

## Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia after first relapse

E Coustan-Smith<sup>1</sup>, A Gajjar<sup>1,4</sup>, N Hijiya<sup>1,4</sup>, BI Razzouk<sup>1,4</sup>, RC Ribeiro<sup>1,4</sup>, GK Rivera<sup>1,4</sup>, JE Rubnitz<sup>1,4</sup>, JT Sandlund<sup>1,4</sup>, M Andreansky<sup>1</sup>, ML Hancock<sup>2</sup>, C-H Pui<sup>1,3,4</sup> and D Campana<sup>1,3,4</sup>

<sup>1</sup>Department of Hematology-Oncology, Children's Research Hospital, Memphis, TN, USA; <sup>2</sup>Department of Biostatistics, St Jude Children's Research Hospital, Memphis, TN, USA; <sup>3</sup>Department of Pathology, Children's Research Hospital, Memphis, TN, USA; and <sup>4</sup>The University of Tennessee Health Science Center, Memphis TN, USA

**Using flow cytometric techniques capable of detecting 0.01% leukemic cells, we prospectively studied minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL) after first relapse. At the end of remission reinduction, 41 patients had a bone marrow sample adequate for MRD studies; 35 of these were in morphologic remission. Of the 35 patients, 19 (54%) had MRD  $\geq$  0.01%, a finding that was associated with subsequent leukemia relapse. The 2-year cumulative incidence of second leukemia relapse was  $70.2 \pm 12.3\%$  for the 19 MRD-positive patients and  $27.9 \pm 12.4\%$  for the 16 MRD-negative patients ( $P=0.008$ ). Among patients with a first relapse off therapy, 2-year second relapse rates were  $49.1 \pm 17.8\%$  in the 12 MRD-positive and 0% in the 11 MRD-negative patients ( $P=0.014$ ); among those who received only chemotherapy after first relapse, the 2-year second relapse rates were  $81.5 \pm 14.4\%$  ( $n=12$ ) and  $25.0 \pm 13.1\%$  ( $n=13$ ), respectively ( $P=0.004$ ). Time of first relapse and MRD were the only two significant predictors of outcome in a multivariate analysis. We conclude that MRD assays should be used to guide the selection of postremission therapy in patients with ALL in first relapse.**

*Leukemia* (2004) 18, 499–504. doi:10.1038/sj.leu.2403283

**Keywords:** acute lymphoblastic leukemia; minimal residual disease; relapse; flow cytometry

### Introduction

In approximately 20% of children with acute lymphoblastic leukemia (ALL), disease recurs despite intensive multiagent chemotherapy.<sup>1</sup> Relapse is the most common cause of treatment failure in childhood ALL; relapsed ALL is as common as most pediatric solid tumors and more common than acute myeloid leukemia in childhood.<sup>2</sup>

With current therapy, second remissions are achieved in the majority of patients, but overall cure rates seldom exceed 30%.<sup>2–14</sup> Clinical features that predict poorer outcome in patients with relapsed ALL include shorter duration of first remission<sup>4,12,15,16</sup> and relapse in the bone marrow.<sup>12,17,18</sup> Adverse biologic features include T-cell immunophenotype<sup>5,18</sup> and Philadelphia chromosome.<sup>19</sup>

Assays of minimal, that is, submicroscopic, minimal residual disease (MRD) provide strong prognostic information in children with newly diagnosed ALL,<sup>20–26</sup> and such assays are increasingly used to select intensity of postremission treatment.<sup>27,28</sup> Retrospective studies with stored samples have suggested that MRD

could also be prognostically important in patients who achieve a second remission,<sup>29,30</sup> but incorporation of MRD assays into treatment protocols for relapsed ALL requires further clarification of its clinical significance and of its relation with other features of the disease. Here, we report the results of a prospective study of MRD assayed by four-color flow cytometry at the end of remission reinduction in patients with ALL in first relapse.

