The multidrug resistance-associated protein 3 (MRP3) is associated with a poor outcome in childhood ALL and may account for the worse prognosis in male patients and T-cell immunophenotype

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Short title: Expression of MRP genes in childhood ALL

Key words: ABCC genes, acute lymphoblastic leukemia, ABC-transporter, stem cell

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Abstract

The family of MRPs (multidrug resistance-associated proteins) belongs to the superfamily of ABC-transporters which have the ability to function as outward pumps for chemotherapeutic drugs and therefore might be involved in drug resistance. In this study the expression of the MRP2, MRP3, MRP4, MRP5 and SMRP genes was measured using TaqMan real time PCR in 103 children with previously untreated ALL (precursor B-ALL: n=71; T-ALL: n=32). All five genes were expressed with a great variability. Only MRP3 expression was associated with a significantly worse prognosis (p=0.008). The median expression of MRP3 was 10-fold higher in T-ALL than in precursor B-ALL (p<0.001) and 4-fold higher in male patients than in female patients (p<0.001). The prognostic impact of MRP3 was independent of immunophenotype or sex. Higher levels of MRP3 were found in patients with a poor in vivo response to prednisone but this could not be confirmed in an independent case control study (40 patients) for prednisone response. In healthy donors, the median expression of MRP4 was 4-fold higher in bone marrow and 8-fold higher in CD34+ stem cells as compared to peripheral blood (p=0.002). Our results suggest that MRP3 is involved in drug resistance in childhood ALL. It therefore represents an interesting target to overcome multidrug resistance. High levels of MRP3 could possibly be the reason for the poorer prognosis of patients with male gender or T-ALL. Similar to other members of the family of ABC-transporters, MRP4 seams to be a marker for immature stem cells.
Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent malignancy in childhood. Major improvements in the treatment of ALL have been achieved over the last 30 years and more than 70% of children with ALL can be cured with current therapeutic regimens [1;2;3]. However, some patients still fail to respond to therapy and others relapse with resistant disease.

Several mechanisms of drug resistance have been identified. One of these is the overexpression of adenosine triphosphate (ATP)-dependent membrane proteins that function as drug efflux pumps [4]. The best-characterized drug efflux pump is the permeability glycoprotein (P-gp) which is encoded by the multidrug resistance gene 1 (MDR1). Expression of P-gp/MDR1 has been identified as an independent adverse prognostic factor for complete remission and survival in patients with acute myeloid leukemia (AML) [5;6;7]. Recent studies suggested that the results in the treatment of AML can be improved by combining chemotherapy with drugs that inhibit the function of P-gp [8;9]. However, the clinical relevance of P-gp seems to be much smaller in childhood ALL than in AML. Some studies found a prognostic impact of P-gp expression in childhood ALL [10;11] but others failed to show such an association [12;13].

Our group could recently demonstrate that the expression of the breast cancer resistance protein (BCRP), which is a close relative of MDR1, was also associated with a poor response to chemotherapy in AML patients [14], but not in ALL patients [15].

Like MDR1 and BCRP, the family of multidrug resistance-associated proteins (MRP) belongs to the superfamily of ATP-binding-cassette (ABC)-transporters. Their structure, function and substrate specificity has been studied intensively [16;17]. In transfection studies, all MRPs were shown to confer resistance to drugs that are used in the treatment of ALL (MRP1, MRP2 and MRP3: doxorubicin, vincristine, methotrexate; MRP4: methotrexate, thioguanine, 6-
mercaptopurin; MRP5 and SMRP: thioguanine, 6-mercaptopurine [18]). So far, little is known about the clinical relevance of MRPs in hematological malignancies. Only the expression of MRP1 has been studied in larger groups of ALL patients. No association with response to chemotherapy was found [13].

The aims of our study were to find out whether the more recently discovered members of the MRP family (MRP2, MRP3, MRP4, MRP5 and SMRP) are expressed in childhood ALL and whether they are associated with a poor response to chemotherapy.

Patients and Methods

Patients, Diagnosis and Therapy

All 103 patients were diagnosed with previously untreated ALL. The main patient characteristics are summarized in Table I. The initial diagnosis of ALL was determined by Pappenheim-stained bone marrow smears and cytochemistry reactions (periodic acid-Schiff reaction, acid phosphatase, alphanaphthyl acetate esterase and myeloperoxidase reaction). Immunophenotype and chromosomal rearrangements were determined by standard methods [1;19]. Written consent was given for the use of all patient samples for this study.

