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Minimal residual disease monitoring in adult T-cell acute lymphoblastic leukemia: a molecular based approach using T-cell receptor γ and δ gene rearrangements

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Background and Objectives. Minimal residual disease (MRD) is important in the measurement of response to treatment in childhood B- and T-cell acute lymphoblastic leukemia (ALL) and in adult B-cell ALL. Little is known about MRD evaluation in adult T-cell ALL. This study aimed to determine the prognostic significance of MRD measurements in adult T-cell ALL.

Design and Methods. T-cell receptor (*TCR*) γ (*G*) and *TCR* δ (*D*) gene analyses were carried out at presentation in 49 patients with *de novo* T-ALL using a polymerase chain reaction (PCR) approach. In 26 of the patients bone marrow (BM) samples were collected at sequential time points (0-2, 3-5, 6-9, 10-24 months) after diagnosis for MRD investigation. The relationship between MRD status and clinical outcome was investigated and correlated with age, gender and white blood cell count at presentation.

Results. *TCRG* clonal gene rearrangements were found in 40 patients (82%). Eleven patients showed *TCRD* rearrangements (22%), in one of them as the sole molecular marker. Vγ I family rearrangements predominated (45 of 65 alleles) together with $V\delta1\text{-}J\delta_{1/2}$ (9 of 13 alleles). Continuous clinical remission (CCR) occurred in 17 patients while nine patients relapsed. MRD analysis showed that negative tests during the first 6 months post-induction, and persisting negative MRD after induction were the best predictors of CCR. A positive test after 5 months was better at predicting relapse. In only four of seven patients was relapse preceded by a positive test the 5 months preceding relapse.

Interpretation and Conclusions. Overall the ability of positive and negative tests to predict relapse or CCR was weaker in this cohort of adult T-ALL patients than in T- and B-lineage childhood ALL and B-lineage adult ALL. *TCRG* and *TCRD* gene analysis provides a clonal marker in the majority of adult T-ALL. These results suggest that caution should be taken in using MRD data based on TCR gene rearrangements to predict prognosis in adult T-ALL. Biological reasons may underlie differences between the performance of MRD tests in B- and T-lineage ALL. Further studies in a larger cohort of patients are needed to determine the exact role that MRD determination has in the management of T-ALL in adults.

Key words: adult lymphoblastic leukemia; T-cell ALL; MRD; TCR γ and δ genes.

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Most patients with acute lymphoblastic leukemia (ALL) enter remission with standard induction therapy. Since many, particularly adults, relapse, it is important to identify those patients at greater risk of disease relapse who might benefit from more intensive therapy or stem cell transplantation in first remission. Presenting white blood cell count (WBC), age, gender, cytogenetics, percentage of bone marrow blasts at day 8 or day 14 post-induction, and prednisone response are established criteria for stratifying patients with ALL into low, intermediate and high risk groups. Molecular assessment during therapy using immunoglobulin (IG), T-cell receptor (TCR) or fusion genes associated with recurrent chromosomal abnormalities has shown that minimal residual disease (MRD) is an independent prognostic factor in childhood B- and T- lineage ALL. It is more reliable for risk stratification than age, gender and WBC.[1-6] Also, MRD monitoring using immunologic tests and three or four color flow cytometry has been shown to be highly sensitive and better at predicting outcome than conventional morphologic assessment in ALL[7,8] and acute myeloid leukemia (AML).[9]

As in children, MRD measurements during treatment in adult B-lineage ALL, albeit in fewer patients[10-15] significantly predict clinical outcome[13-15] and are better than total WBC, age, and gender in a multivariate analysis.[15]

Only a few studies of MRD using polymerase chain reaction (PCR) have been carried out in adults with T-ALL. The results have shown frequent persistence of residual disease until the end of the first year of treatment.[16,17] The most informative time point appears to be at the beginning of maintenance. That is 8-9 months after presentation[16,17] rather than in the immediate post-induction stage observed in adult B-lineage ALL.[15] This also contrasts with the situation in childhood ALL in which early post-induction measurements provide results highly predictive of clinical outcome.[1,2,6]

In the present study we used TCRγ (*TCRG)* and

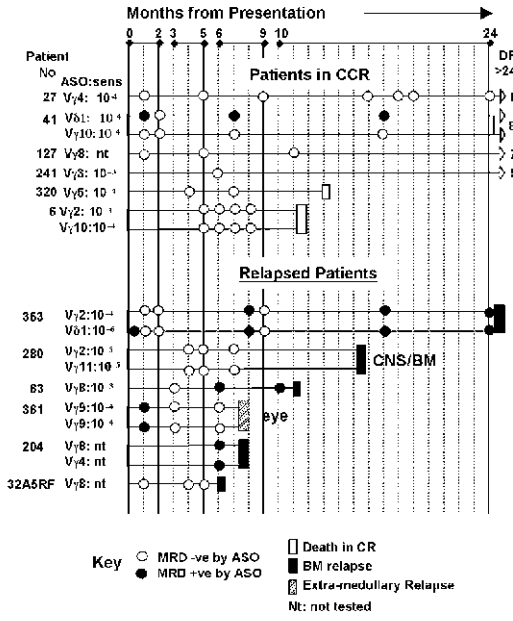

Figure 1. MRD results of adult T-ALL patients receiving chemotherapy only. Six patients relapsed and six remained in CCR. Horizontal lines represent the follow-up period from presentation. The result of each ASO is presented for each patient. *CCR, complete clinical remission; allo, allo-bone marrow transplant; auto, auto-bone marrow transplant; DFS, disease-free survival; ASO, allele-specific oligonucleotide; sens. ASO: sensitivity tested for each individual ASO; CNS, central nervous system.*

