Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies

Authors:
Nicola Gökbuget, Michael Kneba, Thorsten Raff, Heiko Trautmann, Claus-Rainer Bartram, Renate Arnold, Rainer Fietkau, Mathias Freund, Arnold Ganser, Wolf-Dieter Ludwig, Georg Maschmeyer, Harald Rieder, Stefan Schwartz, Hubert Serve, Eckhard Thiel, Monika Brüggemann and Dieter Hoelzer for the German Multicenter Study Group for Adult ALL

MB and DH contributed equally to this work

Author’s affiliation:
1Department of Internal Medicine II, Hematology and Oncology, Goethe University Hospital, Frankfurt, Germany;
2Department of Internal Medicine II, University Hospital of Schleswig Holstein, Campus Kiel, Kiel, Germany;
3Institute of Human Genetics, Ruprecht-Karls-University, Heidelberg, Germany;
4Department of Hematology and Oncology, Charité - University Medicine Berlin, Campus Virchow-Klinikum, Berlin, Germany;
5Institute for Radiotherapy, University Hospital, Erlangen;
6Division of Hematology and Oncology, University of Rostock, Rostock, Germany;
7Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany;
8Department of Hematology, Oncology, and Tumor Immunology, HELIOS Clinic Berlin-Buch, Berlin, Germany;
9Department of Hematology, Oncology and Palliative Care, Ernst von Bergmann Clinic, Potsdam, Germany;
10Cytogenetics, University of Düsseldorf, Düsseldorf, Germany;
11Department of Hematology and Oncology, Charite Campus Benjamin Franklin, Berlin, Germany

Corresponding Author:
Dr. Nicola Gökbuget
Goethe University Hospital, Department of Medicine II
Theodor Stern Kai 7
D-60590 Frankfurt am Main
Phone: +49 (0) 69 6301 6365
Fax: +49 (0) 69 6301 7463
Mail: goekbuget@em.uni-frankfurt.de
ABSTRACT

Quantification of minimal residual disease (MRD) by real-time polymerase chain reaction directed to T-cell-receptor and immunoglobulin gene rearrangements allows a refined evaluation of response in acute lymphoblastic leukemia (ALL). The German Multicenter Study Group for Adult ALL prospectively evaluated molecular response after induction/consolidation chemotherapy according to standardized methods and terminology in patients with Philadelphia chromosome-negative ALL. The cytological complete response (CR) rate was 89% after induction phase I and II. At this time-point the molecular CR rate was 70% in 580 patients with cytological CR and evaluable MRD. Patients with molecular CR after consolidation had a significantly higher probability of continuous complete remission (CCR) (74% vs. 35%; p<0.0001) and of overall survival (80% vs. 42%; p=0.0001) compared to patients with molecular failure. Patients with molecular failure without stem cell transplantation (SCT) in first CR relapsed after a median time of 7.6 months; CCR and survival at 5 years only reached 12% and 33%, respectively. Quantitative MRD assessment identified patients with molecular failure as a new high-risk group. These patients display resistance to conventional drugs and are candidates for treatment with targeted, experimental drugs and allogeneic SCT. Molecular response was shown to be highly predictive for outcome and therefore constitutes a relevant study endpoint. The studies are registered at www.clinicaltrials.gov as NCT00199056 and NCT00198991.
INTRODUCTION

Treatment results in adult acute lymphoblastic leukemia (ALL) have improved over the past three decades with complete remission rates increasing to 85-90% and overall survival rates to 40-50% \(^1\) \(^2\). Nevertheless 40-50% of patients, including standard risk patients without known poor-risk features, still develop relapse, which is associated with an extremely poor survival rate of with less than 10% \(^3\). Reduction of relapse rate is therefore the major aim of treatment optimization in ALL. One approach is to identify patients in clinical CR with high risk of relapse as candidates for early treatment intensification including allogeneic stem cell transplantation (SCT).

Response to standard induction treatment has been proven to be the most important factor in predicting outcome and risk of relapse. Cytological confirmation of complete remission (CR) and time to achievement of CR are accepted as highly relevant prognostic factors and have been used as endpoints for clinical trials, including pivotal trials with new antileukemic agents \(^4\). However, sensitivity of detecting tumor cell reduction on a morphological basis by definition is limited to 5% and microscopic evaluation is prone to high inter-individual variability.

