Title	Clinical significance of minimal residual disease in adult acute lymphoblastic leukemia
Author(s)	Kikuchi, Misato; Tanaka, Junji; Kondo, Takeshi; Hashino, Satoshi; Kasai, Masaharu; Kurosawa, Mitsutoshi; Iwasaki, Hiroshi; Morioka, Masanobu; Kawamura, Tsugumichi; Masauzi, Nobuo; Fukuhara, Takashi; Kakinoki, Yasutaka; Kobayashi, Hajime; Noto, Satoshi; Asaka, Masahiro; Imamura, Masahiro
Citation	International Journal of Hematology, 92(3), 481-489 https://doi.org/10.1007/s12185-010-0670-1
Issue Date	2010-10
Doc URL	http://hdl.handle.net/2115/44159
Rights	The final publication is available at www.springerlink.com
Type	article (author version)
File Information	IJH92-3_481-489.pdf

[Instructions for use](#)

Running title: Minimal residual disease in adult ALL

Clinical significance of minimal residual disease in adult acute lymphoblastic leukemia

Misato Kikuchi ¹⁾, Junji Tanaka ¹⁾, *, Takeshi Kondo ²⁾, Satoshi Hashino ²⁾, Masaharu Kasai ³⁾, Mitsutoshi Kurosawa ⁴⁾, Hiroshi Iwasaki ⁵⁾, Masanobu Morioka ⁶⁾, Tsugumichi Kawamura ⁷⁾, Nobuo Masauzi ⁸⁾, Takashi Fukuhara ⁹⁾, Yasutaka Kakinoki ¹⁰⁾, Hajime Kobayashi ¹¹⁾, Satoshi Noto ¹²⁾, Masahiro Asaka ²⁾, and Masahiro Imamura ¹⁾

¹⁾Department of Hematology and Oncology, ²⁾Third Department of Internal Medicine, Hokkaido University Graduate School of Medicine.

³⁾Department of Hematology, Sapporo Hokuyu Hospital.

⁴⁾Department of Hematology, National Hospital Organization, Hokkaido Cancer Center.

⁵⁾Second Department of Internal Medicine, Sapporo Kosei Hospital.

⁶⁾Department of Internal Medicine, Aiiku Hospital.

⁷⁾Department of Internal Medicine, Hakodate Central Hospital.

⁸⁾Department of Hematology, Hakodate City Hospital.

⁹⁾Department of Internal Medicine, Asahikawa City Hospital.

¹⁰⁾Department of Internal Medicine, Asahikawa Kosei Hospital.

¹¹⁾Fourth Department of Internal Medicine, Obihiro Kosei Hospital.

¹²⁾Department of Internal Medicine, Kushiro Rosai Hospital.

* Corresponding author. Junji Tanaka, Department of Hematology and Oncology,
Hokkaido University Graduate School of Medicine, N15, W7, Kita-ku, Sapporo,
Hokkaido, 060-8648, Japan. Phone: +81 11 706 7214; Fax: +81 11 706 7823.

E-mail address: jutanaka@med.hokudai.ac.jp (J. Tanaka)

Abstract

Monitoring minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL) is a useful way for assessing treatment response and relapse.

We studied the value of MRD and showed a correlation with relapse for 34 adult patients with ALL. MRD was evaluated by real-time quantitative polymerase chain reaction (RQ-PCR) with probes derived from fusion chimeric genes (BCR/ABL) (n=12) or PCR-based detection of clonal immunoglobulin and T-cell receptor gene rearrangements (n=16), or both (n=6). We analyzed 27 of the 34 patients who could be examined for MRD on day 100 after induction therapy. The overall survival (OS) rate (45.0%) and relapse-free survival (RFS) rate (40.0%) at 2 years in CR patients with MRD level $\geq 10^{-3}$ (n=12) were significantly lower than those in CR patients with MRD level $< 10^{-3}$ (n=15) (OS rate: 79.0%, RFS rate: 79.4%) (log-rank test, $P=0.017$ and 0.0007). We also applied multicolor flow cytometry for comparison with MRD results analyzed by PCR methods. The comparison of results obtained in 27 follow-up samples showed consistency in 17 samples (63.0%) ($P=0.057$). MRD analysis on day 100 is important for treatment decision in adult ALL.

Key words: MRD; adult ALL; allogeneic transplantation; polymerase chain reaction; flow cytometry

Introduction

The prognosis of adult patients with acute lymphoblastic leukemia (ALL) is dismal.

