EXPRESSION OF THE OUTCOME PREDICTOR IN ACUTE LEUKEMIA 1 (OPAL1) GENE IS NOT AN INDEPENDENT PROGNOSTIC FACTOR IN PATIENTS TREATED ON COALL OR ST. JUDE PROTOCOLS.


From the Departments of aPediatric Oncology/Hematology, Erasmus MC-Sophia Children’s Hospital, Erasmus University Medical Center Rotterdam, The Netherlands; bPharmaceutical Sciences, cBiostatistics, and dPathology, eHematology/Oncology, St. Jude Children’s Research Hospital, Memphis, TN, USA; fHematology/Oncology, University Children’s Hospital, Hamburg; giPediatric Hematology and Oncology, University Medical Center, Heinrich-Heine-University, Düsseldorf; hiPediatric Oncology, Dr. von Haunersches Children’s Hospital, University of Munich, Munich, Germany, iCOALL study group.

*both authors contributed equally

RUNNING TITLE: OPAL1 Expression and Outcome in Acute Leukemia
SCIENTIFIC HEADING: Neoplasia
WORD COUNT: Abstract: 177 words; Manuscript: 3130 words

Corresponding author:
Dr. Monique L. den Boer
Erasmus MC-Sophia Children’s Hospital
Erasmus University Medical Center
Department of Pediatric Oncology/Hematology
Dr. Molewaterplein 60
3015 GJ Rotterdam
The Netherlands
Phone: +31 10 4636691
Fax: +31 10 4089433
E-mail: m.l.denboer@erasmusmc.nl

Copyright © 2006 American Society of Hematology

M.d.B., J.R.D., G.E.J.-S., U.Gr., U.Gö., C.-H.P, W.E, and R.P. participated in designing and performing the study; A.H., M.H.C., and D.P. controlled and analyzed data; A.H., and M.H.C. wrote the paper; and all authors checked the final version of the manuscript.
ABSTRACT

New prognostic factors may result in better risk classification and improved treatment of children with acute lymphoblastic leukemia (ALL). Recently, high expression of a gene named OPAL1 (Outcome Predictor in Acute Leukemia) was reported to be associated with favorable prognosis in ALL. Therefore, we investigated whether OPAL1 expression was of prognostic importance in two independent cohorts of children with ALL treated on COALL-92/-97 (N=180) and on St. Jude Total 13 protocols (N=257). We observed a consistently higher (2.8-fold) expression of OPAL1 in TEL-AML1-positive ALL compared to TEL-AML1-negative ALL in both cohorts, but higher OPAL1 expression was not consistently associated with other favorable prognostic indicators such as age and white blood cell count, or ALL genetic subtype. Lower OPAL1 expression was also not associated with increased in vitro drug resistance. Multivariate analyses including known risk factors showed that OPAL1 expression was not independently related to prognosis in either the COALL or St. Jude cohorts. In conclusion, OPAL1 expression may not be an independent prognostic feature in childhood ALL and its previously reported prognostic impact appears to be treatment-dependent.
INTRODUCTION

The prognosis of childhood acute lymphoblastic leukemia (ALL) has improved remarkably over the past four decades due to the introduction of effective risk-adapted combination chemotherapies. Conventional factors used to stratify patients are clinical and biological parameters such as age at diagnosis, initial white blood cell count (WBC), immunophenotype, the presence of specific genetic abnormalities\(^1\) and early response to treatment.\(^2\) Newer approaches include *in vitro* drug resistance profiles,\(^3\) and measurement of minimal residual disease after induction of initial remission.\(^4,5\)

The use of DNA microarrays enables investigators to simultaneously assess the expression of thousands of genes. In previous studies in childhood ALL, microarray analysis was successfully applied to identify known genetic and phenotypic subtypes,\(^6-8\) as well as treatment-specific changes in gene expression\(^9\) and genes related to drug resistance.\(^10\) Recently, this technology was used to identify three novel genes, referred to as G0, G1 and G2, that were highly predictive of outcome in 254 patients with childhood ALL enrolled in Pediatric Oncology Group (POG) treatment protocols.\(^11-13\) The top discriminating gene, G0, was fully cloned and named **OPAL1** *(Outcome Predictor in Acute Leukemia 1)*. The function of **OPAL1** is unknown, although the presence of a cytochrome c-like heme-binding site and a transmembrane domain suggested **OPAL1** may be involved in the mitochondrial electron transport chain.\(^14\) We initially identified this gene as one of the top ranked class discriminating genes that was over-expressed in ALL cells positive for the *TEL-AML1* gene fusion.\(^7,8\) In the POG study, **OPAL1** was expressed at higher levels in ALL subgroups with a favorable prognosis (i.e., ALL with t(12;21)/*TEL-AML1*, normal and hyperdiploid karyotypes) compared to a subgroup with an unfavorable prognosis (i.e., ALL with t(9;22)/*BCR-ABL*) and another subgroup previously associated with an unfavorable prognosis in (i.e., ALL with t(1;19)/*E2A-PBX1*).\(^12\) High **OPAL1** expression was shown to be highly predictive of a favorable outcome in the total ALL group, but also in ALL subgroups, such as T-lineage ALL and t(12;21)/*TEL-AML1*-positive B-lineage ALL. Finally, low **OPAL1** was significantly related to induction failures.\(^12\)
To independently validate these interesting results we analyzed in depth the expression pattern of OPAL1 in two independent cohorts of children with newly diagnosed ALL treated on protocols of the Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia (COALL, N=180) and St. Jude Children’s Research Hospital (St. Jude, N=257). OPAL1 expression was investigated in relation to in vitro resistance to four widely used drugs in the treatment of childhood ALL, i.e. prednisolone, vincristine, L-asparaginase and daunorubicin. In addition, OPAL1 expression was tested as a predictor of clinical outcome in childhood ALL, where ALL subtypes as well as other known prognostic factors were included in a multivariate analysis.
MATERIALS AND METHODS