In the past decade, more sensitive and precise methods have been developed, capable of detecting leukemic cells on a molecular level and to identify minimal residual disease (MRD) with a detection limit of \(10^{-4}\) to \(10^{-5}\) (0.01-0.001%) \(^5\) \(^6\). Quantitative measurement of residual leukemic blasts is based on analysis of leukemia-specific characteristics such as individual gene rearrangements, fusion genes or leukemia-specific immunophenotypes \(^5\) \(^6\). Most experience and a high level of standardization have been accumulated for evaluation of leukemia-specific T-cell receptor (TCR) and immunoglobulin (Ig) gene rearrangements by quantitative PCR \(^7\).
The prognostic relevance of MRD for predicting relapse risk and survival has been demonstrated in a number of clinical trials in pediatric\textsuperscript{9,10,11,12,13} and adult ALL\textsuperscript{14,15,16,17,18}. Overall, measurement of MRD a) is a sensitive tool for assessing the response to treatment b) is well standardized and reproducible, if measured in qualified reference laboratories, c) predicts overall outcome and d) has therefore been incorporated into most European ALL treatment protocols for risk stratification and serves as a basis to identify patients to be referred to SCT in first CR. Moreover, standardized definitions for MRD-based response evaluation and monitoring, such as ‘complete MRD response’, ‘MRD persistence’ and ‘MRD reappearance’ have been established by the Consensus Development Workshop on MRD\textsuperscript{8} allowing for comparison of MRD results between different trials.

Since 1999, the German Multicenter Study Group for Adult ALL (GMALL) has conducted two consecutive trials (GMALL 06/99 and 07/03) with prospective MRD evaluation. This report contains an analysis of the up-to-now largest cohort of MRD data in adult ALL. It aims to analyze the molecular response rates in correlation to cytological response rates and to evaluate the prognostic impact of molecular response within risk groups defined according to conventional prognostic factors and the outcome of patients with molecular failure and thereby to establish reference data for future MRD based clinical trials.
MATERIAL AND METHODS

Patients

The analysis includes patients with Philadelphia chromosome (BCR-ABL)-negative ALL aged 15-55 years included in the GMALL trials 06/99 and the ongoing trial 07/03 between April 1999 and July 2009 in order to ensure a follow-up of 1 year in the majority of patients. Patients were diagnosed and treated in 130 participating centers of the GMALL study group. 90 patients with molecular response evaluation had already been reported in a previous publication. The studies were approved by the institutional review board of the University of Frankfurt, Germany, and are registered at clinicaltrials.gov (NCT00199056, NCT00198991). All patients have given signed informed consent in accordance with the Declaration of Helsinki.

Risk stratification and treatment

Both studies are based on a risk-adapted treatment strategy with prospective MRD monitoring at several time points during treatment and follow-up as described elsewhere. The treatment overview and chemotherapy schedule is detailed in figure 1 and supplemental table 1. Patients were allocated to risk groups based on conventional prognostic factors at diagnosis. High risk features were white blood cell count (WBC) above 30,000/µL in B-lineage ALL, pro B-ALL, early or mature T-cell ALL, MLL-AF4 / t(4;11) translocation or no achievement of cytological CR after induction I. In the beginning of study 06/99 also few patients with thymic T-ALL and WBC above 100,000/µL were considered as high risk. Patients with any of these factors were allocated to the high risk (HR) group. The remaining patients were allocated to the standard risk (SR) group.
All patients received intensive 7-drug induction therapy with phase I and II followed by one uniform consolidation mainly based on high-dose methotrexate and high-dose cytarabine starting at day 71 as described earlier. SR patients were scheduled to receive further intensive consolidation chemotherapy. HR patients were candidates for allogeneic SCT. In study 07/2003 SR patients with persistent MRD above $10^{-4}$ until week 16 were candidates for transplantation in first CR as well.

**Evaluation of minimal residual disease and definition of response**

The main cytological response evaluation took place after induction phase II (day 46/day 71). Standard criteria were used to evaluate response. CR was defined as no evidence of leukemic blasts in the bone marrow (< 5%), complete resolution of extramedullary manifestations and recovery of peripheral cell counts. Relapse was defined as reappearance of disease either as unequivocal blasts in the bone marrow (>5%), in the CNS or at extramedullary sites after prior achievement of CR.