Although most adult patients with ALL enter complete remission (CR), only 30% to 40% of patients survive 5 or more years (1). The major cause of treatment failure is relapse, affecting approximately half of the patients who have achieved CR (2). Survival depends on risk factors such as age, white blood cell (WBC) count, time to CR, disease immunophenotype, cytogenetics, and molecular abnormalities (1), and several studies have shown that detection of minimal residual disease (MRD) in childhood and adult ALL is an independent risk parameter of high clinical relevance (3). Early indicators of disease outcome would be particularly useful for the design of new treatments.

The rationale of MRD analysis is to improve estimation of treatment response, to provide independent prognostic information, and to optimize therapeutic strategies.

Established methods for detecting MRD are polymerase chain reaction (PCR) amplification of antigen receptor genes and of fusion transcripts and flow cytometric detection of ectopic or aberrant immunophenotypes (4).

Flow cytometric detection of MRD is based on the identification of immunophenotypic combinations expressed on leukemic cells but not on normal hematopoietic cells (4).

Abnormal antigen expression or leukemia-specific gene rearrangements or fusion

1
2
3 transcripts are suitable for MRD detection; however, they cannot be identified in all
4
5
6 patients. Therefore, the complementary use of both methods might allow monitoring of
7
8
9 virtually all patients for MRD.
10

11
12 We previously reported that molecular MRD status by PCR amplification of antigen
13
14
15 receptor genes is a strong predictor of outcome in adult ALL (5). In this study, we
16
17
18 accumulated more patients and reevaluated the significance of MRD. We also compared
19
20
21 the two methods of MRD detection: PCR amplification and flow cytometry.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Patients and methods

Patient characteristics

A total of 46 adult ALL patients were included in the study during the period from May 2001 to December 2007 at Hokkaido University Hospital and hospitals associated with the Hokkaido Leukemia Study Group. They were registered with the study when the diagnoses of ALL were made. Twelve of the 46 patients who entered the study were excluded (patients with no IGH, TCR δ , or TCR γ clonal marker or fusion transcripts at diagnosis (n=5), patients who died before or during induction chemotherapy (n=5), and patients who did not achieve CR (n=2)). In total, MRD could be monitored in 34 of the 46 initial patients.

The characteristics of the 34 patients are summarized in Table 1. Seventeen (50.0%) of the patients were male and 17 (50.0%) were female. Their median age was 48.5 years (range, 15-79 years) and median duration of follow-up was 567.5 days (range, 49-2040 days). The median WBC count was $10.9 \times 10^9 /L$ (range, $1.7-3272.5 \times 10^9 /L$). Thirty patients had B-precursor ALL and 4 had T-ALL. Eighteen patients were Philadelphia (Ph) chromosome-positive: 13 patients (55.6%) had p190^{BCR/ABL} transcripts, 3 patients (16.7%) had p210^{BCR/ABL} transcripts, and the other 2 (11.1%) had both transcripts. One patient was positive for SIL/TAL1 and another patient was positive for E2A/PBX1, but

these fusion transcripts were not used to detect MRD. Eighteen (52.9%) of the 34 patients received chemotherapy only, and the remaining 16 (47.1%) received allogeneic stem cell transplantation after chemotherapy. Eight patients had related donors and 8 patients had unrelated donors. Clinical characteristics of the patients are shown in Table 2.

Twenty-six patients were treated with intravenous cyclophosphamide, vincristine, daunorubicin or doxorubicin, etoposide, cytosine arabinoside, steroid, l-asparaginase, with or without imatinib, and intrathecal methotrexate for induction therapy. One patient received only imatinib, another patient received etoposide and imatinib, and another patient was treated with idarubicin and cytosine arabinoside because the diagnosis of acute myelogenous leukemia was made at the first time. The induction therapies for other five patients were not known. The conditioning regimen for allogeneic transplantation consisted of total body irradiation in combination with cyclophosphamide and etoposide for 13 patients or fludarabine for 1 patient. The conditioning regimen was unknown for the other three patients.

Clinical decisions concerning treatment of the patients were made by their physicians regardless of MRD results. Enrollment in the study was contingent upon informed consent of the patient. The study was approved by the Institutional Review Board of

Hokkaido University Graduate School of Medicine.

Remission and relapse

Morphologic CR was defined as less than 5% blast cells in a regenerated bone marrow (BM) aspirate, absence of extramedullary leukemia, and peripheral blood (PB) neutrophil and platelet counts of $> 1.5 \times 10^9 /L$ and $> 100 \times 10^9 /L$, respectively. Clinical relapse was defined as detection of at least 5% blast cells in BM or detection of leukemic cells extramedullary.

