6§
Diagnosis/relapse in percent‖	20/7	5/5	5/0	2/5	27/15

*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_H 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 DNJ_H clone-specific primer that was used for minimal residual disease analysis, which indicates that a V_H replacement had occurred.

Immunoglobulin light chain κ-locus. Twenty cases in group I had an *IGK-Kde* 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 *IGK-Kde* 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 *IGK-Kde* rearrangements were present at diagnosis. The latter patient retained three of the five rearrangements at relapse, whereas another patient (patient 18) kept one *IGK* 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 *TCRA* 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 *TCRG* 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 *IGH* 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 *IGH* and *TCRD* rearrangements was found in the initial leukemias of group I. At relapse, *IGH* 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

Immunogenotype patterns	*IG/TCR* locus			
	IGH (%)	*IGK* (%)	*TCRD* (%)	*TCRG* (%)
Changed (group I)				
Diagnosis	93	64	71	93
Relapse	82	68	46	93
Conserved (group II)	69	77	38	77
Overall frequency at diagnosis	85	68	61	88

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 *IGK* 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 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

Immunogenotype patterns					
Changed			Conserved		
Patient no.	Age*	Remission†	Patient	Age*	Remission†
12	1.7	18	38	1.8	56
8	1.9	40	29	2.0	46
3	2.0	52	41	2.7	6
22	2.3	34	33	3.2	25
24	2.3	60	34	3.4	32
2	2.5	56	35	4.7	52
5	2.5	20	31	4.9	16
14	2.5	44	40	6.2	44
21	2.7	42	39	6.9	28
26	2.8	50	37	7.4	40
27	3.0	34	36	9.5	55
11	3.0	47	32	11.8	24
9	3.1	50	30	12.0	18
16	3.1	24			
6	3.2	25			
4	3.4	15			
10	3.9	30			
18	3.9	11			
20	3.9	48			
13	4.0	34			
1	4.3	48			
17	5.3	30			
28	5.3	30			
23	5.4	35			
15	6.0	40			
7	6.1	34			
25	7.9	35			
19	13.4	25			

*Age at diagnosis (years).
†First remission duration (months).

Discussion

Our comparison of the *IG/TCR* gene rearrangement patterns in 41 *TEL-AML1+* BCP-ALL from diagnosis and relapse revealed that relapses are the consequence of clonal evolution and/or selection in approximately two thirds of the children (68%, group I), whereas in the last third of them (32%, group II) the original clones reemerge. Clonal changes were significantly more common in younger children, but correlated neither with the duration of the first remission nor with the age at relapse. In cases in which the rearrangement patterns diverged between diagnosis and relapse (group I), the frequency of rearranged loci as well as the overall number of rearrangements also differed significantly between these two time points, whereas in cases with identical clones (group II) these variables were similar to those of the relapses in group I.

Previous comparisons of the diagnosis and relapse *IG/TCR* gene rearrangement patterns in BCP-ALL were either confined to specific gene loci or did not take potential peculiarities of particular genetic or age groups into consideration (20, 23, 24). Consequently, some authors noted a higher likelihood for clonal changes in patients with longer remission durations (18, 25), whereas others did not (26). In our cohort of *TEL-AML1+* cases, the remission duration was neither associated with particular immunogenotype patterns nor did it depend on whether the relapse was the consequence of clonal evolution or selection. In support of subclone selection are the data from an earlier study demonstrating that in all six cases with a changed immunogenotype, the relapse clone was detectable at diagnosis irrespective of the remission duration (6). Such clones may withstand the initial treatment and may, therefore, also persist at a low level for several months, as was previously shown in two patients with a late recurrence of the disease (8). However, the biology behind the therapy resistance of these relapse clones also seems to differ from the ones that evolve into early relapses because the treatment response of such late relapses is usually as favorable as it is for the initial disease (27).

The incidence of oligoclonal *IGH* rearrangements of the cases reported herein was 20% and was, therefore, not significantly different from the 12% of the 214 previously analyzed *TEL-AML1+* patients. Further, in this study, we could not establish that oligoclonality has a prognostic meaning or represents a more aggressive disease form as has been reported for BCP-ALL (18, 25, 28). It merely seems to be an expression of the high and persistent recombinatorial potential of these preleukemic/leukemic cells, which is also evidenced by their pronounced recombination-activating gene activity (29) and high number of *IG/TCR* gene rearrangements (13).