All patients were treated according to multicenter studies in Germany: ALL-VI/80 (7 patients), ALL-VII/81 (41 patients), ALL-VIII/87 (10 patients) [2;20;21], ALL-BFM-90 (16 patients) [1], ALL-BFM-95 (19 patients) [22] and ALL-BFM-2000 (10 patients). The main drugs that were used in all studies were steroids, methotrexate, cytosine-arabinoside, anthracycline, asparaginase and vincristine. The event-free survival was 41% in ALL-VI/80, 58% in ALL-VII/81, 74% in ALL-VIII/87 [2;20;21], 78% in ALL-BFM-90 [1] and 79% in ALL-BFM-95 [22]. The improvement of the treatment results from 1980 to 1990 was mainly
achieved by an intensification of chemotherapy. The therapy regimens of the studies ALL-BFM-90/95 and 2000 are similar.

To assess the association of prednisone response and MRP3 expression an independent study was performed. This study was designed as case-control study for prednisone response. Cases were patients with poor prednisone response (n=20), controls were patients with good prednisone response (n=20).

According to ALL-BFM criteria [1], prednisone good response was defined as the reduction of leukemic blasts in the peripheral blood to <1000 per µl on day 8 after 7 days monotherapy with prednisone and a single intrathecal application of methotrexate on treatment day one. Prednisone poor-response is defined as the presence of ≥1000 per µl peripheral blood blasts on treatment day 8.

All patients of the case-control study have been treated according to trial ALL-BFM 2000. Matching criteria were initial white blood cell count, age at diagnosis, sex, and immunophenotype (Table II). All samples were negative for BCR/ABL, TEL/AML1 and MLL/AF4 rearrangements.

Healthy Donors

Five samples of peripheral blood CD34+ stem cells (3 male, 2 female; aged 22 to 38) and six samples of bone marrow (3 male, 3 female; aged 30 to 45) were obtained from healthy adults who donated for stem cell transplantation or bone marrow transplantation. Written consent was given for the use of these samples for this study.

Fourteen samples of peripheral blood were donated by laboratory staff. Eight of these samples (5 male, 3 female; aged 25 to 48) were used to analyze all mononuclear cells. The other six samples (3 male; 3 female; aged 23 to 46) were used for a separate analysis of T-lymphocytes and B-lymphocytes.
Sample Collection and Processing

Leukemic cells and mononuclear cells of healthy donors were isolated from bone marrow or peripheral blood by Ficoll-Hypaque density gradient centrifugation. After this procedure the percentage of leukemic cells was more than 90% in all patient samples as determined by May-Gruenwald-Giemsa stained cytospins. All patient samples were collected prior to the beginning of chemotherapy. All samples were cryopreserved in liquid nitrogen.

CD34+ stem cells of healthy donors were isolated using the CliniMACS system. T-lymphocytes (CD3+) and B-lymphocytes (CD19+) of healthy donors were isolated using the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturers instructions. The purity of the cell populations was more than 90% determined by flow cytometry.

Quantitative real time PCR

Total RNA was isolated using RNeasy Mini Kit including DNase digestion (Qiagen, Hilden, Germany). The amount of RNA was measured by photometry and a stock solution of 2µg RNA in 40µl was prepared. RNA was transcribed into cDNA using Omniscript (Qiagen, Hilden, Germany).

Quantitative PCR was performed using the ABI Prism™ 7700 Sequence Detector (Applied Biosystems, Weiterstadt, Germany). Primers and TaqMan probes for MDR1 [23], MRP2, MRP5 and SMRP [24] were used as described previously. Primers and TaqMan probes for MRP3 and MRP4 were:

MRP3: Forward 5´-GCACCATTGCTGGCTACA-3´; Reverse 5´-GCAGGACACCCAGGACCAT-3´; TaqMan probe 5´-CATCCTCTCCACCTGCTCCAAGCTCA-3´

MRP4: Forward 5´-TGGATTCTGTGGCTTTGAACAC-3´; Reverse 5´-AGCCAAAATGAGCGTGCAA-3´; TaqMan probe 5´-CGTACGCTATGCCACGCTGA-3´
Final concentration of the primers was 900nM (300nM for MRP3 and SMRP), final concentration of the TaqMan probes was 300nM (200nM for MRP3 and SMRP). All TaqMan probes were labeled with FAM and TAMRA.