Sample processing

BM or PB was collected at the time of initial diagnosis and at several clinical points (day 30 and day 100 after diagnosis, before the conditioning regimen, after transplantation and any points at the end of each chemotherapy). Some samples were collected after completion of the treatment. The samples were used for PCR study of IGH/TCR rearrangements and BCR/ABL fusion gene transcripts. Some of the samples were also used for flow cytometry study. A total of 231 samples (220 BM and 11 PB samples) were analyzed; the median number of samples analyzed per patient was 6 (range, 2-22) (Table 3). For a total of 27 BM samples from five patients MRD was analyzed by both PCR amplification and flow cytometry. We did not evaluate the difference in values of MRD between BM and PB samples.

1
2
3 *Quantitative real-time PCR analysis of BCR/ABL*
4
5

6 Samples were analyzed for BCR/ABL, TEL/AML1, MLL/AF4, MLL/AF9, MLL/AF6,
7
8
9 MLL/ENL, E2A/PBX1, and SIL/TAL1 chimeric genes. Samples were amplified by
10
11
12 real-time quantitative polymerase chain reaction (RQ-PCR) and quantified by parallel
13
14
15 amplification of serial dilutions of transcript-containing plasmids. A 10^{-5} sensitivity for
16
17
18
19 MRD detection could be obtained.
20
21

22 *PCR analysis of IGH/TCR gene rearrangements*
23
24

25 When a BCR/ABL chimeric fusion gene was not present, leukemia-specific probes were
26
27
28 generated by genomic amplification and sequencing of VDJ regions of the
29
30
31 immunoglobulin heavy chain (IGH) and T-cell receptor δ and γ (TCR δ and TCR γ)
32
33
34 genes. The method of PCR analysis of IGH/TCR gene rearrangements has been
35
36
37 described previously (5). The sensitivity was 10^{-3} . When two MRD probes gave
38
39
40
41 different results in the same patient, the higher MRD level was considered valid for the
42
43
44 purpose of the study.
45
46

47 *Flow cytometry*
48
49

50 Leukemia-associated immunophenotypes were identified by flow cytometry with
51
52
53 four-color combinations of monoclonal antibodies. The flow cytometric method used
54
55
56 has been described previously (6). For each case, one or more marker combinations that
57
58
59
60
61
62
63
64
65

1
2
3 allowed the identification of one leukemic cell in 10^3 - 10^4 normal nucleated cells were
4
5
6 selected at diagnosis and used to study MRD during therapy. This approach reached
7
8
9 sensitivities up to 10^{-4} .
10

11 *Statistical analysis*

12
13 Survival curves were plotted according to the method of Kaplan and Meier, and
14
15
16 comparison of the curves was made using log-rank tests. Overall survival (OS) was
17
18
19 measured from the date of induction therapy until death. Relapse-free survival (RFS)
20
21
22 was measured from the date of induction therapy until the date of relapse or death,
23
24
25 whichever occurred first. Univariate and multivariate analyses were performed to
26
27
28 evaluate the independent prognostic factors for OS and RFS by a Cox regression model.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Results

Identification of MRD-PCR targets

Eighteen patients were Ph chromosome-positive and their MRD was detected by BCR/ABL fusion transcripts. Thirty IGH/TCR gene rearrangements were found at diagnosis in the 22 ALL patients: 19 IGH (two different kinds in five patients), 6 TCR δ , and 5 TCR γ gene rearrangements. Eight patients had two targets and 14 patients had one target (Table 3). MRD was analyzed by both BCR/ABL fusion transcripts and IGH/TCR gene rearrangements in 6 patients. The results obtained by the two methods were concordant. In 5 patients, MRD was also detected by flow cytometry (BCR/ABL, 4; IGH gene rearrangement, 1).

Patients achieved first CR after a median period of 35 days (range, 15–105 days).

Sixteen patients (47.1%) received allogeneic stem cell transplantation following induction and consolidation chemotherapy. Seventeen patients relapsed and 17 patients remained in CR.

We analyzed 27 of the 34 patients who could be examined for MRD on day 100. The overall survival (OS) rate (45.0%) and relapse-free survival (RFS) rate (40.0%) at 2 years in CR patients with MRD level $\geq 10^{-3}$ (n=12) were significantly lower than those

1
2
3 in CR patients with MRD level $<10^{-3}$ (n=15) (OS rate: 79.0%, RFS rate: 79.4%)
4
5
6 (log-rank test, $P=0.017$ and 0.0007). A lower MRD value on day 100 after induction
7
8
9 therapy was associated significantly with longer survival (Fig.1a and b). Two patients
10
11
12 were excluded from the study because of relapse before day 100 after induction therapy.
13
14
15
16 Monitoring of MRD enabled prediction of relapse in 10 of the 14 relapsed patients
17
18
19 whose results of MRD analyses were available before relapse. The median time from
20
21
22 molecular to clinical relapse was 65 days (range, 12-305 days).
23
24