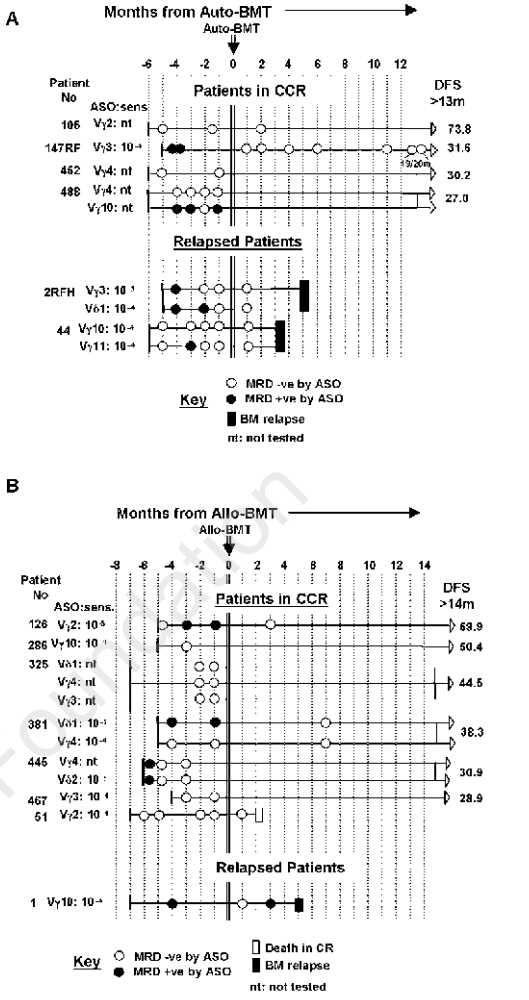

Figure 2. MRD results of adult T-ALL receiving transplantation after chemotherapy. A: MRD results from patients who received chemotherapy and autologous BMT. B: patients who received chemotherapy and allogeneic BMT. *Abbreviations as for Figure 1.*

TCRδ (*TCRD*) gene rearrangements to identify clonal markers in a cohort of 49 adult T-ALL. In 26 of them bone marrow (BM) samples and markers suitable for MRD investigation were available for analysis during the first 24 months of treatment. These tests were assessed for their ability to predict clinical outcome and compared to standard clinical prognostic criteria.

Design and Methods

Patients' samples

Presentation bone marrow samples (fresh, frozen or archival) were obtained from 49 adult patients (15-55 years) with *de novo* T-lineage ALL (according to immunologic criteria) who had been treated with the UKALLXII trial protocol between 1992 and 2000 at the Royal Free Hospital or other participating UK centers.[18] Thirty-seven (75.5%) of the patients were male and 12 (24.5%) were female; their median age was 29 years (range 16-51). The median WBC count was 37×10^9/L (range 0.9-536×10^9/L). Thirty-three (67%) patients had $\leq100\times10^9$/L and 16 (33%) had $\geq100\times10^9$/L. Forty-eight of the 49 patients achieved first continuous clinical remission (CCR) after a median period of 22 days (range 14-137 days).

Patients were all treated according to the UKALLXII protocol that randomized patients between chemotherapy and autologous stem cell transplant (SCT), unless a sibling donor was available for allogeneic transplant.[18] Twelve patients received chemotherapy (CHT) only. The remaining 14 received an autologous stem cell transplant (SCT)(n=6) or allogeneic (n=8) SCT following induction CHT treatment. Results are illustrated for the three therapy groups separately, because of the different effects the treatment would have on outcome (Figures 1 and 2). Patients in the three groups did not differ in age (median: 32 years, 22 years

and 26 years, respectively) or WBC count (median: 44, 22 and 57×10^9/L, respectively).

Nine patients relapsed between 5.5 and 24.3 months after diagnosis (median 11.5 months). Six had received CHT only, two autologous and one allogeneic SCT. Two patients (#280 and 361) in the CHT group suffered an extra-medullary relapse in the CNS and eye, respectively, but only the former was associated with a medullary leukemic infiltration. Seventeen patients remained in CCR (median: 31.6 months; range 8.5-82.4). Seven patients showed features associated with poor prognosis (WBC ≥100×10^9/L) but only three relapsed, while four remained in CCR. Notably, two of the latter had received allogeneic SCT.

DNA extraction procedures

Mononuclear cell separation and DNA extraction from fresh and archival material was carried out as previously described.[15] All samples were amplified for the constitutional β-actin gene (Table 1) to ensure DNA integrity.[19]

Identification of clonal TCRG and TCRD gene rearrangements at diagnosis

Samples obtained at presentation were analyzed by PCR using the primers listed in Table 1.[20-25]

Four *TCRD* gene rearrangements were studied: Vδ1-Jδ$_{1-2}$, Vδ2-Jδ$_{1-2}$, Vδ2-Dδ3 and Dδ2-Dδ3. *TCRG* gene rearrangements were investigated in twelve single PCR reactions using the four sense FR1-Vg family specific primers and three anti-sense Jγ primers. Conditions for amplification have been previously reported.[22] The cycling protocol used in this study consisted of 5 min of initial denaturation at 94° C, followed by 35 cycles at 94°C for 1 min, 58° C (for the β-actin) or 60° C (for the *TCRG*) or 66° C (for the *TCRD*) for 1 min, 72° C for 1 min 30 sec and a final extension at 72° C for 10 min. Eight microliters of the PCR product were examined on a 1.5% agarose gel and heteroduplex analysis was performed in a non-denaturing PAGE 6% gel for positive PCR reactions, as previously described.[26] Homoduplex bands were excised from the polyacrylamide gel, eluted overnight and following re-amplification the individual PCR products were cloned and sequenced as previously described.[27] The cloned sequences were analyzed using the ImMunoGeneTics database (IMTG)[28] that identified the closest matching functional V, D or J segment.