### Materials and methods

#### Patients

We prospectively studied bone marrow samples obtained from patients with ALL in first relapse enrolled in the St Jude Children's Research Hospital studies for first relapse ALL between March 1993 and March 2003. The only criteria for inclusion in the study was the availability of a sample at diagnosis and/or relapse (to identify suitable immunophenotypes for flow cytometric studies of MRD) and of an adequate bone marrow at the end of remission induction for MRD studies. We studied the leukemia immunophenotypes in 57 patients (40 with B-lineage and 17 with T-lineage ALL) aged 8 months to 21 years (median, 10 years). Cells in 52 of the 57 patients (91%) expressed phenotypes suitable for flow cytometric studies of MRD.<sup>31,32</sup> Of these, 41 had a sample obtained at the end of remission reinduction (day 36) with sufficient cells for flow cytometric studies of MRD. Rate of remission failure and second ALL relapse for these 41 patients were not significantly different than those of the 16 patients who could not be studied because of the lack of suitable phenotype or lack of adequate day 36 sample. These studies were approved by the St Jude Institutional Review Board.

#### Treatment protocol

Patients were enrolled in successive St Jude treatment protocols for relapsed ALL.<sup>10,33,34</sup> Remission induction treatment included dexamethasone, vincristine and asparaginase, as well as etoposide or topotecan. Intrathecal injection of methotrexate, hydrocortisone and cytarabine were also administered during remission induction as CNS-directed therapy. The patients who achieved morphologic remission ( $n=35$ ) received two courses of consolidation therapy followed by continuation chemotherapy. Of these, 10 received hematopoietic stem cell transplantation, three from a related donor and seven from an unrelated donor. It should be noted that MRD results were not communicated to the physicians and were not a factor in treatment decisions.

Correspondence: D Campana, Department of Hematology-Oncology, St Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105-2794, USA; Fax: +1 901 495 3749; E-mail: dario.campana@stjude.org  
Received 3 October 2003; accepted 5 November 2003

### Flow cytometric assessment of MRD

Bone marrow aspirates were collected in preservative-free heparin. Leukemia-associated immunophenotypes (patterns of cell markers expressed on leukemic cells but not on normal bone marrow cells) were identified by flow cytometry with various combinations of monoclonal antibodies and/or heterologous antisera conjugated to fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein and allophycocyanin.<sup>22,24,31,32,35,36</sup> Matched nonreactive fluorochrome-conjugated antibodies were used as controls. The staining procedure has been described.<sup>32</sup> For each case, one or more marker combinations that allowed the identification of one leukemic cell in  $10^4$  or more normal nucleated bone marrow cells were selected at diagnosis or at first relapse and applied to study MRD at the end of remission induction.<sup>32</sup> In the early part of the study, we used three-color analysis with a single-laser FACScan flow cytometer; after August 1998, all samples were assayed by four-color analysis and analyzed with a dual laser-FACScalibur flow cytometer (both cytometers were from Becton Dickinson, San Jose, CA, USA). The flow cytometry protocol used for MRD detection has been described in detail previously.<sup>32</sup> In each test of each sample, we acquired data from more than  $10^5$  mononuclear cells. Detectable MRD was defined as 0.01% or more leukemic cells among mononuclear cells in the sample.

### Statistical analysis

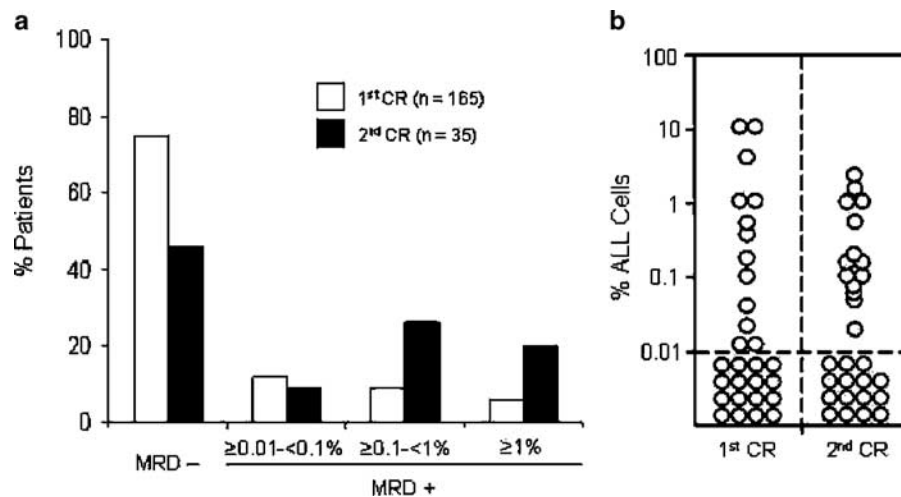
Differences in the distributions of clinical and cellular features by the presence or absence of MRD at day 36 were compared by the Fisher's exact test. The cumulative incidence of second ALL relapse was estimated by the method of Kalbfleisch and Prentice,<sup>37</sup> which accounts for competing risks (second malignancies and deaths during remission) and compared by Gray's test stratified for treatment protocol, and for chemotherapy alone or hematopoietic stem cell transplantation.<sup>38</sup> The cutoff date of follow-up observations was May 2003. Time at risk was calculated from the date of second remission until the date of