MRD evaluation took place during induction I (day 11), after induction I (day 26), after induction II (day 46), before consolidation I (day 71), after consolidation I (week 16) and at further time points during consolidation treatment and follow-up. Analysis for the essential time-points day 71 and week 16 was based on the patients with evaluable MRD-results at the respective time-point irrespective of prior or later MRD results. Bone marrow samples were sent to central reference laboratories at the University of Heidelberg and at the University of Kiel. MRD was determined by real-time quantitative PCR of leukemia-specific Ig and TCR gene rearrangements using clone-specific primers and a set of different germ-line TaqMan probes and germ line primers. Quality control and standardized interpretation of quantification data was achieved in the frame of the European Study Group on MRD detection in ALL (EuroMRD, formerly ESG MRD ALL).
Molecular response is only reported for patients in complete cytological remission, with at least one marker for MRD analysis and samples available at the respective time points. Feasibility of MRD evaluation in the multicenter setting will be reported separately. Results were classified as 'molecular CR' (molCR) in case of MRD negativity at the respective time point with an assay sensitivity of at least $10^{-4}$. Persistent quantifiable MRD positivity within the quantitative range ($\geq 10^{-4}$) was defined as 'molecular failure' (molFail) and reappearance of MRD within the quantitative range ($>10^{-4}$) after prior achievement of molecular CR was defined as molecular relapse. MRD positivity below quantitative range or below $10^{-4}$ was considered to be non-evaluable for molecular response assessment.

**Statistical analysis**

Data are presented as percentages for categorical variables and medians for continuous variables. Categorized variables were compared using the Chi-square-test and medians with the Wilcoxon test to estimate significant differences. Survival analysis was performed with the Kaplan-Meier method. Overall survival was calculated from the date of diagnosis to the date of death or date of last follow-up in all included patients. Probability of continuous complete remission was calculated from the date of CR to the date of relapse or date of last follow-up. Patients who died in CR or who were withdrawn from study treatment were censored at the respective dates. Disease free survival was calculated from the date of CR to the date of last follow-up in case of continuous complete remission. Patients with withdrawal, relapse, death in CR or secondary malignancy were counted as events. Comparisons of survival curves were performed with log-rank tests. The Cox model was used to test the prognostic role of molecular response in correlation to conventional prognostic factors. In order to evaluate the effect of SCT compared to
chemotherapy, a landmark analysis was performed. For this analysis, all chemotherapy patients with remission duration shorter than the median time to SCT were excluded. A calculated p-value below 0.05 was established to indicate statistical significance. All analyses were performed with the SAS program (SAS-PC, Version 9, SAS Institute, Cary, NC).

RESULTS

Overall response after induction

A total of 1648 patients from 130 centers, with standard risk (N=975) and high risk (N=673) features were evaluable. Overall, cytological CR was achieved in 89% of patients, with significant differences between SR and HR patients (92% vs. 85%; p <0.0001). Significant differences in cytological CR were also observed for immunophenotypic subtypes and age groups, whereas the WBC at diagnosis had no impact on cytological CR (Table 1).

A total of 580 CR patients were evaluable for analysis of molecular response defined by the level of MRD before consolidation I (day 71). The proportion of standard risk patients was higher in the group of patients with evaluable MRD (75%) results compared to the total patient cohort (59%) (table 1). MolCR was achieved in 70% of patients at this time point with significant differences between SR and HR patients (77% vs. 51%; p<0.0001). In addition molCR rates for B-lineage and T-lineage ALL differed significantly (66% vs. 79%; p=0.001). A superior molCR rate was observed for thymic T-ALL compared to other subgroups of T-ALL (89% vs. 65%; p<0.0001). A higher molCR rate was also documented for B-lineage ALL patients with WBC below 30,000/μl compared to patients with higher WBC (68% vs. 56%; p=0.06) (Table 1).
**Time to molecular CR**

Molecular response to chemotherapy was assessed at different time points during and after induction and after first consolidation. MolCR rates increased from 6% on day 11 to 36% on day 26 and to 70% on day 71 during or after induction chemotherapy and reached 76% in week 16 after consolidation I (Figure 1). The molCR rate was significantly lower in HR patients compared to SR patients at all time points. Patients with T-ALL reached a significantly higher molCR rate on day 71 (79% vs. 66%; p=0.001) compared to patients with B-lineage ALL, whereas no difference was observed on day 26 (36% vs. 36%) and in week 16 (75% vs. 78%). Nine percent of patients with molFail after induction on day 71 (N=87) achieved molecular CR after first consolidation. This proportion was higher in SR than in HR patients (13% vs. 0%; p=0.06), whereas no difference was observed in B-lineage as compared to T-lineage ALL.