25 *Comparison of the results obtained by flow cytometry and PCR analysis*

26

27
28 We compared MRD levels determined by both flow cytometry and PCR analysis in 27
29
30
31 follow-up BM samples from 5 patients (Table 4). Four of them had Ph-positive ALL
32
33
34 and MRD was analyzed by BCR/ABL fusion transcripts. The other patient was
35
36
37 analyzed by IGH gene rearrangement.
38
39
40

41 Concordance between the flow cytometric and PCR results was obtained in 17 (63.0%)
42
43
44 of the 27 samples ($P=0.057$). No significant changes regarding immunophenotype were
45
46
47 observed when MRD phenotypes at relapse were compared to the original phenotypes
48
49
50 at diagnosis.
51
52

53
54 Of the 10 samples that were MRD-positive by flow cytometry but MRD-negative by
55
56
57 PCR, very low levels (below 10^{-4}) could be detected by flow cytometry in 5 samples
58
59
60
61

1
2
3 from 2 patients. The other 5 samples from 3 patients were MRD-positive by flow
4
5
6 cytometry (0.18%, 0.16%, 0.39%, 0.42%, and 2.53%) but MRD-negative by PCR.
7
8

9
10 *Other prognostic factors*
11

12
13 In addition, we examined factors correlated with OS and RFS by the log-rank test. In
14
15
16 the log-lank test, a lower MRD value on day 100 was associated significantly with
17
18
19 longer survival (Table 5). However, we could not determine other factors, such as age,
20
21
22 WBC count at diagnosis, Ph chromosome, immunophenotype, sex, days to achieve CR,
23
24
25 and type of treatment (chemotherapy or transplantation). Therefore, our data provide
26
27
28 evidence that molecular MRD status on day 100 is a strong predictor of outcome in
29
30
31 adult ALL.
32

33
34
35 *Relative risk of relapse*
36

37
38 Looking for informative predictors of the achievement of molecular CR on day 100, we
39
40
41 used univariate and multivariate analyses to investigate the role of conventional clinical
42
43
44 findings such as age younger than 55 years or not, WBC count at diagnosis, Ph
45
46
47 chromosome, immunophenotype, sex, days to achieve CR, and MRD levels on day 30.
48
49
50 Univariate analysis showed that MRD positivity on day 100 was associated with age
51
52
53 older than 55 years and MRD positivity on day 30, but there were no significant
54
55
56 associations in multivariate analysis.
57
58
59
60
61
62
63
64
65

Discussion

There is increasing evidence that MRD has strong prognostic significance in adult patients with ALL. Mortuza et al. found that MRD positivity detected especially at 3 to 6 months after induction therapy in adults with B-ALL was associated with an increased risk of relapse (7). Holowiecki et al. reported that MRD equal or greater than 0.1% of bone marrow cells after induction was a strong and independent predictor for relapse in both standard and high-risk groups (2).

We also showed that OS and RFS at 2 years in CR patients positive for MRD by PCR-based detection of BCR/ABL transcripts or IGH/TCR gene rearrangements were significantly lower than those in CR patients negative for MRD.

Three highly specific and sensitive methodologies for MRD detection are available: multiparameter flow cytometric immunophenotyping, RQ-PCR-based detection of fusion gene transcripts or breakpoints, and PCR-based detection of clonal immunoglobulin and T-cell receptor gene rearrangements (8).

PCR-based detection of rearranged IGH/TCR genes is currently the most broadly applied MRD technique owing to its high level of standardization, its well-defined quantitative range and good sensitivity, as well as applicability in the majority of ALL

1
2
3 patients (3). To reach a higher level of sensitivity, DNA sequencing of the junctional
4
5
6 regions is required in order to design tumor-specific primers and/or probes.
7

8
9 A major drawback of using rearranged immune genes as MRD-PCR targets is the
10
11 possible occurrence of continuing rearrangements during the course of therapy and
12
13 during follow-up, which can lead to false-negative PCR results (9).
14
15
16

17
18 Relapse could not be predicted in 4 of the 14 relapsed patients in this study. In two of
19
20 them, TCR δ or IGH gene rearrangement was not detected even at the time of relapse.
21
22
23

24
25 Clonal TCR δ rearrangement in particular can be lost during the follow-up period
26
27 preceding relapse (10), and continuing rearrangements during the disease course occur
28
29 in 10-40% of cases depending on the target used (11).
30
31
32

33
34 One specific advantage of flow cytometry over PCR-based assays is that it allows direct
35
36 quantification of MRD, rather than extrapolating it from amounts of PCR product (9).
37
38
39