relapse, occurrence of a competing event or the date of last contact for patients who experienced no event. The significance of the presence or absence of MRD and other factors of potential prognostic value (time of relapse, site of relapse, cell lineage and percentage of circulating blasts at first relapse) were analyzed in the proportional-hazards model suggested by Fine and Gray.<sup>39</sup> The factors found significant in the univariate analyses were then analyzed jointly in a multivariate manner. Hazard ratios and their associated 95% confidence intervals were calculated from the coefficients and standard errors estimated by the model.

### Results

#### *Prevalence of MRD after remission reinduction and its relation with clinical and cellular features*

We prospectively measured the percentage of residual leukemic cells among bone marrow mononuclear cells collected after remission reinduction (day 36) in the 41 patients with a leukemia-associated immunophenotype. Six of these patients failed to achieve remission with the scheduled course of remission induction therapy and all had leukemic cells detectable by flow cytometry. Of the remaining 35 patients who achieved a second morphologic remission, 19 (54%) had  $\geq 0.01\%$  leukemic cells identifiable by flow cytometry in the bone marrow. Among these patients, the levels of leukemia were  $0.01 - < 0.1\%$  in three patients (9%),  $0.1 - < 1\%$  in nine (26%) and  $\geq 1\%$  in seven (20%). In the remaining 16 patients (46%), leukemic cells were below the limit of detection of our technique (0.01%). Collectively, these results contrast with those obtained in patients with newly diagnosed ALL, 75% of whom were MRD negative at the end of remission induction; among those who were MRD positive, the levels of MRD  $\geq 1\%$  were infrequent (Figure 1a).<sup>24</sup> However, a direct comparison of MRD levels measured at first and second remission date in the same patients indicated similar rates of residual disease: of the 29 patients in whom both measurements were available, MRD



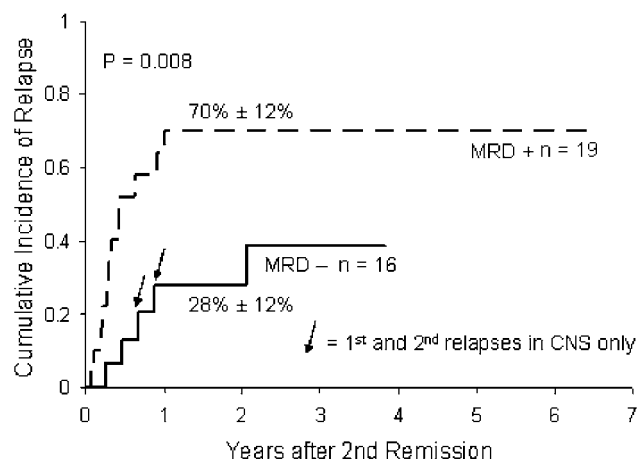
**Figure 1** Prevalence of MRD after remission induction therapy in patients with relapsed ALL compared to that of patients with newly diagnosed ALL. All patients included in the analysis were in morphologic remission. Identical MRD methodology was used to study MRD in the two groups. (a) Bars show the proportion of patients with no detectable MRD (MRD negative) and with various levels of MRD (MRD positive) at first and second complete remission (CR). MRD data in patients with newly diagnosed ALL have been reported previously.<sup>24</sup> (b) Prevalence of MRD in a group of 29 patients studied at first and second CR.