Allele specific oligoprimers (ASO): design and sensitivity tests

Thirty-nine ASO were designed from the CDR3 region for 26 patients: 14 patients were tested with one ASO, 11 patients with two and one patient with three ASO (Figures 1 and 2). Thirty-three ASO were designed in the *TCRG* junctional region and six in the *TCRD* junctional region. The specificity of each primer was tested using five different polyclonal bone marrow DNA samples. The sensitivity of each ASO was determined by testing serial dilutions of patients' DNA in 1 μg of bone marrow DNA obtained from a normal donor. In total, seven ASO showed a 10^{-5} sensitivity (four *TCRG* and 3 *TCRD*), 18 (16 *TCRG* and two *TCRD*) showed a sensitivity of 10^{-4} and two showed a sensitivity of 10^{-3} (two *TCRG*) (Figures 1 and 2). In 17 patients the sensitivity of detection of MRD was at least 10^{-4}, in two at least 10^{-3}. In seven patients (12 ASO) not enough presentation DNA was available to assess the ASO sensitivity.

Table 1. Primer sequences used to analyze *TCRD* and *TCRG* loci.

TCRδ primers	5' to 3' sequence
FR1/Vδ1	TAG GAATTC ACT CAA GCC CAG TCA TCA GT
FR1/Vδ2	TAG GAATTC GGA CAT CGA GTC ATG TCA GCC
Jδ1/2	TAG AAGCTT GG/TG TTC CT/GT TTC CAA AGA T/AGA G
Dδ2	TAG GAATTC AGA AGA GGG TTT TTA TAC TGA TGT G
Dδ3	TAG AAGCTT AGG GAA ATG GCA CTT TTG CC
TCRγ primers	
FR1/Vγ1-8	TAG AAGCTT TGC AGC CAG TCA GAA ATC TTC C
FR1/Vγ9	TAG AAGCTT CAG CCC GCC TGG AAT GTG TGG
FR1/Vγ10	TAG AAGCTT AGC AGT TCC AGC TAT CCA TTT CC
FR1/Vγ11	TAG AAGCTT TGC AAT TGC ACT TGG GCA
Jγ1.3/2.3	TAG GGATCC CAA CAA GTG TTG TTC CAC TGC C
Jγ1.1/2.1	TAG GGATCC TTA CCA GGT GAA GTT ACT ATG AGC
Jγ1.2	TAG GGATCC AAG AAA ACT TAC CTG TAA TGA TAA GC
β-Actin primers	
B127	GAT GGA GTT GAA GGT AGT TT
B1	TGC TAT CCA GGC TGT GCTAT

Individual oligoprimer restriction sites used for cloning are underlined. TCRD primers were adapted from those described in ref.[21] except Dδ2 primer.[22] The Jδ$_{1/2}$ primer included redundant bases in order to amplify both Jδ1 and Jδ2 gene segments. TCRG primers were designed based on previously published sequences.[23-26] β-actin primers were adapted from those described in ref.[19]

MRD-PCR analysis of the bone marrow DNA

MRD-PCR amplification of the remission marrow samples was performed using denaturing conditions, as previously described.[27] Results were grouped for 4 time-points during treatment. The first time point (1-2 months) corresponds to the induction treatment phase and the second (3-5 months) to the intensification and CNS therapy phases.[18] For patients receiving allogeneic or autologous SCT this time point corresponds to the time of bone marrow harvesting. For patients receiving CHT, consolidation therapy was administered from 6-9 months and maintenance therapy from 10-24 months.[18] The result of the last sample tested was used for MRD analysis in patients with more than one test at any time-point.

Criteria for exclusion from the MRD study were the following: (i) patients with no *TCRG* or *TCRD* clonal marker at presentation (n=8); (ii) Philadelphia positive disease (n=1); (iii) patients who failed to achieve CCR (n=1); (iv) patients with no follow-up samples (n=8); (v) patients with no junctional region of their TCR clonal rearrangement (n=4); (vi) patients whose ASO gave a background signal on polyclonal normal DNA (n=1). In total, MRD could be monitored in 26 of the 49 initial patients. Samples for follow-up monitoring were sent to our laboratory on a voluntary basis from each of the participating centers and compliance was higher than that in any previously reported study (83%). The end-point for MRD analysis was death in CCR or clinical relapse based on morphologic assessment. Eighty-nine follow-up BM samples from 26 patients (range 1-8 BM) were analyzed for MRD (average, three samples per patient). No significant difference was found (with respect to age, gender, WBC, days to first CCR and outcome) between patients eligible for MRD analysis and the remaining 23 patients who were excluded for the reasons listed above (*data not shown*).

Results

TCRG and TCRD gene rearrangements at diagnosis

Forty-one (84%) of the 49 patients analyzed at presentation had at least one γ and/or δ gene rearrangement. Thirty (73.2%) patients had only *TCRG* gene rearrangements, ten (24.4%) had at least one *TCRG* and one *TCRD* rearrangement and one (2.4%) had only *TCRD* gene rearrangement. Bi-allelic *TCRG* gene rearrangements were most common (23/40 patients; 57.5%). In nine of the 11 patients with a *TCRD* clone only one allele was rearranged.