**Prognostic impact of molecular response**

Due to initial treatment stratification, the prognostic impact of molCR and molFail was first analyzed within risk groups defined by conventional risk factors.

**Standard risk patients**

The probability of continuous complete remission (CCR) after 5 years was significantly higher for patients with molCR compared to patients who had molFail on day 71 (69±3% vs. 42±6%; p<0.0001) and in week 16 (74±3% vs. 37±6%; p<0.0001). When patients who underwent SCT in first CR were excluded from the analysis, the probability of CCR after 5 years further decreased for patients who had molFail on
Similar results were observed for disease free survival.

At both time points, overall survival at 5 years was significantly higher for patients with molCR compared to patients with molFail. Based on MRD evaluation in week 16, the probability of overall survival was $81\pm3\%$ vs. $43\pm6\%$ ($p<0.0001$). When patients who underwent SCT in first CR were excluded, survival of patients with molFail further decreased and was most unfavorable for patients with molecular failure in week 16 ($31\pm7\%$) (Table 2).

**High risk patients**

Within the high risk group, the probability of CCR after 5 years was significantly higher for patients with molCR at day 71 ($78\pm8\%$ vs. $41\pm7\%; p<0.0001$) and in week 16 ($75\pm8\%$ vs. $29\pm12\%; p<0.0001$) compared to patients with molFail. When patients with SCT in first CR were excluded from the analysis, the probability of CCR after 5 years further decreased for patients with molFail on day 71 ($36\pm26\%$ vs. $21\pm10\%; p=0.001$) and in week 16 ($50\pm35\%$ vs. $19\pm17\%; p<0.0001$). Results were similar for disease free survival. Overall survival at 5 years was significantly better for patients with molCR compared to patients with molFail at both time points. When patients with SCT in first CR were omitted, no significant difference was observed between molFail and molCR patients, but patient numbers were very small (Table 2).

**Total patient cohort**

Analyzing all patients from both risk groups, molFail after consolidation chemotherapy (week 16) had the worst prognostic impact on CCR after 5 years (Table 2). The probability of CCR after 5 years was $74\pm3\%$ for patients with molCR
versus 35±5% for patients with molFail (p<0.0001) (Figure 2A). When patients who underwent SCT in first CR were omitted from analysis, the probability of CCR after 5 years decreased to 12±5% (p<0.0001) (Figure 2B). Results for disease free survival were similar. Overall survival was significantly inferior in molFail compared to molCR (42±5% vs. 80±3%; p<0.0001) (Figure 3A) and decreased further when patients with SCT in first CR were omitted from the analysis (33±7% vs. 81±3%; p<0.0001) (Figure 3B).

A multivariate analysis of prognostic factors including age, immunophenotype, risk group and molecular response status in week 16 revealed that molecular response was the only parameter with significant prognostic impact on CCR after 5 years with a hazard ratio of 4.5 (p<0.0001). In addition, the multivariate analysis confirmed age with a hazard ratio of 1.3 (p=0.0007) and molecular remission status with a hazard ratio of 4.0 (p<0.0001) to have significant impact on overall survival.
Effect of allogeneic stem cell transplantation in patients with molecular failure

After first consolidation (week 16) 120 patients showed molFail (89 SR and 31 HR patients). In 47% of these patients with molFail, SCT was realized in first CR. The SCT performance rate was significantly higher in HR patients compared to SR patients (71% vs. 39%; p<0.002). The median time from CR to SCT was 6.7 months (2.4 – 44 months) and significantly shorter in HR patients than in SR patients (4.4 vs. 9.1 months; p<0.0001).

The probability of CCR after 5 years was significantly higher for patients with molFail and SCT in first CR compared to those without SCT in first CR (66±7% vs. 12±5%; p<0.0001). For landmark analysis, patients with remission duration shorter than 232 days (median time to SCT plus 1 month) were excluded. The difference remained significant for the comparison of patients with (N=29) versus without SCT (N=40) (74±9% vs. 15±6%; p<0.0001), which also translated into a better survival in patients with SCT compared to those without (54±8% vs. 33±7%; p=0.06). Results were similar for disease free survival (table 3).

In patients with molFail without allogeneic SCT in first CR, the median time from detection of molFail to cytological relapse was 7.6 months (Figure 4A). The median remission duration decreased to 4.9 months in patients (N=41) with a higher MRD level (above $10^{-3}$) (Figure 4B).