40
41 However, the immunophenotype of leukemic cells may change during progression of
42
43 the disease, and if these changes affect markers used for monitoring MRD, a
44
45 false-negative finding may result (12, 13).
46
47
48

49
50 In this study, concordance between the flow cytometric and PCR results was obtained in
51
52 17 (63.0%) of the 27 samples. Comparative analyses showed that more concordant
53
54 results could be obtained for both methods at the level of 10^{-3} and 10^{-4} (14, 15). In three
55
56
57
58
59
60
61
62
63
64
65

1
2
3 cases, some samples showed discordant results. In these cases, MRD might be
4
5
6 overestimated by flow cytometry because normal cells cannot be easily distinguished
7
8
9 from leukemic cells in studies of MRD (6).
10

11
12 The combination use of the two methods may offset the possibility of false-negative and
13
14
15 false-positive MRD results due to these events (16).
16
17

18
19 We did not find a significant prognostic value of MRD in patients who received
20
21
22 allogeneic transplantation. It has been shown that MRD detected before transplantation
23
24
25 was a significant predictor of failure after transplantation (17, 18). However, Patel et al.
26
27
28 suggested that molecularly determined MRD pre-transplant was not a risk factor for
29
30
31 relapse in patients receiving allogeneic stem cell transplantation in the first CR (19). It
32
33
34 has also been shown that MRD status after allogeneic transplantation was an important
35
36
37 predictor of outcome in adults with ALL (7, 18). The MRD status before and after
38
39
40 transplantation was not correlated with survival in this study, which may be explained
41
42
43 by the small number of patients who received transplantation.
44
45
46

47
48 Results of log-rank tests showed that age, WBC count at diagnosis, sex, Ph-positivity,
49
50
51 and immunophenotype were not associated with prognosis or relapse. Generally,
52
53
54 advanced age of the patient and high WBC count at diagnosis with acute leukemia are
55
56
57 related to poor prognosis. In this study, we did not intervene in clinical decisions
58
59
60
61
62
63
64
65

concerning treatment of the patients, but MRD level on day 100 after induction chemotherapy was only a good prognostic factor to monitor relapse and classify the groups as good or poor prognosis.

Although limitation of MRD assessment for prediction of extramedullary relapse is recognized (19), MRD methods can be used to predict outcome on the basis of early response to therapy and to recognize leukemia relapse (20).

Thorn et al. suggested that MRD levels calculated by the quantification of BCR/ABL transcripts were higher than levels obtained by flow cytometry and by quantitative PCR of rearranged IGH/TCR genes (21). However, whether the results of these methods coincide with those of PCR amplification of fusion transcripts is not yet established (22). Further progress in how to assess MRD and improve the prognosis in adult patients with ALL in association with MRD is expected.

In this study, we investigated the prognostic value of MRD in adult patients with ALL.

We found that ALL patients with MRD level $<10^{-3}$ on day 100 had significantly better

OS and RFS than those of ALL patients with MRD level $\geq 10^{-3}$. Therefore, MRD

analysis is useful for monitoring the prognosis of ALL patients. PCR analysis and flow

cytometry were both useful for the detection of MRD. However, both methods can yield

false-negative and false-positive results, and improvements are needed for further

1
2
3 optimization and standardization to assess MRD. It is important to investigate an
4
5
6 appropriate way to choose among these methods or use of them in tandem.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Acknowledgments

This work was supported in part by Kyowa-Kirin, Japan (Tokyo, Japan).

References

1. Bassan R, Spinelli O, Oldani E, Intermesoli T, Tosi M, Peruta B, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood 2009; 113:4153-62.
2. Holowiecki J, Krawczyk-Kulis M, Giebel S, Jagoda K, Stella-Holowiecka B, Piatkowska-Jakubas B, et al. Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4-2002 MRD Study. Br J Haematol. 2008; 142:227-37.
3. Brüggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. Leukemia 2010; 24:521-35.
4. Campana D, Coustan-Smith E. Minimal residual disease studies by flow cytometry in acute leukemia. Acta Haematol. 2004; 112:8-15.
5. Toubai T, Tanaka J, Ota S, Fukuhara T, Hashino S, Kondo T, et al. Minimal residual

disease (MRD) monitoring using rearrangement of T-cell receptor and immunoglobulin H gene in the treatment of adult acute lymphoblastic leukemia patients. Am J Hematol. 2005; 80:181-7.

6. Chen JS, Coustan-Smith E, Suzuki T, Neale GA, Mihara K, Pui CH, et al.

Identification of novel markers for monitoring minimal residual disease in acute lymphoblastic leukemia. Blood 2001; 97:2115-20.

7. 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.

8. Szczepański T. Why and how to quantify minimal residual disease in acute lymphoblastic leukemia? Leukemia 2007; 21:622-6.