Sixty-five *TCRG* gene rearrangements were identified (Table 2A). The VγI family was involved in the majority (69%). Individual V gene rearrangements most frequently involved Vγ10 (12 alleles; 18,5%) and Vγ4 (12 alleles; 18.5%) followed by Vγ2, Vγ3 and Vγ8 (15.5%, 12.5% and 11%). In one patient a bi-allelic Vγ8-Jγ1.3/2.3 rearrangement was detected with deletion of the CDR2-FR3 regions in one of the alleles and insertion of 18 N nucleotides in the junctional region. There was a preferential use of the Jγ1.3/2.3 segments (56 alleles, 86%) (Table 2A). Analysis of the *TCRD* locus revealed a higher fre-

Table 2. *TCRG* and *TCRD* gene rearrangements in T-ALL.

TCRG gene rearrangements (40 patients)

Vγ family	*VγI*						*VγII*	*VγIII*	*VγIV*	*Total*
A										
	Vγ2	Vγ3	Vγ4	Vγ5	Vγ8	$V\gamma_{1\text{-}8}$ (ns)	Vγ9	Vγ10	Vγ11*	
Jγ1.3/2.3	9 (14%)	8 (12.5%)	12 (18.5%)	3 (4.5%)	7 (11%)	1 (1.5%)	3 (4.5%)	9 (14%)	4 (6%)	56 (86.5%)
Jγ1.1/2.1	1 (1.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (6%)	0 (0%)	3 (4.5%)	1 (1.5%)	9 (13.5%)
Total alleles	10 (15.5%)	8 (12.5%)	12 (18.5%)	3 (4.5%)	7 (11%)	5 (7.5%)	3 (4.5%)	12 (18.5%)	5 (7.5%)	65 (100)%

**One patient had 2 Vγ11 and 1 Vγ2. ns: not sequenced.*

TCRD gene rearrangements (11 patients)

B					
	$V\delta1\text{-}J\delta_{1/2}$	$V\delta2\text{-}J\delta_{1/2}$	Vδ2-Dδ3*	Dδ2-Dδ3**	Total
Number of alleles	9 (69%)	2 (15.5%)	2 (15.5%)	0 (0%)	13 (100%)

***Only 41 of the 49 patients were screened for Vδ2-Dδ3 and Dδ2-Dδ3. 2A) Vγ and Jγ usage in 65 alleles (40 patients). Individual Vγ and Jγ segments were identified by sequencing (Vγ family I segments) or by PCR (VγFII, VγFIII, VγFIV and Jγ segments). 2B) TCRD gene rearrangements detected by PCR in 11/49 cases (13 alleles) and their respective frequency.*

quency of the Vδ1-Jδ$_{1-2}$ (9 alleles; 69%) rearrangement compared to Vδ2-Jδ$_{1-2}$ (two alleles only; 15.5%)(Table 2B). Forty-one of the 49 T-ALL patients were also screened for incomplete *TCRD* gene rearrangements (Vδ2-Dδ3 and Dδ2-Dδ3) but only one patient had a bi-allelic rearrangement for Vδ2-Dδ3.

Junctional regions

Of the 51 *TCRG* rearrangements sequenced, 18 (35%) were in-frame and 33 (65%) out-of-frame (Table 3). The average number of junctional region (N+p nucleotides) base pairs (bp) was 7.9 (range: 0-26)(Table 3). The average number of deleted nucleotides was higher in the Jγ gene segment (4.8 bp) than in the Vγ segment (2.8 bp).

Eight *TCRD* gene rearrangements were sequenced and three were in-frame and five were out-of-frame (Table 3). In these eight Vδ1-Jδ$_{1/2}$ rearrangements an average junctional region (N+D+p) of 30 (14-40) nucleotides was observed (Table 3). The average number of deleted nucleotides was slightly higher in the Vδ segments (3.1; range 0-8) than in the Jδ segments (2.5; range 0-5).

MRD tests and outcome

In 26 patients molecular markers and follow-up samples were available for MRD investigation (Figures 1 and 2).

Despite intensive therapy, persistence of MRD was observed during the first year of treatment. Six (40%) of 15 patients were still positive post-induction; four (19%) of 21 at 3-5 months; five (27.7%) of 18 at 5-9 months and 3 (37.5%) of 8 beyond 9 months. The persistence of residual disease was associated with an increased incidence of relapse among MRD positive patients. Two (33%) of 6 patients positive post-induction and 2 (50%) of 4 patients positive at 3-5 months relapsed. After 5 months, however, four (80%) of 5 tested patients later relapsed, while all three (100%) patients positive after 9 months later relapsed. This indicates that the ability of a positive test to predict relapse was better after 5 months of follow-up than at earlier time points. Thirteen patients showed only negative tests at all times and only two of them (15%) relapsed (#280 and 32A5RF). This suggests that early eradication of detectable disease together with persistence of long-term molecular remission is a good indicator of future CCR.

However, single-time point negative tests had a poor ability to predict outcome. Among patients who had a BM relapse (n=8) seven had been tested between 1 and 5 months earlier, and although four of them tested positive, three tested negative (one on CHT and 2 following autologous SCT).

Table 3. Molecular analysis of *TCRG* and *TCRD* rearrangements. Average number of deleted nucleotides (V+J), p nucleotides, N nucleotides and germinal D nucleotides in 51 *TCRG* and eight *TCRD* junctional regions sequenced.