The expression of the resistance genes was standardized for expression of beta-2-microglobulin which was measured using Pre-Developed Assay Reagents (Applied Biosystems, Weiterstadt, Germany).

The final volume for each PCR was 30µl including 1.5µl of the investigated sample. Universal PCR Master Mix (Applied Biosystems, Weiterstadt, Germany) was used according to the manufacturers instructions.

Serial dilutions of cDNA of reference cell lines were used to generate standard curves. The reference cell lines were: MCF7/CH1000 (MRP2 and MRP3), K562 (MRP4, MRP5 and SMRP) and CEM-ADR5000 (MDR1). The expression of each gene in each sample was analyzed in duplicate. The regression coefficients of the standard curves ranged between 0.994 and 0.999. The variation of the duplicate measurements was extremely small compared to the variation between different samples. In the few cases where there was a substantial difference between the two values, the sample was re-analyzed.

In 20 samples the measurement of the expression of MRP3 was repeated using the probe and primers supplied by Assays-on-Demand from Applied Biosystems, Weiterstadt, Germany. The Spearman’s correlation coefficient of the two measurements was 0.985 with p<0.001.

**Statistical Methods**

Cox regression analysis was used to estimate the prognostic relevance of each MRP gene. In order to prevent a bias due to the different outcomes in the six studies, all Cox regression analyses were calculated with the number of the study as a stratification variable. Kaplan-Mayer statistics and Log-rank tests were calculated to estimate the significance of differences
between survival curves. Since the levels of MRP expression did not follow a normal
distribution, expression of MRP in different groups of patients was compared using the Mann-
Whitney test for two groups and the Kruskal-Wallis test for more than two groups. The
correlation between MRP expression and other pretherapeutic findings was investigated by
means of Spearman’s correlation coefficient and the MRP expression at presentation and at
relapse was compared using the Wilcoxon test. All p-values are given for two-sided tests. All
calculations were performed using the SPSS 11.0 program (SPSS Inc, Chicago, IL, USA).

**Results**

**Expression of MRPs in healthy controls**

Measurable amounts of all five MRP genes were found in all samples from healthy controls
(Fig. 1). For MRP2, MRP5 and SMRP we found no significant differences between the
different types of controls that were analyzed (peripheral blood mononuclear cells, bone
marrow mononuclear cells, peripheral blood T-lymphocytes (CD3+), peripheral blood B-
lymphocytes (CD19+) and peripheral blood stem cells (CD34+)).

The median expression of MRP4 was 4-fold higher in bone marrow and 8-fold higher in
CD34+ stem cells as compared to peripheral blood (p=0.002; Fig. 1).

The median expression of MRP3 was 10-fold higher in samples that contained all
mononuclear cells from bone marrow or peripheral blood as compared to samples that only
contained isolated T-lymphocytes or B-lymphocytes (p-values: <0.01; Fig. 1). This result
suggests that the expression of MRP3 in blood and bone marrow is mainly attributed to high
levels in monocytes or myeloid precursor cells.
None of the five MRP genes differentiated significantly between male and female individuals in any type of control samples. None of the five MRP genes was associated with the age of the donor.

**Expression of MRPs in childhood ALL**

All five genes were expressed with a great variability (Fig. 1). Measurable amounts of MRP2, MRP4, MRP5 and SMRP were found in all patients. MRP3 was not detectable in 29 patients. The variation from the 10th percentile to the 90th percentile was 8-fold for MRP2, 15-fold for MRP4, 9-fold for MRP5 and 18-fold for SMRP. The variation from the lowest measurable value for MRP3 to the 90th percentile was 30-fold. The median levels of all five MRP genes were higher in ALL samples than in healthy B or T-lymphocytes (Fig. 1; p-values: <0.05).