$\geq 0.01\%$  was detected in 13 patients at first remission and in 14 patients at second remission (Figure 1b).

The presence or absence of residual leukemia by flow cytometry at day 36 was not significantly related to age, gender, time of relapse or presence of circulating blasts at relapse, although four out of five patients with peripheral blood lymphoblasts  $\geq 10 \times 10^9/l$  had MRD (Table 1). All four patients with first extramedullary relapse (central nervous system (CNS)=3; testicular=1) were MRD negative at day 36 (Table 1). Rates of detection did not differ significantly according to cell lineage, presence of the Philadelphia chromosome, 11q23 abnormalities or *TEL/AML1* transcripts, or ploidy. It is worth noting, however, that four of eight patients with B-lineage ALL and hyperdiploidy (51–65 chromosomes) or *TEL* gene rearrangements were MRD positive after remission induction. Among patients with newly diagnosed ALL, only 10 of 57 with those abnormalities were MRD positive ( $P=0.058$ ).<sup>24</sup>

### Detection of MRD and risk of second leukemia relapse

We determined the prognostic impact of MRD in the cohort of 35 patients who achieved morphologic remission after the scheduled remission reinduction therapy. The 2-year cumulative incidence of relapse for the 19 patients with detectable ( $\geq 0.01\%$ ) leukemic cells at day 36 was  $70.2 \pm 12.3$  vs  $27.9 \pm 12.4\%$  for the 16 patients with negative findings ( $P=0.008$ ; Figure 2). All 12 second relapses in the MRD-positive group were hematological and nine of these occurred within 6 months after first relapse. By contrast, of the five relapses in the MRD-negative group, three were hematological and occurred 4, 7 and 25 months after first relapse; the other two second relapses in this group were limited to the CNS and occurred in patients who had isolated CNS first relapses. After excluding the four patients who had an isolated extramedullary first relapse (all of whom were MRD negative at day 36) from the analysis, the 2-year cumulative incidence of relapse for the 12 MRD-negative patients was  $18.2 \pm 12.3\%$  ( $P=0.013$ ). The leukemia-associated immunophenotypes used to monitor MRD were retained in all 14 patients tested at the time of second hematological relapse (11 of the 12 in the MRD-positive group and all three in the MRD-negative group). Therefore, negative



**Figure 2** Cumulative incidence of relapse in patients with relapsed ALL according to MRD after remission induction therapy. All patients included in the analysis were in morphologic remission at the time of the MRD assay.

MRD findings at day 36 in these patients could not be attributed to immunophenotypic shifts.

MRD was a significant predictor of outcome in patients who had their first relapse after cessation of therapy: in this group, 2-year cumulative incidence of relapse for the 12 patients with a positive MRD test was  $49.1 \pm 17.8$  vs  $0\%$  for the 11 MRD-negative patients ( $P=0.014$ ). Five of 12 patients with detectable MRD relapsed, all within 1 year of the MRD assay. By contrast, only one of the 11 MRD-negative patients relapsed 25 months after the negative MRD assay. The 2-year relapse rate did not change if the two patients who had an isolated extramedullary relapse off therapy were removed from the MRD-negative group ( $P=0.026$ ). As expected, the 12 patients who relapsed on therapy had a dismal outcome: all seven who were MRD positive at day 36 relapsed, as did four of five who were MRD negative. Therefore, relapse on therapy carries a high risk of second relapse irrespective of achievement of MRD negativity after remission reinduction.

Of the 35 patients included in the study, 25 were treated with chemotherapy only and 10 received subsequent hematopoietic

**Table 1** MRD after remission induction and clinical and cellular features of the disease in patients with relapsed ALL