	V+J deleted nucleotides	*p nucleotides*	*N nucleotides*	*D nucleotides*
TCRG				
In-frame (18)	8.8 (0-19)	0.4 (0-2)	6.6 (0-18)	–
Out-of-frame (33)	6.9 (0-24)	0.5 (0-2)	7.8 (0-26)	–
Total (51)	7.6 (0-24)	0.5 (0-2)	7.4 (0-26)	–
TCRD				
In-frame (3)	4.3 (3-5)	0.0	25.0 (22-27)	14.3 (12-18)
Out-of-frame (5)	6.4 (3-12)	1.6 (0-4)	11.0 (6-15)	12.0 (7-16)
Total (8)	5.6 (3-12)	1.1 (0-4)	16.3 (6-27)	12.9 (7-18)

In six of the nine patients who relapsed, BM samples were available at the time of relapse. In five (#353, 280, 204, 44, and 1) a clone identical to that at presentation was detected, while in one patient the BM tested negative at the time of the extra-medullary relapse. This patient never showed BM morphologic involvement. The clones comprised eight *TCRG* gene rearrangements and one *TCRD* target. Thus false negative tests due to clonal evolution could be excluded in all cases tested. However, at no time was there any statistically significant association between the MRD result and outcome (Fisher's exact test; p=1.00, p=1.00, p=1.00, p=0.40 at 0-2, 3-5, 6-9 and 10-24 months, respectively) or any association between the survival rates and the MRD results (Figure 3).

MRD tests in patients undergoing SCT

Six patients received autologous SCT. There was no association between positive tests prior to transplant or post-transplant and outcome, whether residual disease was detected or not (Figure 2A). Among patients receiving allogeneic transplant (Figure 2B), three (#126, 381 and 1) of eight tested positive during the 4 months prior to transplant. However, only one patient (#1) relapsed. This patient was the only one of three patients tested for MRD 3 and 5 months after transplant. Three of the four patients tested post-transplant were negative for MRD and they all remained in CCR.

Concordance between different clonal markers

For analysis of outcome, the positive test was taken into consideration if discordant results were obtained. In 12 patients, 42 BM samples were ana-

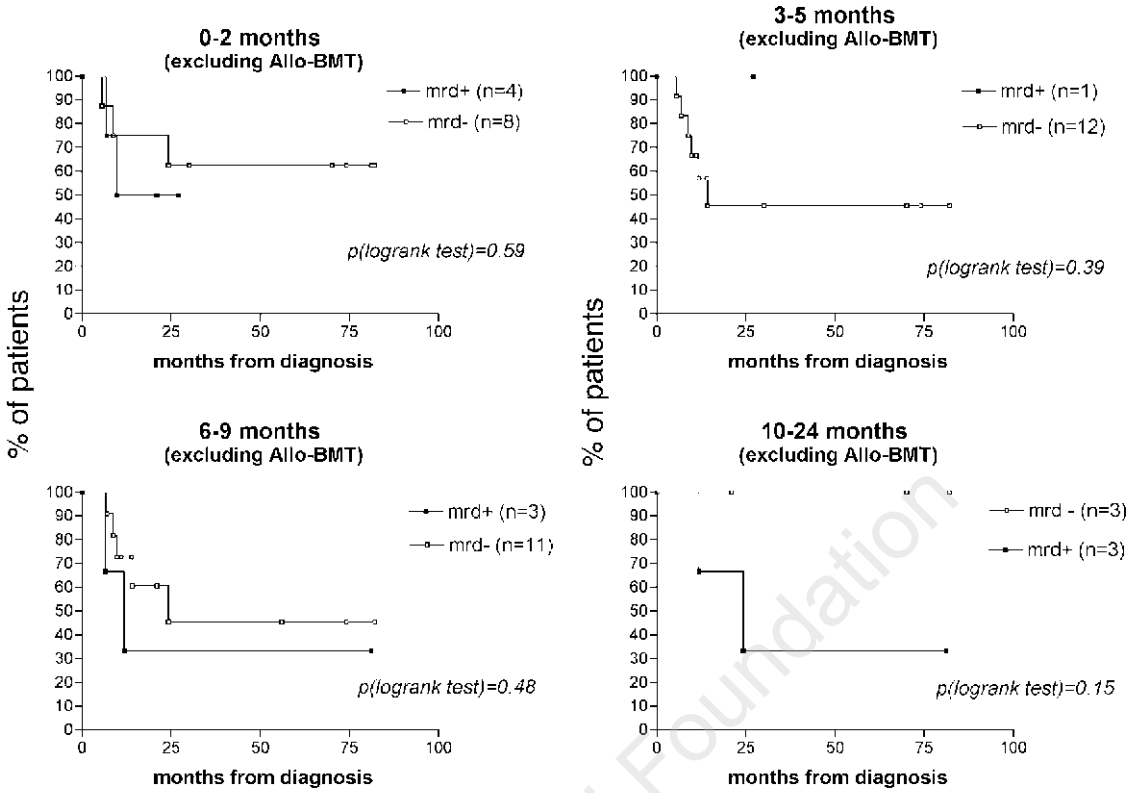

Figure 3. Disease-free survival curve according to MRD-positivity and MRD-negativity at different times of assessment. The patients included in this study were the same as those represented in Figure 2.

lyzed with at least two markers. In seven patients (58%) (corresponding to 76% of the samples-32/42), concordant results were obtained between markers. In the remaining 5 patients (41%) (#2RFH, 41, 44, 381, and 488) (10 samples) discrepant results were observed. Vδ1 primers were consistently more sensitive than Vγ primers (compared to either Vγ10: #41; or Vγ3: #RFH2; or Vγ4: #381) when the two markers were tested in the same sample.