**Association of MRPs with other diagnostic features of ALL**

All genes were investigated for their association with sex, age, immunophenotype, initial white blood cell (WBC) count and the percentage of leukemic cells in peripheral blood. The median expression of MRP2, MRP5 and SMRP was about two times higher in patients with T-ALL as compared to precursor B-ALL (p-values: <0.01). Median expression of MRP3 was even 10 times higher in T-ALL (p<0.001; Fig. 1).

Higher levels for MRP2, MRP3, MRP5 and SMRP were associated with higher initial WBC counts and higher percentages of leukemic cells. However, the Spearman’s correlation coefficients were relatively small (range: 0.24 to 0.40; p-values: <0.05).

Surprisingly, the median expression of MRP3 was 4-fold higher in male patients than in female patients (p<0.001; Fig. 2). There was a higher proportion of T-ALLs in male patients (Tab. 1) but this did not explain the difference between MRP3 expression in boys and girls. When analyzing precursor B-ALL and T-ALL separately, the expression of MRP3 was still significantly higher in male patients (only precursor B-ALL: p=0.035; only T-ALL: p=0.003).
None of the other MRP genes differentiated between male and female patients. None of the MRP genes was associated with the age of the patients.

In a previous study we measured the expression of MDR1 gene in 38 patients who were also included in the present study. None of the MRP genes was significantly associated with the expression of MDR1.

Only in the more recent studies ALL-BFM-90/95 and 2000, patients were routinely investigated for the chromosomal rearrangements BCR/ABL and TEL/AML1 [1]. Out of the 45 patients one was found positive for BCR/ABL and four patients were found positive for TEL/AML1. As far as we can tell from these small numbers, there was no trend for a particularly high or low expression of the MRP genes in these patients.

**Expression of MRPs and survival**

A univariate Cox regression analysis was calculated to estimate the prognostic relevance of each MRP gene. Only the expression of MRP3 was associated with a significantly worse prognosis (p=0.008). Table IIIA provides the data for overall survival in patients with high levels of MRP3 and in patients with low levels of MRP3 as defined by different cutoffs for high and low expression. Survival curves using the 75th percentile as cutoff are given in Figure 3A. The results were similar when relapse-free survival was calculated instead of overall survival.

Forty-five patients included in the main study were treated according to the more recent studies ALL-BFM-90, ALL-BFM 95 and ALL-BFM 2000. In these studies very similar therapy regimens have been used and the results were much better than in the preceding studies. So far, only four of those forty-five patients succumbed to their disease. This number is too small for statistically significant analyses of risk factors. However, the data presented in Table IIIB and Figure 3B suggest that the prognostic impact of MRP3 was not or only in part overcome by the more intensive therapy in recent studies.
MRP3 expression was strongly correlated with T-cell immunology and male gender, both of which are known to be indicators of a poor prognosis [1;25]. However, when sex or immunophenotype were used as stratification variables, Cox regression analysis still showed a significant association between MRP3 expression and poor outcome (p-values: <0.01). This illustrates that the prognostic impact of MRP3 is independent of immunophenotype or sex.

In a multivariate stepwise Cox regression analysis with the continuous variables: MRP3 expression, WBC count, enlargement of liver below coastal arch, enlargement of spleen below coastal arch; and the dichotomous variables: sex, immunophenotype (T-ALL versus precursor B-ALL) and age (under 2 years versus older than 2 years); only the WBC count and the expression of MRP3 sustained independent prognostic relevance. The inclusion of the other variables did not significantly improve the prognostication compared to those two variables alone.

None of the other four MRP genes was associated with a poor prognosis. Even when testing a large set of different cutoffs for high and low expression, we found no convincing trend for a prognostic implication of MRP2, MRP4, MRP5 or SMRP.

Expression of MRPs and response to prednisone

As the initial treatment response to prednisone is the strongest prognostic factor of outcome within the experience of the BFM study group, response to prednisone was determined in all patients in whom the therapy was started after January 1988 (n=53). Nine patients showed a poor response, 44 patients where classified as prednisone good responders.

No association with response to prednisone was found for MRP2, MRP4, MRP5 or SMRP. The median expression of MRP3 was four times higher in patients with a poor response to prednisone (p=0.01) but the proportions of patients with male gender (67% versus 48%) and with T-ALL (44% versus 25%) were also higher in patients with a poor response to prednisone than in patients with a good response. In order to analyze whether the association
of MRP3 with the response to prednisone was independent of these factors an independent case-control study for prednisone response was performed.