9. Brüggemann M, Pott C, Ritgen M, Kneba M. Significance of minimal residual disease in lymphoid malignancies. Acta Haematol. 2004; 112:111-9.

10. Foroni L, Hoffbrand AV. Molecular analysis of minimal residual disease in adult acute lymphoblastic leukaemia. Best Pract Res Clin Haematol. 2002; 15:71-90.

11. Szczepański T, Willemse MJ, Brinkhof B, van Wering ER, van der Burg M, 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

1
2
3 stable PCR targets for monitoring of minimal residual disease. Blood 2002; 99:2315-23.

4
5
6 12. Brisco J, Hughes E, Neoh SH, Sykes PJ, Bradstock K, Enno A, et al. Relationship
7
8
9 between minimal residual disease and outcome in adult acute lymphoblastic leukemia.
10
11
12 Blood 1996; 87:5251-6.

13
14
15 13. Radich J, Gehly G, Lee A, Avery R, Bryant E, Edmands S, et al. Detection of
16
17
18 bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia
19
20
21 after marrow transplantation. Blood 1997; 89:2602-9.

22
23
24 14. Kerst G, Kreyenberg H, Roth C, Well C, Dietz K, Coustan-Smith E, et al.
25
26
27 Concurrent detection of minimal residual disease (MRD) in childhood acute
28
29
30 lymphoblastic leukaemia by flow cytometry and real-time PCR. Br J Haematol. 2005;
31
32
33 128:774-82.

34
35
36 15. Robillard N, Cavé H, Méchinaud F, Guidal C, Garnache-Ottou F, Rohrlich PS, et al.
37
38
39 Four-color flow cytometry bypasses limitations of IG/TCR polymerase chain reaction
40
41
42 for minimal residual disease detection in certain subsets of children with acute
43
44
45 lymphoblastic leukemia. Haematologica 2005; 90:1516-23.

46
47
48 16. Neale GA, Coustan-Smith E, Stow P, Pan Q, Chen X, Pui CH, et al. Comparative
49
50
51 analysis of flow cytometry and polymerase chain reaction for the detection of minimal
52
53
54 residual disease in childhood acute lymphoblastic leukemia. Leukemia 2004; 18:934-8.

- 1
2
3 17. Sánchez J, Serrano J, Gómez P, Martínez F, Martín C, Madero L, et al. Clinical
4
5
6 value of immunological monitoring of minimal residual disease in acute lymphoblastic
7
8
9 leukaemia after allogeneic transplantation. *Br J Haematol.* 2002; 116:686-94.
10
11
12 18. Spinelli O, Peruta B, Tosi M, Guerini V, Salvi A, Zanotti MC, et al. Clearance of
13
14
15 minimal residual disease after allogeneic stem cell transplantation and the prediction of
16
17
18 the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia.
19
20
21
22 *Haematologica* 2007; 92:612-8.
23
24
25 19. Patel B, Rai L, Buck G, Richards SM, Mortuza Y, Mitchell W, et al. Minimal
26
27
28 residual disease is a significant predictor of treatment failure in non T-lineage adult
29
30
31 acute lymphoblastic leukaemia: final results of the international trial UKALL
32
33
34 XII/ECOG2993. *Br J Haematol.* 2010; 148:80-9.
35
36
37 20. Campana D. Role of minimal residual disease monitoring in adult and pediatric
38
39
40 acute lymphoblastic leukemia. *Hematol Oncol Clin North Am.* 2009; 23:1083-98.
41
42
43 21. Thörn I, Botling J, Hermansson M, Lönnerholm G, Sundström C, Rosenquist R, et
44
45
46 al. Monitoring minimal residual disease with flow cytometry, antigen-receptor gene
47
48
49 rearrangements and fusion transcript quantification in Philadelphia-positive childhood
50
51
52 acute lymphoblastic leukemia. *Leuk Res.* 2009; 33:1047-54.
53
54
55 22. Campana D. Minimal residual disease in acute lymphoblastic leukemia. *Semin*
56
57
58
59
60
61
62
63
64
65

Hematol. 2009; 46:100-6.

Figure legends

Figure 1. Comparison of overall survival (OS, a) and relapse-free survival (RFS, b) by MRD detection on day 100 after induction therapy.

Table 1. Characteristics of the patients.