Outcome after molecular relapse

We observed a total of 34 molecular relapses in patients with continuous cytological remission and without parallel extramedullary relapse. Of these, 38% (N=13) occurred during the first year of treatment and 62% (N=21) subsequently. All except one patient were assigned to the SR group. The probability of continuous cytological...
remission from the date of molecular relapse was 21±9% at 5 years. If patients with SCT in first cytological remission were excluded, the probability of CCR decreased to 5±5% at 3 years (Figure 5A) compared to 80±18% in the few patients (N=10) who underwent SCT in ongoing first cytological remission (p<0.0001).

The median time from detection of molecular relapse in patients without SCT in first CR to subsequent clinical relapse (N=24) was 2.6 months (0.8-43 months). Overall survival of patients with molecular relapse was 36±14% at 5 years. In patients without SCT in first CR survival was 15±12% only (Figure 5B) compared to 80±18% in patients (N=10) with SCT in first CR (p=0.02).

**DISCUSSION**

This is the largest analysis to date of the prognostic impact of MRD on the outcome of adult ALL. MRD results are reported according to new internationally approved standards. We feel that it is essential to have a well-defined methodology and terminology to create reference data for future trials and achieve comparability between different trials. The strict definition of molecular response criteria was one reason that only a proportion of the total patient cohort could be considered for the molecular response assessment. Patients without MRD test at the respective time-point, with non-evaluable MRD e.g. low positive, non quantifiable or insufficient sensitivity were excluded. This may have contributed to an overall positive selection of the analyzed patient cohort since in addition patients are excluded who relapsed or died before reaching the respective time points. Poorer results in patients without MRD testing have also been reported by others. The conclusion that MRD is a highly relevant prognostic factor in patients with MRD testing at the respective time
points is not diluted by this effect. Furthermore MRD testing was more frequently performed in standard risk patients compared to high risk patients. The interpretation of results is however not hampered by this fact, because prognostic relevance was tested first within risk groups first and then – after similar prognostic effects had been demonstrated - in the total cohort.

In this study, response to treatment significantly differed depending on the method of response assessment. A complete remission as assessed by cytological evaluation was achieved in 89% of all patients which is in line with other published ALL trials showing CR rates of 80-90% 17,20,21. Measurement of MRD allowed a more sensitive assessment of response and demonstrated that 30% of the patients with cytological CR did not achieve molecular CR. In addition considerable differences across specific ALL subtypes became apparent. HR patients achieved a lower molecular CR rate compared to SR patients (51% vs. 71%). Patients with thymic T-ALL showed a significantly better molecular response to treatment than other T-lineage subgroups (89% vs. 65%) which correlates with the more favorable outcome of this subtype 22, 23. Poorer MRD response of early T-ALL compared to other subtypes of T-ALL has also been demonstrated in pediatric patients by MRD evaluation using flow cytometry 24.

MRD assessment at several early time points during treatment allowed evaluating the kinetics of molecular response. Additional patients with incomplete molecular response after induction I achieved a molecular CR after induction II (36% versus 70% molecular responses). However, with only 9% of patients who additionally entered molecular CR during first consolidation, the effect of further standard chemotherapy was limited. Future clinical application of molecular response evaluation may include the assessment of treatment modifications during induction.
and early consolidation therapy. Thus, it has been recently demonstrated that the addition of rituximab to induction treatment in CD20-positive ALL may contribute to an improved molecular CR rate \(^{25}\).

The most important time points for MRD evaluation were in our hands after induction (day 71) because it parallels the cytologic response evaluation and after consolidation I (week 16). Patients without a molecular CR at this time-point have a high risk of relapse and little chance to obtain a molecular CR with conventional treatment. The most relevant time points have to be defined, however, depending on treatment protocol.

Detectable MRD after induction and first consolidation was associated with an unfavorable course of disease regardless of pre-existing conventional risk factors, clearly validating the strong independent prognostic impact of molecular response as demonstrated in several other smaller cohorts of adult ALL patients \(^{18,14,15,16,17}\). In this study only 12% of the patients with molecular failure after first consolidation who did not undergo SCT in first CR remained in continuous complete remission. Despite continued chemotherapy the median time to cytological relapse was 7.6 months in patients with MRD above \(10^{-4}\). In patients with MRD above \(10^{-3}\) the median time to relapse was 4.9 months only.

A multivariate analysis proved that molecular response was the only significant prognostic factor for remission duration and survival throughout both conventional risk groups. Likewise, studies in pediatric ALL have demonstrated that molecular response is the most important independent prognostic factor \(^{13}\). Even when using gene expression assays and testing for the presence of poor molecular aberrations
such as Ikaros/IKZF1 deletions, MRD persistence after induction maintained its
prognostic relevance as a functional biologic parameter \(^{26}\).