We found no trend for an association of MRP3 gene expression with response to prednisone in this group of patients (Table II). This finding suggests that the association of MRP3 with response to prednisone in the original study population was mainly due to the higher proportion of T-ALL and male patients in the group with a poor response.

In consistence with the original study population we found that within the independent case-control study the median expression of MRP3 was five times higher in male patients than in female patients (p=0.019) and four times higher in T-ALL than in precursor B-ALL (p=0.045).

**Expression of MRPs at the time of diagnosis and at relapse**

In eleven patients the expression of the five MRP genes was measured at the time of diagnosis as well as in first relapse. The expression levels of all five genes were very similar at both time points. None of the five genes was significantly upregulated at the time of relapse.

**Discussion**

To our knowledge this study provides first data on the association between the response to chemotherapy and the expression of MRP2, MRP3, MRP4, MRP5 and SMRP in ALL patients. Our results suggest that MRP3 is involved in drug resistance in ALL. This is consistent with our observations in childhood AML, where MRP3 showed a strong prognostic impact [26]. The observation that there is no association of MRP3 expression and prednisone response does not contradict that conclusion, as MRP3 has not been described as being specifically associated with steroid resistance. MRP3 therefore represents an interesting marker for risk-adapted therapy and a possible target for the development of specific drugs to
overcome multidrug resistance. Sirotnak et al. [27] found that the function of MRPs can be inhibited by probenecid and that the efficacy of chemotherapeutics in mice could be enhanced by the co-administration of probenecid.

One of the most interesting results of this study is that MRP3 strongly differentiates between male and female patients. Many large studies have indicated that boys who suffer from ALL do worse than girls [1;25]. A reason for this difference could not yet be found. Our results suggest that higher levels of MRP3 expression might account for the poorer prognosis in male patients. Future studies should address the biological mechanism for this differential expression. Likewise, MRP3 seems to be one reason for the worse prognosis of patients with T-ALL compared to precursor B-ALL.

It was recently described that the ABC-transporters, MDR1 and BCRP are indicators of immature stem cells and that these proteins probably play an important role in cell differentiation [28;29]. Our results show that MRP4 is highly expressed in CD34+ stem cells with intermediate levels in bone marrow and low levels in peripheral blood (Fig.1). These findings suggest that MRP4 could be another member of the family of ABC-transporters which is an indicator of immature stem cells and is involved in cell differentiation.

Prospective studies, including specific functional assays and the analysis of protein expression are necessary to confirm our results. In a study on lung cancer cell lines, Young et al. [30] could demonstrate a good correlation between mRNA and protein levels for MRP2 and MRP3.

Our results do not indicate a clinical relevance of MRP2, MRP4, MRP5 or SMRP in childhood ALL. Either the expression of these genes in the leukemic cells is to small to cause a significant efflux of chemotherapeutic drugs or the levels of mRNA do not strongly correlate with the amount of functional protein in the cell membrane.
Acknowledgement

We thank Dr. Douglas D Ross from the University of Maryland Greenebaum Cancer Center for providing the cell line MCF7/CH1000 which was used to generate standard curves for MRP2 and MRP3.


27 Sirotnak FM, Wendel HG, Bornmann WG et al. Co-administration of probenecid, an inhibitor of a cMOAT/MRP-like plasma membrane ATPase, greatly enhanced the


Table I. Initial patient data

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>103</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>6.8 (0.2 - 17.1)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>54/49</td>
</tr>
<tr>
<td>Median WBC in 10^9/l (range)</td>
<td>48 (2 - 688)</td>
</tr>
<tr>
<td>Median percentage of leukemic cells in WBC (range)</td>
<td>83 (1-100)*</td>
</tr>
<tr>
<td>BCR/ABL (yes/no/n.a.)</td>
<td>1/44/58</td>
</tr>
<tr>
<td>MLL/AF4 (yes/no/n.a.)</td>
<td>0/45/58</td>
</tr>
<tr>
<td>TEL/AML1 (yes/no/n.a.)</td>
<td>4/41/58</td>
</tr>
<tr>
<td>Liver &gt; 3cm below costal margin (yes/no/n.a.)</td>
<td>52/48/3</td>
</tr>
<tr>
<td>Spleen &gt; 3cm below costal margin (yes/no/n.a.)</td>
<td>51/49/3</td>
</tr>
<tr>
<td>Response to prednisone (good**/poor***/n.a.)</td>
<td>44/9/50</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td></td>
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<tr>
<td>precursor B lineage (male/female)</td>
<td>71 (34/37)</td>
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<tr>
<td>T lineage (male/female)</td>
<td>32 (20/12)</td>
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</table>