Disease feature	MRD <0.01%	MRD $\geq 0.01\%$	P-value <sup>a</sup>
Age (years)			
<1	1	1	
1–9	9	5	
$\geq 10$	6	13	0.149
Gender			
Female	3	8	
Male	13	11	0.167
Time of relapse			
On therapy	5	7	
Off therapy	11	12	1.000
Site of relapse			
Bone marrow	7	15	
Combined	5	4	
Extramedullary	4	0	0.027
Circulating blasts <sup>b</sup>			
< $10 \times 10^9/l$	7	11	
$\geq 10 \times 10^9/l$	0	4	0.263
Lineage			
B	12	15	
T	4	4	1.000
<i>BCR/ABL</i> <sup>c</sup>			
Yes	1	2	
No	9	13	1.000
11q23 abnormalities <sup>c</sup>			
Yes	3	2	
No	7	13	0.358
<i>TEL</i> rearrangements <sup>c</sup>			
Yes	2	2	
No	8	13	1.000
51–65 chromosomes <sup>c</sup>			
Yes	2	2	
No	8	13	1.000

<sup>a</sup>Fisher's exact test.

<sup>b</sup>Isolated bone marrow relapses only.

<sup>c</sup>B-lineage only; not done on extramedullary relapses ( $n=2$ ).

**Table 2** Univariate and multivariate analysis of factors prognostic of second leukemic relapse

Adverse feature	Univariate				Multivariate			
	Coefficient	Standard error	Risk ratio (95% CI) <sup>a</sup>	P-value	Coefficient	Standard error	Risk ratio (95% CI) <sup>a</sup>	P-value
Relapse on therapy	1.801	0.5128	6.01 (2.22, 16.55)	0.001	1.845	0.5321	6.32 (2.23, 17.96)	0.001
MRD $\geq 0.01\%$	1.171	0.4987	3.23 (1.21, 8.57)	0.02	1.223	0.4516	3.40 (1.40, 8.23)	0.007
Bone marrow relapse	0.005	0.2536	1.01(0.61, 1.65)	0.98				
T-lineage	-0.113	0.5327	0.89 (0.31, 2.54)	0.83				
Circulating blasts $> 10 \times 10^9/l$	-1.443	0.9217	0.24 (0.039, 1.44)	0.12				

<sup>a</sup>Risk ratio and its 95% confidence interval.

stem cell transplant. Among patients who received chemotherapy alone after first relapse, 2-year cumulative incidence of second relapse was  $81.5 \pm 14.4\%$  for the 12 MRD-positive patients and  $25.0 \pm 13.1\%$  for the 13 MRD-negative patients ( $P=0.004$ ). Seven of the 10 patients who received hematopoietic stem cell transplantation were MRD positive at the end of remission reinduction: three relapsed post-transplant, two died of transplant-related complications and two were in remission at the time of this analysis. The remaining three patients were MRD negative: one relapsed post-transplant, one died of complications and one is in remission.

As shown in Table 2, on-therapy relapse and the presence of MRD at day 36 were independently associated with subsequent leukemia relapse. MRD at the end of remission reinduction after adjusting for the effect of time of relapse was associated with a risk ratio of 3.40 (95% confidence interval: 1.40–8.23;  $P=0.007$ ).

## Discussion

MRD assays are increasingly used to adjust the intensity of treatment in patients with ALL and have been incorporated in frontline protocols.<sup>40</sup> The results of this prospective study indicate that MRD monitoring by flow cytometry could also be helpful in the clinical management of patients with relapsed ALL. We found that approximately half of patients with relapsed ALL who achieved a morphologic remission have 0.01% or more residual leukemic cells in bone marrow and that this finding was associated with a significantly higher risk of a second leukemia relapse. In particular, MRD was a strong predictor of outcome among patients who had a first relapse off therapy. By contrast, patients who had a first relapse on therapy did poorly irrespective of achieving MRD-negative status at the end of remission induction.

The reported experience on the clinical significance of MRD after second remission induction in patients with relapsed ALL is scanty. Steenbergen *et al*<sup>29</sup> used polymerase chain reaction (PCR) amplification of antigen-receptor genes and archived samples to compare MRD after remission induction in patients with newly diagnosed and relapsed B-lineage ALL. MRD was detectable in only two of the 18 newly diagnosed patients but in 10 of the 16 with relapsed disease. We also found that the prevalence of MRD at the time of second remission was markedly higher than that determined at first remission (54 vs 25%), and that the levels of residual leukemia among MRD-positive patients in second remission were higher. However, because MRD in first remission was more prevalent in the group of patients who subsequently relapsed, MRD measurements at first and second remission were similar when directly compared in the same group of patients. In a retrospective study of bone