Results similar to those in molecular failure were observed for patients with molecular
relapse \(^{27}\). Without transplantation in first CR, the median time to cytological relapse
was 2.6 months and the probability of continuous complete remission was 5% only.
Regular MRD follow-up tests are a pre-requisite to detect molecular relapses. Since
the overall relapse rate decreases after more than 3 years from diagnosis, it may be
sufficient to limit MRD follow-up to the first 2-3 years.

Molecular failure and relapse are not only associated with high relapse risk but also
with significantly poorer survival. Since molecular failure indicates resistance to
conventional chemotherapy, these patients also frequently respond poorly to salvage
therapies after subsequent cytological relapse. If no SCT is performed in first CR, the
overall survival in molecular failure is only 33% at five years. This new high risk group
represents until now one of the most unfavorable subgroups within adult ALL.

Due to its prognostic relevance MRD has been implemented for treatment decisions
in clinical trials. One option is to offer SCT to patients with molecular failure as to
other HR patients. In this study, patients with molecular failure undergoing SCT in
first CR had a significantly better probability of CCR than those without SCT (66% vs.
11%), which again translated into a better survival (54% vs. 32%). The Northern
Italian Study group also reported an advantage for MRD-positive patients treated with
SCT (N=36) compared to non SCT (N=18) with approximately 50% compared to 10%
long-term disease-free survival \(^{17}\). These results indicate that despite chemotherapy-
resistant disease the combination of conditioning regimen and donor-versus-
leukemia effects could induce continuous remissions in MRD-positive patients. In our
study, however, SCT in first CR could only be accomplished in 47% of the patients with molecular failure, often due to rapidly occurring relapses. Furthermore it has been demonstrated by several groups that a high level of MRD before SCT is associated with a higher relapse risk after allogeneic \textsuperscript{28,29} or autologous transplantation \textsuperscript{30}.

There are two major options which may improve the outcome of patients with molecular failure. One is to optimize study logistics and perform donor search in all patients in order to realize SCT as soon as possible after detection of molecular failure. The other option is to offer an interim therapy to reduce the tumor load and prohibit overt relapse. Treatment with drugs that employ different mechanism of action is of particular interest in these chemotherapy-resistant patients. This may include subtype-specific chemotherapy such as the T-cell specific purine analog nelarabine \textsuperscript{31}, antibody treatment or other immunologic therapies such as donor lymphocyte infusions in molecular relapse after SCT \textsuperscript{32}. Recently, it has been demonstrated in a small pilot study that in patients with persistent MRD a molecular remission rate of 80% could be achieved with a bispecific antibody targeting CD19 \textsuperscript{33}.

Treatment of patients with molecular failure is currently an unmet medical need. These patients are candidates for evaluation of new antileukemic agents using MRD response as primary outcome. The results presented here for outcome of patients with molecular failure provide a reference database for future clinical trials enrolling patients with molecular failure for alternative treatment modalities to improve the patient’s remission duration and overall survival.

In summary, the prospectively collected MRD data provide the proof of principle that evaluation of MRD using to standardized quantitative PCR as a measure of
molecular response was feasible and clinically applicable in a multicenter treatment setting. The measurement of molecular response allowed the identification of a new subgroup of patients with an inadequate initial response and a very high risk of relapse. Early assessment of MRD has not only been proven to be a strong and independent prognostic factor to predict the patients’ outcome, but also provided useful information for further treatment decisions and modifications. Since MRD was shown to directly correlate with clinical outcome, it is an also appropriate primary endpoint for future clinical trials that are evaluating new drugs.

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AUTHORSHIP CONTRIBUTIONS

NG and DH designed the research and coordinated the study. NG performed the statistical analysis and wrote the manuscript. MB, MK, HT, TR and CRB surveyed and conducted the MRD analysis. SS and ET surveyed and conducted the immunophenotyping. All co-authors supported the study conduct as members of the protocol committee and approved the final report.