Discussion

These results show the incidence of detectable *TCRD* and *TCRG* clonal gene rearrangements in 49 adult patients with T-ALL and the predictive value of MRD assessment in 26 patients during therapy.

Molecular markers were detected in 84% of patients tested. *TCRG* was the commonest clonal marker (40/49; 81%) and rearrangements were predominantly (58%) biallelic. Vγ family I rearrangements (VfγI-Jγ1.3/2.3) were the most common rearrangements detected, in agreement with previous studies.[29,30] Overall *TCRG* rearrangements were less frequent than previously reported in adult T-ALL[29,30] but in line with the *TCRG* germline configuration being more frequent in adults than in childhood T-ALL.[29] *TCRD* (Vδ1-$J\delta_{1/2}$ or Vδ2-$J\delta_{1/2}$) gene rearrangements were detected in 11 (22%) of the 49 T-ALL patients, a lower incidence than previously reported.[31,32] This could be because we did not estimate deletions of the δ locus that could account for the lower numbers, as we did not apply Southern blotting to our investigation and deletions would not be detected by our PCR strategy. We detected two incomplete Vδ2-Dδ3 rearrangements in one patient, which are rarely found in T-ALL.[32,33]

Overall, analysis of MRD data in T-ALL showed some similarities but more differences with our MRD data from B-cell lineage adult ALL.[15] First, our data show persistence of residual disease in adult T-ALL patients: a third of all patients carry MRD during the first year of treatment, as do patients with B-cell ALL.[15] However, the association between positive tests and relapse was poor in T-ALL during the first 5 months of treatment (33%

and 50%) although it improved beyond this time point (80% and 100%) while the association was statistically significant in B-cell adult ALL.[15]

Second, we found a poor association between MRD results taken before or after autologous or allogeneic SCT and outcome, unlike the prognostically significant results in B-cell ALL, although caution is advised in interpreting results in T-ALL given the limited number of patients analyzed in our study.

Our results in adult T-ALL also differ from those in a recent report on childhood T-ALL, in which Willemse *et al*.[6] detected a significantly higher number of patients and higher levels of residual disease in childhood T-ALL during the first six months of treatment compared to B-cell ALL and pointed at differences in disease type and treatment response between B- and T-cell childhood ALL. These authors did, however, report that MRD levels were valid predictors of outcome at all time points in T-cell ALL as already demonstrated in childhood B-cell ALL. We only confirmed this correlation at a later time point in adults, in agreement with previous molecular assessments of MRD in adult T-ALL.[16,17]

Our data in adult T-ALL patients undergoing SCT differ from those reported for childhood ALL by Knechtli *et al.*,[34] who found that almost 80% of patients MRD positive before transplant later relapse. However, differences in age and transplant procedure (T-cell depletion in childhood but not in adult ALL) may influence outcome. We previously observed that MRD post-allo SCT but not pre-allo SCT was a good indicator of outcome in adult B-cell ALL,[15] further highlighting differences between adult and childhood B-cell ALL. These data indicate that MRD tests obtained from childhood ALL studies cannot be directly applied to adult ALL and that B- and T-cell lineage disease must also be evaluated separately, as we have done in this study and others[6] are now doing for childhood ALL.

How can we explain the differences in the relevance of MRD tests in T-cell ALL and B-cell ALL? First, the number of patients studied was small making statistical analysis difficult and further larger studies in adult T-cell ALL should be carried out.

Second, technical factors, such as poor sensitivity and clonal evolution, could play a role. However, we measured the level of sensitivity of 70% (27/39) of the ASO primers used and 92% of the ASO tested had at least a 10^{-4} sensitivity (Figures 1 and 2), in agreement with published guidelines.[2,35,36] Unfortunately, insufficient material was available for the remaining patients. False negative results due to clonal evolution can be ruled out since only one of six patients analyzed at relapse was ASO negative, most probably due to the clinical extramedullary relapse with lack of BM involvement. In 76% (32/42) of the samples analyzed with two markers, concordant results were obtained. Although rarely, discrepancies between results obtained when multiple markers are investigated have been previously described in a similar proportion of patients (30%) by other investigators using immunophenotyping for MRD assessment.[8] We, therefore, feel that technical faults do not play a major role. The recent development of real time PCR technology, with high levels of sensitivity and excellent reproducibility,[37,38] may contribute to a more rapid and accurate estimate of MRD in childhood and adult ALL and may help to understand the differences in MRD detection between adult T-cell and B-cell ALL.

Finally, we believe that biological differences between B-ALL and T-ALL in adults and children may account for differences in performance of the MRD tests.

T-ALL is now recognized as a disease of the thymus and not of the BM and therefore level of disease in the BM may vary in B- and T-cell leukemia. Indeed, in childhood B-lineage ALL the BM can have a 10-fold higher level of residual disease than the peripheral blood (PB). Recently, Coustain-Smith *et al*.[39] demonstrated that only 8 of 37 B-lineage ALL patients with MRD in the BM had detectable disease in the PB, but showed that in T-ALL MRD in the PB is detected at levels similar to or higher than those detected in the BM.