marrow samples collected from 26 patients with relapsed ALL treated in the ALL-REZ BFM studies, Eckert *et al*<sup>30</sup> found that levels of MRD  $\geq 0.1\%$  at the end of remission reinduction, as determined by PCR amplification of clonal immunoglobulin or T-cell receptor genes, was associated with a poorer event-free survival. Our results generally agree with this finding and demonstrate that the presence of MRD after remission reinduction is directly and independently associated with a second recurrence of leukemia. In our study, the 0.01% cutoff appeared to be more informative than the 0.1% level use by Eckert *et al*; all three patients with MRD  $\geq 0.01\%$  but  $< 0.1\%$  subsequently relapsed.

Bone marrow relapse is the most frequent indication for allogeneic hematopoietic stem cell transplantation in children.<sup>41</sup> As only 10 patients received transplantation, our study does not clarify the significance of MRD at the end of remission reinduction in this context. However, other studies have shown that sequential MRD monitoring before and after transplant may be useful to help select clinical strategies.<sup>29,42–46</sup> Owing to the toxicities associated with transplantation, it is important to identify patients who can be cured with chemotherapy alone. We suggest that MRD assays postremission induction could be particularly useful in patients with 'standard risk' relapsed ALL (ie those who have a hematologic relapse off therapy). In our current treatment plan for relapsed ALL, a high level of MRD ( $\geq 1\%$ ) after a 6-week remission induction is an indication for allogeneic hematopoietic stem cell transplant, even for patients with off-therapy relapse. MRD is then sequentially monitored and transplant is performed as soon as MRD is reduced by intensive consolidation therapy and a suitable donor is available. Patients with off-therapy relapse and lower levels of detectable MRD ( $\geq 0.01$ – $< 1\%$ ) after remission will be closely monitored and offered a transplant if MRD persists.

## Acknowledgements

We thank Peixin Liu and Mo Mehrpooya for technical assistance and Yinmei Zhou for assistance with the statistical analysis. This work was supported by Grants CA60419 and CA21765 from the National Cancer Institute and by the American Lebanese Syrian Associated Charities (ALSAC). C-H Pui is supported by the American Cancer Society FM Kirby Clinical Research Professorship.

## References

- 1 Pui CH, Campana D, Evans WE. Childhood acute lymphoblastic leukemia – current status and future perspectives. *Lancet Oncol* 2001; **2**: 597–607.
- 2 Chessells JM. Relapsed lymphoblastic leukaemia in children: a continuing challenge. *Br J Haematol* 1998; **102**: 423–438.