DISCLOSURE OF CONFLICTS OF INTEREST

Nothing to declare.
References


### Table 1  Cytological and molecular response rates after induction therapy (day 71) in correlation to prognostic factors

<table>
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<tr>
<th></th>
<th>Cytological CR rate</th>
<th>Molecular CR rate</th>
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<tr>
<td></td>
<td>N (%) (^a)</td>
<td>n (%)</td>
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<tr>
<td><strong>Total</strong></td>
<td>1648</td>
<td>1467 (89%)</td>
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<td><strong>Risk groups</strong></td>
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<tr>
<td>Standard risk</td>
<td>975 (59%)</td>
<td>895 (92%)</td>
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<td>High risk</td>
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<td>572 (85%)</td>
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<td><strong>Immunophenotype</strong></td>
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<tr>
<td>B-lineage</td>
<td>1076 (65%)</td>
<td>961 (89%)</td>
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<tr>
<td>T-lineage</td>
<td>569 (35%)</td>
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<td>c-ALL</td>
<td>912 (56%)</td>
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<td>169 (10%)</td>
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<td>mature T ALL</td>
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<td>Thymic T ALL</td>
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<tr>
<td><strong>Age</strong></td>
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</tr>
<tr>
<td>15-35 years</td>
<td>982 (60%)</td>
<td>890 (91%)</td>
</tr>
<tr>
<td>35-55 years</td>
<td>666 (40%)</td>
<td>577 (87%)</td>
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<td><strong>Leukocytes</strong></td>
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<tr>
<td>B-lineage (&lt;30000)</td>
<td>783 (73%)</td>
<td>706 (90%)</td>
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<tr>
<td>(&gt;30000)</td>
<td>288 (27%)</td>
<td>251 (87%)</td>
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<tr>
<td>T-lineage (&lt;100000)</td>
<td>416 (76%)</td>
<td>372 (89%)</td>
</tr>
<tr>
<td>(&gt;100000)</td>
<td>134 (24%)</td>
<td>116 (87%)</td>
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</table>

\(^a\) Percentages show distribution of characteristics within the patient group analysed for cytological CR rate

\(^b\) Percentages show distribution of characteristics within the patient group analysed for cytological CR rate

\(^c\) Chi\(^2\) test

CR, complete response; cytCR, cytological complete response; molCR, molecular complete response; n.s., not significant
Table 2  Outcome after 5 years according to molecular response in ALL patients after induction (day 71) and consolidation (week 16)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 71 after Induction</th>
<th>Week 16 after Consolidation</th>
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<tbody>
<tr>
<td></td>
<td>MolCR*</td>
<td>MolFail*</td>
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<tr>
<td></td>
<td>N</td>
<td>n</td>
</tr>
<tr>
<td>Standard Risk</td>
<td></td>
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<tr>
<td>Continuous complete remission (CCR)</td>
<td>434 335 69±3 99 42±6 p&lt;0.0001</td>
<td>424 335 74±3 89 37±6 p&lt;0.0001</td>
</tr>
<tr>
<td>CCR (SCT excluded)</td>
<td>384 317 69±3 67 32±7 p&lt;0.0001</td>
<td>376 322 74±3 54 13±5 p&lt;0.0001</td>
</tr>
<tr>
<td>Disease free survival (DFS)</td>
<td>434 335 62±3 99 35±6 p&lt;0.0001</td>
<td>424 335 68±3 89 28±5 p&lt;0.0001</td>
</tr>
<tr>
<td>DFS (SCT excluded)</td>
<td>384 317 62±3 67 27±6 p&lt;0.0001</td>
<td>376 322 68±3 54 11±5 p&lt;0.0001</td>
</tr>
<tr>
<td>Overall survival</td>
<td>434 335 80±3 99 47±6 p&lt;0.0001</td>
<td>424 335 81±3 89 43±6 p&lt;0.0001</td>
</tr>
<tr>
<td>Overall survival (SCT excluded)</td>
<td>384 317 80±3 67 38±7 p&lt;0.0001</td>
<td>376 322 81±3 54 31±7 p&lt;0.0001</td>
</tr>
<tr>
<td>High Risk</td>
<td></td>
<td></td>
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<tr>
<td>CCR</td>
<td>145 72 78±8 73* 41±7 p&lt;0.0001</td>
<td>80 49 75±8 31 29±12 p&lt;0.0001</td>
</tr>
<tr>
<td>CCR (SCT excluded)</td>
<td>40 16 36±26 24 21±10 p&lt;0.0001</td>
<td>20 11 50±35 9 19±17 p&lt;0.0001</td>
</tr>
<tr>
<td>Disease free survival (DFS)</td>
<td>145 72 66±8 73 32±6 p&lt;0.0001</td>
<td>80 49 64±6* 31 18±8* p&lt;0.0001</td>
</tr>
<tr>
<td>DFS (SCT excluded)</td>
<td>40 16 18±15* 24 6±5* p&lt;0.0001</td>
<td>20 11 34±26* 9 11±10* p&lt;0.0009</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>146 72 72±8 74 49±7 p&lt;0.0005</td>
<td>80 49 71±9 31 41±10 p&lt;0.003</td>
</tr>
<tr>
<td>Overall survival (SCT excluded)</td>
<td>40 16 56±17 24 21±9 p&lt;0.0001</td>
<td>20 11 83±15 9 44±17 p&lt;0.000</td>
</tr>
<tr>
<td>Overall</td>
<td>579*</td>
<td>407 70±3 172 39±5 p&lt;0.0001</td>
</tr>
<tr>
<td>CCR (SCT excluded)</td>
<td>424 333 68±3 91 26±6 p&lt;0.0001</td>
<td>396 333 74±3 63 12±5 p&lt;0.0001</td>
</tr>
<tr>
<td>Disease free survival (DFS)</td>
<td>579 407 63±3 172 31±4 p&lt;0.0001</td>
<td>504 384 67±3 120 25±5 p&lt;0.0001</td>
</tr>
<tr>
<td>DFS (SCT excluded)</td>
<td>424 333 60±3 91 20±5 p&lt;0.0001</td>
<td>396 333 68±3 63 10±4 p&lt;0.0001</td>
</tr>
<tr>
<td>Overall survival</td>
<td>580 407 79±3 173 47±5 p&lt;0.0001</td>
<td>504 384 80±3 120 42±5 p&lt;0.0001</td>
</tr>
<tr>
<td>Overall survival (SCT excluded)</td>
<td>424 333 80±3 91 36±6 p&lt;0.0001</td>
<td>396 333 81±3 63 33±7 p&lt;0.0001</td>
</tr>
</tbody>
</table>