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).

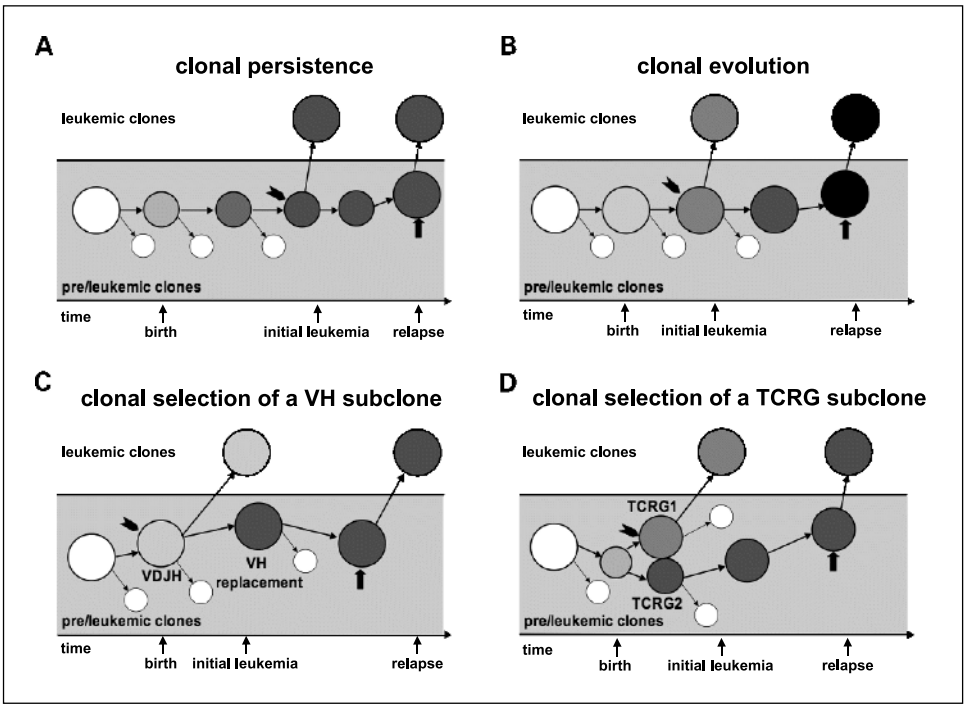

Fig. 2. Schematic representation of four distinct modes of disease recurrence that became apparent from the diverse immunogenotype patterns detected at diagnosis and relapse. Circles with the different gray shades, *TEL-AML1*+ clones with their individual immunogenotype. Their respective location in 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 intial 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 VDJ_H 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 unique capability of these cells for continuing recombination. The shorter the latency period of a transformed cell pool is, the more likely is its probability for producing clonal progenies with a multitude of different *IG/TCR* rearrangements. Owing to the continuous "maturation" of these rearrangements and clonal selection processes, the chance for the detection of such polyclonal populations, however, shrinks with the length of the latency period and, therefore, also with the increasing age of the respective patients at diagnosis.

Finally, we want to briefly refer to the potential consequences of our data for minimal residual disease analyses. As we have pointed out above, the presence of a PCR-detectable oligoclonality does not predispose to a higher relapse rate. Nevertheless, the higher number of rearrangements in younger children, together with the particular propensity of the occurrence of rearrangements in the relapse, which differs from those in the initial clone, requires specific attention. In our study, the frequency and stability of *IG/TCR* rearrangements (Table 2), with the exception of a higher instability of *TCRD* rearrangements, concur with those reported by Szczepansky et al. (23) on unselected childhood BCP-ALL. Therefore, our findings reinforce the currently used recommendation for the hierarchical order of applying the individual immunoreceptor loci in minimal residual disease studies according to their frequency and stability: *IGK*, *TCRG*, *IGH*, and *TCRD* (23, 30). Moreover, our data corroborate that preferentially two markers from different gene loci should be used. The remarkable instability of the individual *IGH* rearrangements can, to a large extent, be bypassed with the already widely applied approach to design primers that cover the DNJ_H region (26). Nevertheless, the lack of appropriate stable rearrangements together with the unpredictable clonal fluctuation prevents the use of the DNJ_H or any other rearrangement as an exclusive marker. Although Vδ2-Jα and *TCRB* rearrangements have only been analyzed in a few cases, the preliminary results indicate that the ensuing configurations are also very heterogeneous as they are secondary rearrangements and ontogenetically younger (22, 40).