- 3 Rivera GK, Buchanan G, Boyett JM, Camitta B, Ochs J, Kalwinsky D *et al*. Intensive retreatment of childhood acute lymphoblastic leukemia in first bone marrow relapse. A Pediatric Oncology Group Study. *N Engl J Med* 1986; **315**: 273–278.
- 4 Buchanan GR, Rivera GK, Boyett JM, Chauvenet AR, Crist WM, Vietti TJ. Reinduction therapy in 297 children with acute lymphoblastic leukemia in first bone marrow relapse: a Pediatric Oncology Group Study. *Blood* 1988; **72**: 1286–1292.
- 5 Henze G, Fengler R, Hartmann R, Kornhuber B, Janka-Schaub G, Niethammer D *et al*. Six-year experience with a comprehensive approach to the treatment of recurrent childhood acute lymphoblastic leukemia (ALL-REZ BFM 85). A relapse study of the BFM group. *Blood* 1991; **78**: 1166–1172.
- 6 Sadowitz PD, Smith SD, Shuster J, Wharam MD, Buchanan GR, Rivera GK. Treatment of late bone marrow relapse in children with acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood* 1993; **81**: 602–609.
- 7 von der Weid N, Wagner B, Angst R, Arnet B, Baumgartner C, Beck D *et al*. Treatment of relapsing acute lymphoblastic leukemia in childhood. III. Experiences with 54 first bone marrow, nine isolated testicular, and eight isolated central nervous system relapses observed 1985–1989. *Med Pediatr Oncol* 1994; **22**: 361–369.
- 8 Miniero R, Saracco P, Pastore G, Zurlo MG, Terracini B, Rosso P *et al*. Relapse after first cessation of therapy in childhood acute lymphoblastic leukemia: a 10-year follow-up study. Italian Association of Pediatric Hematology-Oncology (AIEOP). *Med Pediatr Oncol* 1995; **24**: 71–76.
- 9 Feig SA, Harris RE, Sather HN. Bone marrow transplantation versus chemotherapy for maintenance of second remission of childhood acute lymphoblastic leukemia: a study of the Children's Cancer Group (CCG-1884). *Med Pediatr Oncol* 1997; **29**: 534–540.
- 10 Rivera GK, Hudson MM, Liu Q, Benaim E, Ribeiro RC, Crist WM *et al*. Effectiveness of intensified rotational combination chemotherapy for late hematologic relapse of childhood acute lymphoblastic leukemia. *Blood* 1996; **88**: 831–837.
- 11 Giona F, Testi AM, Rondelli R, Amadori S, Arcese W, Meloni G *et al*. ALL R-87 protocol in the treatment of children with acute lymphoblastic leukaemia in early bone marrow relapse. *Br J Haematol* 1997; **99**: 671–677.
- 12 Gaynon PS, Qu RP, Chappell RJ, Willoughby ML, Tubergen DG, Steinhilber PG *et al*. Survival after relapse in childhood acute lymphoblastic leukemia: impact of site and time to first relapse – the Children's Cancer Group Experience. *Cancer* 1998; **82**: 1387–1395.
- 13 Yumura-Yagi K, Hara J, Horibe K, Tawa A, Komada Y, Oda M *et al*. Outcome after relapse in childhood acute lymphoblastic leukemia. *Int J Hematol* 2002; **76**: 61–68.
- 14 Buchanan GR, Rivera GK, Pollock BH, Boyett JM, Chauvenet AR, Wagner H *et al*. Alternating drug pairs with or without periodic reinduction in children with acute lymphoblastic leukemia in second bone marrow remission: a Pediatric Oncology Group Study. *Cancer* 2000; **88**: 1166–1174.
- 15 Schroeder H, Garwicz S, Kristinsson J, Siimes MA, Wesenberg F, Gustafsson G. Outcome after first relapse in children with acute lymphoblastic leukemia: a population-based study of 315 patients from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). *Med Pediatr Oncol* 1995; **25**: 372–378.
- 16 Morris EC, Harrison G, Bailey CC, Hann IM, Hill FG, Gibson BE *et al*. Prognostic factors and outcome for children after second central nervous system relapse of acute lymphoblastic leukaemia. *Br J Haematol* 2003; **120**: 787–789.
- 17 Buhner C, Hartmann R, Fengler R, Dopfer R, Gadner H, Gerein V *et al*. Superior prognosis in combined compared to isolated bone marrow relapses in salvage therapy of childhood acute lymphoblastic leukemia. *Med Pediatr Oncol* 1993; **21**: 470–476.
- 18 Wheeler K, Richards S, Bailey C, Chessells J. Comparison of bone marrow transplant and chemotherapy for relapsed childhood acute lymphoblastic leukaemia: the MRC UKALL X experience. Medical Research Council Working Party on Childhood Leukaemia. *Br J Haematol* 1998; **101**: 94–103.
- 19 Beyersmann B, Agthe AG, Adams HP, Seeger K, Linderkamp C, Goetze G *et al*. Clinical features and outcome of children with first marrow relapse of acute lymphoblastic leukemia expressing BCR-ABL fusion transcripts. BFM Relapse Study Group. *Blood* 1996; **87**: 1532–1538.
- 20 Brisco MJ, Condon J, Hughes E, Neoh SH, Sykes PJ, Seshadri R *et al*. Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction. *Lancet* 1994; **343**: 196–200.
- 21 Cave H, van der Werff ten Bosch J, Suci 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**: 591–598.
- 22 Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC *et al*. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet* 1998; **351**: 550–554.
- 23 van Dongen JJ, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemsse MJ, Corral L *et al*. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet* 1998; **352**: 1731–1738.
- 24 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**: 2691–2696.
- 25 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**: 1253–1258.
- 26 Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N *et al*. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood* 2002; **99**: 1952–1958.
- 27 Pui CH, Campana D. New definition of remission in childhood acute lymphoblastic leukemia. *Leukemia* 2000; **14**: 783–785.
- 28 Campana D. Determination of minimal residual disease in leukemia patients. *Br J Haematol* 2003; **121**: 823–838.
- 29 Steenbergen EJ, Verhagen OJ, van Leeuwen EF, van den Berg H, Behrendt H, Slater RM *et al*. Prolonged persistence of PCR-detectable minimal residual disease after diagnosis or first relapse predicts poor outcome in childhood B-precursor acute lymphoblastic leukemia. *Leukemia* 1995; **9**: 1726–1734.
- 30 Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Beyersmann B *et al*. Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. *Lancet* 2001; **358**: 1239–1241.
- 31 Coustan-Smith E, Sancho J, Behm FG, Hancock ML, Razzouk BI, Ribeiro RC *et al*. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. *Blood* 2002; **100**: 52–58.
- 32 Campana D, Coustan-Smith E. Advances in the immunological monitoring of childhood acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol* 2002; **15**: 1–19.
- 33 Wall AM, Gajjar A, Link A, Mahmoud H, Pui CH, Relling MV. Individualized methotrexate dosing in children with relapsed acute lymphoblastic leukemia. *Leukemia* 2000; **14**: 221–225.
- 34 Edick MJ, Gajjar A, Mahmoud HH, van de Poll ME, Harrison PL, Panetta JC *et al*. Pharmacokinetics and pharmacodynamics of oral etoposide in children with relapsed or refractory acute lymphoblastic leukemia. *J Clin Oncol* 2003; **21**: 1340–1346.
- 35 Coustan-Smith E, Sancho J, Hancock ML, Razzouk BI, Ribeiro RC, Rivera GK *et al*. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. *Blood* 2002; **100**: 2402.
- 36 Coustan-Smith E, Ribeiro RC, Rubnitz JE, Razzouk BI, Pui CH, Pounds S *et al*. Clinical significance of residual disease during treatment in childhood acute myeloid leukemia. *Br J Haematol* 2003; **123**: 243.
- 37 Kalbfleisch JD, Prentice RL. *The Statistical Analysis of Failure Time Data*. New York: John Wiley & Sons, 1980.
- 38 Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988; **16**: 1141–1154.
- 39 Fine JP, Gray J. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
- 40 Gadner H, Haas OA, Masera G, Pui CH, Schrappe M. 'Ponte di Legno' Working Group – report on the Fifth International Child-