n.a. = not available
* peripheral blood was only used to analyze MRP expression in patients with more than 80% of leukemic cells before and more than 90% after Ficoll-Hypaque density gradient centrifugation
** <10^9 leukemic cells/l on day 8
*** >10^9 leukemic cells/l on day 8
Table II. Initial data of patients included in the independent case control study for the analysis of the association of MRP3 expression and prednisone response. Cases and controls were matched for age, sex, immunophenotype, and initial WBC count. All samples were negative for BCR/ABL, TEL/AML1 and MLL/AF4 rearrangements.

<table>
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<th>poor response to prednisone</th>
<th>good response to prednisone</th>
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<td>number of patients</td>
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<td>BCR/ABL</td>
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<tr>
<td>TEL/AML1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>MRP3</td>
<td>median (range)</td>
<td>0.00022 (0 - 0.0033)</td>
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Table III A and B. Prognostic impact of MRP3 expression using quartiles and thirds as cutoffs for high and low expression.

A: all patients (n=103)

<table>
<thead>
<tr>
<th>Percentile used as cutoff</th>
<th>Low expression of MRP3</th>
<th>High expression of MRP3</th>
<th>Log-Rank test p-value</th>
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<tr>
<td></td>
<td>Overall survival after 10 years in %</td>
<td>Standard error in %</td>
<td>Overall survival after 10 years in %</td>
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<tr>
<td>25</td>
<td>64</td>
<td>11</td>
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<td>6</td>
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</tr>
<tr>
<td>75</td>
<td>64</td>
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B: only patients who were treated in the studies ALL-BFM 90/95 or 2000 (n=45)

<table>
<thead>
<tr>
<th>Percentile used as cutoff</th>
<th>Low expression of MRP3</th>
<th>High expression of MRP3</th>
<th>Log-Rank test p-value</th>
</tr>
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<tr>
<td></td>
<td>Overall survival after 5 years in %</td>
<td>Standard error in %</td>
<td>Overall survival after 5 years in %</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>*</td>
<td>87</td>
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<tr>
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<tr>
<td>75</td>
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<td>5</td>
<td>82</td>
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</table>

* Standard errors can not be computed if survival is 100%
precursor B-ALL   T-ALL   PB   BM   SC   BL   TL

Figure 1. Expression of MRP2 and MRP3 relative to the cell line MCF7/CH1000 and expression of MRP4, MRP5 and SMRP relative to the cell line K562 in 71 samples of precursor B-ALL, 32 samples of T-ALL, 8 samples of healthy peripheral blood mononuclear cells (PB), 6 samples of healthy bone marrow mononuclear cells (BM), 5 samples of healthy peripheral blood CD34+ stem cells (SC), 6 samples of healthy peripheral blood B-lymphocytes (BL), and 6 samples of healthy peripheral blood T-lymphocytes (TL).

Mann Whitney test for MRP3 in T-ALL versus precursor B-ALL: p<0.001
Kruskal-Wallis test for MRP4 in PB versus BM versus SC: p=0.002
Figure 2. Expression of MRP3 (10th, 25th, 50th, 75th and 90th percentile) relative to the cell line MCF7/CH1000 in 54 female and 49 male patients. Mann-Whitney test: p<0.001
Figure 3. A: Overall survival in patients with high levels of MRP3 and in patients with low levels of MRP3. The 75th percentile was used as cutoff for high and low expression. 

N = number of patients; OS = overall survival; SE = standard error

Log-Rank test: p=0.002

B: Same analysis as in A, but restricted to patients who were treated in the more recent studies ALL-BFM 90/95 and 2000.

Log-Rank test: p=0.071
The multidrug resistance-associated protein 3 (MRP3) is associated with a poor outcome in childhood ALL and may account for the worse prognosis in male patients and T-cell immunophenotype

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