Characteristics	N
Number of patients	34
Gender (%)	
Male	17 (50.0%)
Female	17 (50.0%)
Median age, years (range)	48.5 (15-79)
Age	
< 35 years	14 (41.2%)
≥35 years	20 (58.8%)
< 55 years	21 (61.8%)
≥55 years	13 (38.2%)
Median follow-up period, days (range)	567.5 (49-2040)
Immunophenotype	
B-ALL	30 (88.2%)
T-ALL	4 (11.8%)
Median WBC, $\times 10^9/L$ (range)	10.9 (1.7-3272.5)
WBC $< 30 \times 10^9/L$ (B), $< 100 \times 10^9/L$ (T)	25 (73.5%)
WBC $\geq 30 \times 10^9/L$ (B), $\geq 100 \times 10^9/L$ (T)	5 (14.7%)
unknown	4 (11.8%)
Median hemoglobin, g/dL (range)	10.3 (4.1-14.9)
Median platelet, $\times 10^9/L$ (range)	50.5 (3-302)
Median LDH, IU/L (range)	730 (206-11730)
Philadelphia chromosome	
negative	16 (47.1%)
positive	18 (52.9%)
p190 ^{BCR/ABL}	13 (38.2%)
p210 ^{BCR/ABL}	3 (8.8%)
p190 ^{BCR/ABL} and p210 ^{BCR/ABL}	2 (5.9%)

Treatment	
Chemotherapy	18 (52.9%)
Chemotherapy→Transplantation	16 (47.1%)
Conditioning	
Myeloablative	12 (75.0%)
Reduced intensity	2 (12.5%)
unknown	2 (12.5%)
Stem cell source	
Bone Marrow	6 (37.5%)
Peripheral Blood	7 (43.8%)
Cord Blood	3 (18.8%)
Median time to CR, days (range)	35 (15-105)
Outcome at 2 years	
Overall survival	54.6%
Relapse-free survival	51.8%

Table 2. Clinical characteristics and outcome of the patients.

Patient no.	Sex/ age	Immuno- phenotype	WBC (X10 ⁹ /L)	Ph chorosome	MRD markers	Transplan- tation	MRD day100	Outcome	
1	M/28	B	16.6	No	IgH	No	positive	REL d195	D d218
2	F/59	B	13	Yes	IgH, p190 ^{BCR/ABL}	No	positive	REL d245	D d617
3	M/53	B	1.7	Yes	IgH, p210 ^{BCR/ABL}	No	*	REL d159	D d280
4	M/50	B	12.2	No	TCRδ	Yes	negative	REL d494	D d518
5	M/77	B	16.8	Yes	IgH, p190 ^{BCR/ABL}	No	positive	REL d807	D d1003
6	F/25	T	38	No	TCRγ	Yes	negative	CR d2040	A d2040
7	F/47	B	3.7	Yes	IgH, p190 ^{BCR/ABL}	Yes	negative	CR d1989	A d1989
8	M57	B	23	No	IgH	No	positive	REL d141	D d502
9	F/37	B	3	Yes	TCRδ, p190 ^{BCR/ABL}	Yes	negative	CR d769	A d769
10	F/60	B	3	No	TCRδ	No	positive	REL d995	A d1892
11	F/63	B	3272.5	Yes	p210 ^{BCR/ABL}	No	positive	REL d49	D d309
12	F/65	B	141.1	Yes	TCRδ, γ, p190 ^{BCR/ABL}	No	positive	REL d47	D d320
13	M/52	B	10.4	Yes	p210 ^{BCR/ABL}	No	negative	CR d483	A d483
14	M/23	T	8.4	No	TCRδ, γ	Yes	negative	CR d1604	A d1604
15	M/21	T	10	No	TCRγ	Yes	positive	REL d280	D d385
16	F/60	B	2	No	IgH	No	negative	CR d1463	A d1463
17	F/31	B	6	No	IgH	Yes	negative	REL d382	D d501

18	M/15	B	6.3	No	IgH	Yes	negative	CR d1339	A d1339
19	M/62	B	2.2	No	IgH	No	positive	REL d889	D d1138
20	F/19	T	3.3	No	TCR δ , γ	Yes	positive	CR d1169	A d1169
21	F/79	B	*	No	IgH	No	positive	REL d346	D d360
22	F/19	B	155.2	No	IgH	Yes	negative	CR d469	D d469
23	M/72	B	3.8	Yes	p190 ^{BCR/ABL}	No	negative	CR d948	A d948
24	M/46	B	*	Yes	p190 ^{BCR/ABL}	Yes	*	REL d399	A d964
25	F/50	B	4	Yes	p190 ^{BCR/ABL}	Yes	negative	CR d840	A d840
26	M/33	B	230	Yes	p190 ^{BCR/ABL}	No	negative	CR d720	A d720
27	M/72	B	*	Yes	p190 ^{BCR/ABL}	No	-	CR d49	D d49
28	F/26	B	24.9	Yes	p190 ^{BCR/ABL}	Yes	*	CR d738	A d738
29	F/68	B	*	Yes	p190 ^{BCR/ABL} , p210 ^{BCR/ABL}	No	*	REL *	D d352
30	M/25	B	11.3	No	IgH	Yes	negative	CR d640	A d640
31	F/26	B	8.6	No	IgH	Yes	*	REL d158	D d354
32	F/31	B	24.1	Yes	p190 ^{BCR/ABL} , p210 ^{BCR/ABL} ,	Yes	negative	REL d262	A d325
33	M/28	B	16.8	Yes	p190 ^{BCR/ABL}	No	negative	CR d120	A d120
34	M/77	B	36.7	Yes	p190 ^{BCR/ABL}	No	positive	CR d125	A d125

*The data was unknown.