At present, no data are available in adults to compare levels of disease between BM and PB. The relatively poor performance of BM assessment in our study suggest that BM might not be the most appropriate site for evaluation and this may also relate to the role of the thymus in this disease depending on age. Although recent investigations have demonstrated that the thymus retains a residual ability to generate T-cell progeny in adults,[40] thymopoiesis decreases after 25-30 years of age[41] having peaked to its highest level between 2 and 20 years of age. Monitoring of T-cell receptor excision circles (TREC) molecules and CD3 positive cells in the BM post-transplant always shows a lower level in adults than in children. These changes may translate into different rates of proliferation and cell infiltration of T-ALL cells in the BM in adults and children during progression of the disease and explain some of the differences in the performance of MRD tests.

In conclusion, results from MRD tests using *TCRG* and *TCRD* gene rearrangements were relatively poor indicators of outcome in T-ALL particularly for those tests carried out early after induction therapy. The results suggest that the best information is provided at a late stage of treatment (beyond 6-8 months). Larger studies of adult T-ALL are, however, required to validate these preliminary findings and to investigate the possible cause of the poor overall performance of molecular MRD tests. At present caution should be exercised in basing therapy on data derived from MRD analysis in adult T-ALL patients.

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Disclosures

Conflict of interest: none.

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References

1. Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. N Engl J Med 1998; 339:591-8.
2. van Dongen JJ, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998; 352:1731-8.
3. Biondi A, Valsecchi MG, Seriu T, D'Aniello E, Willemse MJ, Fasching K, et al. Molecular detection of minimal residual disease is a strong predictive factor of relapse in childhood B-lineage acute lymphoblastic leukemia with medium risk features. A case control study of the International BFM study group. Leukemia 2000;14:1939-43.
4. Panzer-Grumayer ER, Schneider M, Panzer S, Fasching K, Gadner H. Rapid molecular response during early induction chemotherapy predicts a good outcome in childhood acute lymphoblastic leukemia. Blood 2000; 95:790-4.
5. Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N, et al. Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood 2002; 99:1253-8.
6. Willemse MJ, Seriu T, Hettinger K, d'Aniello E, Hop WC, Panzer-Grumayer ER, et al. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 2002; 99: 4386-93.
7. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000; 96:2691-6.
8. Ciudad J, San Miguel JF, Lopez-Berges MC, Vidriales B, Valverde B, Ocqueteau M, et al. Prognostic value of immunophenotypic detection of minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol 1998; 16: 3774-81.
9. San Miguel JF, Vidriales MB, Lopez-Berges C, Diaz-Mediavilla J, Gutierrez N, Canizo C, et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 2001; 98:1746-51.
10. Nizet Y, Van Daele S, Lewalle P, Vaerman JL, Philippe M, Vermylen C, et al. Long-term follow-up of residual disease in acute lymphoblastic leukemia patients in complete remission using clonogeneic IgH probes and the polymerase chain reaction. Blood 1993; 82:1618-25.
11. Salo A, Pakkala S, Jansson SE, Helminen P, Palotie A. Monitoring of adult B-cell lineage acute lymphoblastic leukemia: validation of a simple method for detecting immunoglobulin heavy chain gene clonality. Leukemia 1993; 7:1459-65.
12. Scholten C, Fodinger M, Mitterbauer M, Laczika K, Mitterbauer G, Haas OA, et al. Kinetics of minimal residual disease during induction/consolidation therapy in standard-risk adult B-lineage acute lymphoblastic leukemia. Ann Hematol 1995; 71:155-60.
13. Brisco J, Hughes E, Neoh SH, Sykes PJ, Bradstock K, Enno A, et al. Relationship between minimal residual disease and outcome in adult acute lymphoblastic leukemia. Blood 1996; 87:5251-6.
14. Foroni L, Coyle LA, Papaioannou M, Yaxley JC, Sinclair MF, Chim JS, et al. Molecular detection of minimal residual disease in adult and childhood acute lymphoblastic leukaemia reveals differences in treatment response. Leukemia 1997; 11:1732-41.
15. Mortuza FY, Papaioannou M, Moreira IM, Coyle LA, Gameiro P, Gandini D, et al. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol 2002; 20:1094-104.
16. Dibenedetto SP, Lo Nigro L, Di Cataldo A, Schiliro G. Detection of minimal residual disease: methods and relationship to outcome in T-lineage acute lymphoblastic leukemia. Leuk Lymphoma 1998; 32:65-75.
17. Neale GA, Menarguez J, Kitchingman GR, Fitzgerald TJ, Koehler M, Mirro J Jr, et al. Detection of minimal residual disease in T-cell acute lymphoblastic leukemia using polymerase chain reaction predicts impending relapse. Blood 1991; 78:739-47.
18. Durrant IJ, Richards SM, Prentice HG, Goldstone AH. The Medical Research Council trials in adult acute lymphocytic leukemia. Hematol Oncol Clin North Am 2000; 14: 1327-52.
19. Porfiri E, Secker-Walker LM, Hoffbrand AV, Hancock JF. DCC tumor suppressor gene is inactivated in hematologic malignancies showing monosomy 18. Blood 1993; 81: 2696-701.
20. Yokota S, Hansen-Hagge TE, Ludwig WD, Reiter A, Ragha-