Acknowledgments

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

References

1. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med 2004;350:1535–48.
2. Greaves M. Molecular genetics, natural history and the demise of childhood leukaemia. Eur J Cancer 1999;35:173–85.
3. Mori H, Colman SM, Xiao Z, et al. Chromosome translocations and covert leukemic clones are generated during normal fetal development. Proc Natl Acad Sci U S A 2002;99:8242–7.
4. Wiemels JL, Ford AM, van Wering ER, Postma A, Greaves M. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion *in utero*. Blood 1999;94:1057–62.
5. Ford AM, Fasching K, Panzer-Grumayer ER, Koenig M, Haas OA, Greaves MF. Origins of "late" relapse in childhood acute lymphoblastic leukemia with TEL-AML1 fusion genes. Blood 2001;98:558–64.
6. Peham M, Konrad M, Harbott J, Konig M, Haas OA, Panzer-Grumayer ER. Clonal variation of the immunogenotype in relapsed ETV6/RUNX1-positive acute lymphoblastic leukemia indicates subclone formation during early stages of leukemia development. Genes Chromosomes Cancer 2004;39:156–60.
7. Zuna J, Ford AM, Peham M, et al. TEL deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukemia. Clin Cancer Res 2004;10:5355–60.
8. Konrad M, Metzler M, Panzer S, et al. Late relapses evolve from slow-responding subclones in t(12;21)-positive acute lymphoblastic leukemia: evidence for the persistence of a preleukemic clone. Blood 2003;101:3635–40.
9. van Dongen JJ, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003;17:2257–317.
10. Wasserman R, Galili N, Ito Y, Reichard BA, Shane S, Rovera G. Predominance of fetal type DJH joining in young children with B precursor lymphoblastic leukemia as evidence for an *in utero* transforming event. J Exp Med 1992;176:1577–81.
11. Steenbergen EJ, Verhagen OJ, van Leeuwen EF, et al. B precursor acute lymphoblastic leukemia third complementarity-determining regions predominantly represent an unbiased recombination repertoire: leukemic transformation frequently occurs in fetal life. Eur J Immunol 1994;24:900–8.
12. Brumpt C, Delabesse E, Beldjord K, et al. The incidence of clonal T-cell receptor rearrangements in B-cell precursor acute lymphoblastic leukemia varies with age and genotype. Blood 2000;96:2254–61.
13. Hubner S, Cazzaniga G, Flohr T, et al. High incidence and unique features of antigen receptor gene rearrangements in TEL-AML1-positive leukemias. Leukemia 2004;18:84–91.
14. van Dongen JJ, Seriu T, Panzer-Grumayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998;352:1731–8.
15. Schrappe M. Evolution of BFM trials for childhood ALL. Ann Hematol 2004;83 Suppl 1:S121–3.
16. Pui CH, Relling MV, Campana D, Evans WE.

Childhood acute lymphoblastic leukemia. Rev Clin Exp Hematol 2002;6:161–80; discussion 200–2.

17. Cave H, Guidal C, Rohrlich P, et al. Prospective monitoring and quantitation of residual blasts in childhood acute lymphoblastic leukemia by polymerase chain reaction study of δ and γ T-cell receptor genes. Blood 1994;83:1892–902.

18. Beishuizen A, Verhoeven MA, van Wering ER, Hahlen K, Hooijkaas H, van Dongen JJ. Analysis of Ig and T-cell receptor genes in 40 childhood acute lymphoblastic leukemias at diagnosis and subsequent relapse: implications for the detection of minimal residual disease by polymerase chain reaction analysis. Blood 1994;83:2238–47.

19. Wasserman R, Yamada M, Ito Y, et al. V_H gene rearrangement events can modify the immunoglobulin heavy chain during progression of B-lineage acute lymphoblastic leukemia. Blood 1992;79:223–8.

20. Germano G, del Giudice L, Palatron S, et al. Clonality profile in relapsed precursor-B-ALL children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. Leukemia 2003; 17:1573–82.

21. Pongers-Willemse MJ, Seriu T, Stolz F, et al. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and TAL1 deletions as PCR targets: report of the BIOMED-1 CONCERTED ACTION: investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:110–8.