Ph indicates philadelphia, REL; relapse; D, dead; A, alive.

Table 3. MRD studies.

Total	34
BCR/ABL transcripts	12
p190 ^{BCR/ABL}	8
p210 ^{BCR/ABL}	2
p190 ^{BCR/ABL} and p210 ^{BCR/ABL}	2
IGH, TCR δ , TCR γ gene rearrangement patterns	16
Monoclonal	10
IGH	6
TCR δ	2
TCR γ	2
Diclonal	6
2 clones of IGH	4
TCR δ and TCR γ	2
BCR/ABL and IGH/TCR rearrangements	6
p190 ^{BCR/ABL} and IGH	2
p190 ^{BCR/ABL} and 2 clones of IGH	1
p210 ^{BCR/ABL} and IGH	1
p190 ^{BCR/ABL} and TCR δ	1
p190 ^{BCR/ABL} and TCR δ , TCR γ	1
Number of samples	231
Median (range)	6 (2-22)
Bone marrow	220
Peripheral blood	11

Figure

Figure 1a

Figure 1b

Table 4. Informative CD combinations used for flow cytometry (FC).

Case	Type	Marker combinations used at follow-up	MRD results		
			PCR + FC +	PCR - FC -	PCR - FC +
9	ALL(B)	CD58/CD45/CD34/CD19 CD13/CD10/CD34/CD19 TdT/CD10/CD34/CD19	1		
23	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD58/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	5	3	7
24	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD33/CD34/CD19	3		2
25	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	1	1	1
26	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	3		

Table 5. Univariate and multivariate analyses of prognostic factors associated with overall survival and relapse-free survival (log-rank test).

Variables	Univariate								Multivariate					
	Overall survival				Relapse-free survival				Overall survival			Relapse-free survival		
	N	Hazard ratio	95% CI	<i>p</i> value	N	Hazard ratio	95% CI	<i>p</i> value	Hazard ratio	95% CI	<i>p</i> value	Hazard ratio	95% CI	<i>p</i> value
Age (years)														
<35	14	1.59	(0.55-4.59)	0.39	14	1.83	(0.63-5.29)	0.26						
≥35	20				19									
<55	21	2.34	(0.87-6.30)	0.09	21	2.28	(0.85-6.14)	0.10	0.42	(0.01-14.67)	0.63	0.64	(0.04-10.26)	0.75
≥55	13				12									
WBC (×10 ⁹ /L)														
<30 (B), <100 (T)	25	3.60	(0.93-13.91)	0.06	25	3.05	(0.82-11.38)	0.10	4.03	(0.12-134.40)	0.44	4.00	(0.24-66.21)	0.33
≥30 (B), ≥100 (T)	5				5									
Gender														
Female	17	0.88	(0.33-2.36)	0.80	16	1.10	(0.41-2.92)	0.85						
Male	17				17									
Ph chromosome														
negative	16	1.03	(0.37-2.83)	0.95	16	1.11	(0.40-3.04)	0.84						
positive	18				17									
Time to CR (days)														
<30	10	1.18	(0.38-3.73)	0.77	10	0.62	(0.22-1.76)	0.37						
≥30	23				22									
Immunophenotype														
B-lineage	30	0.30	(0.04-2.34)	0.25	29	0.27	(0.04-2.10)	0.21						
T-lineage	4				4									
Transplantation														
No	18	0.34	(0.12-0.97)	0.04	17	0.40	(0.14-1.10)	0.08	0.74	(0.03-21.18)	0.86	0.75	(0.06-10.16)	0.83

Yes	16				16									
MRD on day 30														
negative	7	1.13	(0.35-3.70)	0.84	7	1.64	(0.51-5.25)	0.41						
positive	18				17									
MRD on day 100														
negative	15	4.54	(1.17-17.67)	0.03	15	7.14	(1.91-26.66)	0.003	7.46	(1.21-45.91)	0.03	9.60	(1.83-50.49)	0.008
positive	12				12									

Ph indicates Philadelphia.