*Probability (%) ± standard deviation
MolCR, molecular complete response; molFail, molecular failure; SD, standard deviation; SCT, stem cell transplantation.
a 5 years not reached
Table 3  Effect of stem cell transplantation on 5-year outcome of standard and high risk patients with molecular failure after consolidation (week 16)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No SCT N</th>
<th>% ± SD*</th>
<th>SCT n</th>
<th>% ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous complete remission</td>
<td>120</td>
<td>63</td>
<td>57</td>
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<tr>
<td>(Landmark analysis)</td>
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<td></td>
<td></td>
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<tr>
<td>Disease free survival</td>
<td>120</td>
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<tr>
<td>Overall Survival</td>
<td>120</td>
<td>63</td>
<td>57</td>
<td></td>
</tr>
</tbody>
</table>

*Probability ± standard deviation
SCT, stem cell transplantation.
FIGURE LEGENDS

Figure 1 Molecular response rate in relationship to chemotherapeutic treatment phases

Figure 2 Probability of continuous complete remission in standard and high risk patients according to molecular response status in week 16 (P<0.0001), (A) overall and (B) excluding SCT in first CR (P<0.0001)

Figure 3 Probability of survival in standard and high risk patients according to molecular response status in week 16 (P<0.0001), (A) overall and (B) excluding SCT in first CR (P<0.0001)

Figure 4 Probability of continuous complete remission in standard and high risk patients with molecular failure with (A) MRD level >10^{-4} (Median: 7.6 months) and (B) MRD level >10^{-3} in week 16 excluding SCT in first CR (Median: 4.9 months)

Figure 5 Probability of (A) continuous complete remission and (B) survival after molecular relapse excluding SCT in first CR
FIGURES

Figure 1

[Graph showing molecular response rate over time for Overall, Standard Risk, and High Risk categories]
Figure 2A

Figure 2B
Figure 3A

![Figure 3A](image)

- MolCR: 80% (N=384)
- MolFail: 42% (N=120)

Figure 3B

![Figure 3B](image)

- MolCR: 81% (N=333)
- MolFail: 33% (N=63)
Figure 4A

Figure 4B
Figure 5A

Figure 5B
Adults with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies

Nicola Gökbüget, Michael Kneba, Thorsten Raff, Heiko Trautmann, Claus-Rainer Bartram, Renate Arnold, Rainer Fietkau, Mathias Freund, Arnold Ganser, Wolf-Dieter Ludwig, Georg Maschmeyer, Harald Rieder, Stefan Schwartz, Hubert Serve, Eckhard Thiel, Monika Brüggemann and Dieter Hoelzer