- hood Acute Lymphoblastic Leukemia Workshop: Vienna, Austria, 29 April–1 May 2002. *Leukemia* 2003; **17**: 798–803.
- 41 Borgmann A, Von Stackelberg A, Hartmann R, Ebell W, Klingebiel T, Peters C *et al*. Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: a matched-pair analysis. *Blood* 2003; **101**: 3835–3839.
- 42 Radich J, Gehly G, Lee A, Avery R, Bryant E, Edmands S *et al*. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. *Blood* 1997; **89**: 2602–2609.
- 43 Knechtli CJC, Goulden NJ, Hancock JP, Grandage VLC, 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**: 4072–4079.
- 44 van der Velden V, Joosten SA, Willemsse MJ, Van Wering ER, Lankester AW, van Dongen JJ *et al*. Real-time quantitative PCR for detection of minimal residual disease before allogeneic stem cell transplantation predicts outcome in children with acute lymphoblastic leukemia. *Leukemia* 2001; **15**: 1485–1487.
- 45 Bader P, Hancock J, Kreyenberg H, Goulden NJ, Niethammer D, Oakhill A *et al*. Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. *Leukemia* 2002; **16**: 1668–1672.
- 46 Goulden N, Bader P, van der Velden V, Moppett J, Schilham M, Masden HO *et al*. Minimal residual disease prior to stem cell transplant for childhood acute lymphoblastic leukaemia. *Br J Haematol* 2003; **122**: 24–29.