vachar A, Kleihauer E, et al. Use of polymerase chain reactions to monitor minimal residual disease in acute lymphoblastic leukemia patients. Blood 1991; 77:331-9.
21. Takihara Y, Tkachuk D, Michalopoulos E, Champagne E, Reimann J, Minden M, et al. Sequence and organization of the diversity, joining, and constant region genes of the human T-cell delta-chain locus. Proc Natl Acad Sci USA 1988; 85:6097-101.
22. Pongers-Willemse MJ, Seriu T, Stolz F, d'Aniello E, Gameiro P, Pisa P, 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.
23. LeFranc MP, Forster A, Baer R, Stinson MA, Rabbitts TH. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. Cell 1986; 45:237-46.
24. Huck S, Lefranc MP. Rearrangements to the JP1, JP and JP2 segments in the human T-cell rearranging γ gene (TRG γ) locus. FEBS Lett 1987; 224:291-6.
25. Huck S, Dariavach P, Lefranc MP. Variable region genes in the human T-cell rearranging γ (TRG) locus: V-J junction and homology with the mouse genes. Embo J 1988; 7:719-26.
26. Langerak AW, Szczepanski T, van der Burg M, Wolvers-Tettero IL, van Dongen JJ. Heteroduplex PCR analysis of rearranged T cell receptor genes for clonality assessment in suspect T cell proliferations. Leukemia 1997; 11:2192-9.
27. Chim JC, Coyle LA, Yaxley JC, Cole-Sinclair MF, Cannell PK, Hoffbrand VA, et al. The use of IgH fingerprinting and ASO-dependent PCR for the investigation of residual disease (MRD) in ALL. Br J Haematol 1996; 92:104-15.
28. Lefranc MP, Giudicelli V, Ginestoux C, Bodmer J, Muller W, Bontrop R, et al. IMGT, the international ImMunoGeneTics database. Nucleic Acids Res 1999; 27:209-12.
29. Szczepanski T, Langerak AW, Willemse MJ, Wolvers-Tettero IL, van Wering ER, van Dongen JJ. T cell receptor γ (TCRG) gene rearrangements in T cell acute lymphoblastic leukemia refelct 'end-stage' recombinations: implications for minimal residual disease monitoring. Leukemia 2000; 14:1208-14.
30. Delabesse E, Burtin ML, Millien C, Madonik A, Arnulf B, Beldjord K, et al. Rapid, multifluorescent TCRG Vγ and Jγ typing: application to T cell acute lymphoblastic leukemia and to the detection of minor clonal populations. Leukemia 2000; 14:1143-52.
31. Szczepanski T, Langerak AW, Wolvers-Tettero IL, Ossenkoppele GJ, Verhoef G, Stul M, et al. Immunoglobulin and T cell receptor gene rearrangement patterns in acute lymphoblastic leukemia are less mature in adults than in children: implications for selection of PCR targets for detection of minimal residual disease. Leukemia 1998; 12:1081-8.
32. Breit TM, Wolvers-Tettero IL, Beishuizen A, Verhoeven MA, van Wering ER, van Dongen JJ. Southern blot patterns, frequencies, and junctional diversity of T-cell receptor-delta gene rearrangements in acute lymphoblastic leukemia. Blood 1993;82(10):3063-74.
33. Seeger K, Taube T, Eckert C, Hanel C, Pogodda M, Henze G. Unusual T-cell receptor-delta gene rearrangement patterns revealed by screening of a large series of childhood acute lymphoblastic leukaemia by multiplex polymerase chain reaction. Br J Haematol 2001; 113:318-22.
34. Knechtli CJ, Goulden NJ, Hancock JP, Grandage VL, Harris EL, Garland RJ, et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood 1998; 92:4072-9.
35. Pui CH, Boyett JM, Rivera GK, Hancock ML, Sandlund JT, Ribeiro RC, et al. Long-term results of Total Therapy studies 11, 12 and 13A for childhood acute lymphoblastic leukemia at St Jude Children's Research Hospital. Leukemia 2000; 14:2286-94.
36. Foroni L, Harrison CJ, Hoffbrand AV, Potter MN. Investigation of minimal residual disease in childhood and adult acute lymphoblastic leukaemia by molecular analysis. Br J Haematol 1999; 105:7-24.
37. Bruggemann M, Droese J, Bolz I, Luth P, Pott C, von Neuhoff N, et al. Improved assessment of minimal residual disease in B cell malignancies using fluorogenic consensus probes for real-time quantitative PCR. Leukemia 2000; 14:1419-25.
38. van der Velden VH, Willemse MJ, van der Schoot CE, Hahlen K, van Wering ER, van Dongen JJ. Immunoglobulin kappa 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.
39. Coustan-Smith E, Sancho J, Behm FG, Hancock ML, Razzouk B, Ribeiro RC, et al. Comparative analysis of minimal residual disease in peripheral blood versus bone marrow in childhood acute lymphoblastic leukaemia. Blood, 2001 American Society of Haematology, Orlando, USA, Abstract 3497.
40. Mackall CL, Punt JA, Morgan P, Farr AG, Gress RE. Thymic function in young/old chimeras: substantial thymic T cell regenerative capacity despite irreversible age-associated thymic involution. Eur J Immunol 1998; 28:1886-93.
41 Steffens CM, Al-Harthi L, Shott S, Yogev R, Landay A. Evaluation of thymopoiesis using T cell receptor excision circles (TRECs): differential correlation between adult and pediatric TRECs and naive phenotypes. Clin Immunol 2000; 97:95-101.

Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received April 29, 2002; accepted September 4, 2002.

What is already known on this topic

Minimal residual disease is important in the measurement of response to treatment in childhood acute lymphoblastic leukemia (ALL) but little is known about its evaluation in adult T-cell ALL.

What this study adds

Evaluation of minimal residual disease using TCRG and TCRD gene rearrangements does not allow a reliable prediction of prognosis in adult T-cell ALL.

Potential implications for clinical practice

Caution should be exercised in basing therapy on data derived from minimal residual disease analysis in adult T-ALL patients.

Mario Cazzola, Editor-in-Chief (Pavia, Italy)