22. Szczepanski T, van der Velden VH, Hoogeveen PG, et al. Vδ2-Jα rearrangements are frequent in precursor-B-acute lymphoblastic leukemia but rare in normal lymphoid cells. Blood 2004;103:3798–804.

23. Szczepanski T, Willemse MJ, Brinkhof B, van Wering ER, van Dongen JJ. Comparative analysis of Ig and TCR gene rearrangements at diagnosis and at relapse of childhood precursor-B-ALL provides improved strategies for selection of stable PCR targets for monitoring of minimal residual disease. Blood 2002;99: 2315–23.

24. Li A, Zhou J, Zuckerman D, et al. Sequence analysis of clonal immunoglobulin and T-cell receptor gene rearrangements in children with acute lymphoblastic leukemia at diagnosis and at relapse: implications for pathogenesis and for the clinical utility of PCR-based methods of minimal residual disease detection. Blood 2003;102:4520–6.

25. Green E, McConville CM, Powell JE, et al. Clonal diversity of Ig and T-cell-receptor gene rearrangements identifies a subset of childhood B-precursor acute lymphoblastic leukemia with increased risk of relapse. Blood 1998;92:952–8.

26. Steward CG, Goulden NJ, Katz F, et al. 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. Blood 1994; 83:1355–62.

27. Seeger K, von Stackelberg A, Taube T, et al. Relapse of TEL-AML1-positive acute lymphoblastic leukemia in childhood: a matched-pair analysis. J Clin Oncol 2001; 19:3188–93.

28. Kitchingman GR, Mirro J, Stass S, et al. Biologic and prognostic significance of the presence of more than two μ heavy-chain genes in childhood acute lymphoblastic leukemia of B precursor cell origin. Blood 1986; 67:698–703.

29. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133–43.

30. van der Velden VH, Szczepanski T, Wijkhuijs JM, et al. Age-related patterns of immunoglobulin and T-cell receptor gene rearrangements in precursor-B-ALL: implications for detection of minimal residual disease. Leukemia 2003;17:1834–44.

31. van der Velden VH, Willemse MJ, van der Schoot CE, Hahlen K, van Wering ER, van Dongen JJ. Immunoglobulin κ deleting element rearrangements in precursor-B acute lymphoblastic leukemia are stable targets for detection of minimal residual disease by real-time quantitative PCR. Leukemia 2002;16:928–36.

32. Steenbergen EJ, Verhagen OJ, van Leeuwen EF, van den BH, dem Borne AE, van der Schoot CE. Frequent ongoing T-cell receptor rearrangements in childhood B-precursor acute lymphoblastic leukemia: implications for monitoring minimal residual disease. Blood 1995;86:692–702.

33. Tsuzuki S, Seto M, Greaves M, Enver T. Modeling first-hit functions of the t(12;21) TEL-AML1 translocation in mice. Proc Natl Acad Sci U S A 2004;101:8443–8.

34. Steenbergen EJ, Verhagen OJ, van Leeuwen EF, dem Borne AE, van der Schoot CE. Distinct ongoing Ig heavy chain rearrangement processes in childhood B-precursor acute lymphoblastic leukemia. Blood 1993;82:581–9.

35. Hotfilder M, Rottgers S, Rosemann A, Jurgens H, Harbott J, Vormoor J. Immature $CD34^+CD19^-$ progenitor/stem cells in TEL/AML1-positive acute lymphoblastic leukemia are genetically and functionally normal. Blood 2002;100:640–6.

36. Height SE, Swansbury GJ, Matutes E, Treleaven JG, Catovsky D, Dyer MJ. Analysis of clonal rearrangements of the Ig heavy chain locus in acute leukemia. Blood 1996;87:5242–50.

37. Davi F, Gocke C, Smith S, Sklar J. Lymphocytic progenitor cell origin and clonal evolution of human B-lineage acute lymphoblastic leukemia. Blood 1996;88: 609–21.

38. Vineis P. Causality in epidemiology. Soz Praventivmed 2003;48:80–7.

39. Greaves M. Childhood leukaemia. BMJ 2002;324: 283–7.

40. van der Velden VH, Bruggemann M, Hoogeveen PG, et al. TCRB gene rearrangements in childhood and adult precursor-B-ALL: frequency, applicability as MRD-PCR target, and stability between diagnosis and relapse. Leukemia 2004;18:1971–80.