Leukemia samples

Bone marrow and peripheral blood samples were obtained after informed consent from children with newly diagnosed ALL who were enrolled on either the Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia protocols COALL-92/97 (N=180)\textsuperscript{10,15,30} or the St. Jude Children’s Research Hospital (St. Jude) protocols Total Therapy 13 (N=257).\textsuperscript{7,8,16,17} These two independent trials used similar chemotherapeutic agents. Approval was obtained from the Erasmus MC/Sophia Children’s Hospital or SJCRH institutional review board for these studies. If necessary peripheral blood or diagnostic bone marrow samples were enriched for leukemic blasts to be $\geq 90\%$ as previously described.\textsuperscript{10,18}

\textbf{In vitro drug resistance assay}

In COALL patients, responsiveness of leukemia cells to prednisolone (PRED; Bufa Pharmaceutical Products, Uitgeest, The Netherlands), vincristine (VCR; TEVA Pharma, Mijdrecht, The Netherlands), L-asparaginase (ASP; Paronal, Christiaens, Breda, The Netherlands), and daunorubicin (DNR; Cerubidine, Rhône-Poulenc Rorer, Amstelveen, The Netherlands) was determined by the 4-day \textit{in vitro} MTT drug resistance assay.\textsuperscript{3} The concentration ranges tested for these drugs were: PRED: 0.008-250 $\mu$g/ml (N=167); VCR: 0.05-50 $\mu$g/ml (N=166); ASP: 0.003-10 IU/ml (N=166) and DNR: 0.002-2.0 $\mu$g/ml (N=140). The drug concentration lethal to 50\% of the ALL cells (LC$_{50}$ value) was used as the measure of cellular drug resistance.\textsuperscript{3,19}

\textbf{Real-time quantitative PCR}

The mRNA expression levels of \textit{OPAL1} and glyceraldehyde-3-phosphate dehydrogenase (\textit{GAPDH}) as a reference, were determined using quantitative real-time PCR (RTQ-PCR) analysis on the ABI Prism 7700 sequence detection system as previously
described.\textsuperscript{20-23} All PCR reactions were performed with an amplification efficiency of more than 95%. OLIGO 6.22 software (Molecular Biology Insights, Cascade, CO, USA) was used to design primer and probe combinations within \textit{OPAL1} (NM_017787) and \textit{GAPDH} (NM_2046). Primer sequences used were: 5'-TCCTTTGGGTCTTAGACAG-3' (sense) 5'-TGGCAAAAACCTGAAAT-3' (antisense) and 5'-ACAGTCTCAGTGCCTCACTACTATGAA-3' for \textit{OPAL1} and 5'-GTGGAGTCAACGGATT-3' (sense), 5'-AAGCTTCCGTTTCAGAT-3' (antisense) and 5'-TCAACTACATGGTTACATGTCCA-3' for \textit{GAPDH}. Probe sequences used were: 5'-ACAGTCTCAGTGCCTCACTACTATGAA-3' for \textit{OPAL1} and 5'-TCAACTACATGGTTACATGTCCA-3' for \textit{GAPDH}. For each sample, the comparative cycle time (C\textsubscript{t}) value of the \textit{OPAL1} PCR was normalized by subtracting the C\textsubscript{t} value of the \textit{GAPDH} PCR (\Delta C\textsubscript{t}).\textsuperscript{20} From this \Delta C\textsubscript{t} value the relative \textit{OPAL1} expression to \textit{GAPDH} in arbitrary units (AU) was calculated using the following formula: relative mRNA expression = \(2^{-\Delta C_t} \times 100\%\). We observed a significant correlation between \textit{OPAL1} mRNA expression assessed by RT-PCR and microarray (\(r_s=0.35, P=0.003, N=72\)).

**Microarray analysis**

Total RNA was hybridized to U133A (COALL) and U95Av2 (St. Jude) GeneChip\textsuperscript{®} oligonucleotide microarrays, according to the manufacturer’s protocol (Affymetrix, Santa Clara, CA, USA). Data analysis was performed as described before and gene-expression data of the leukemic samples included in this present study were previously published.\textsuperscript{8,10} Briefly, gene expression values were scaled to the target intensity of 2500, using Affymetrix Microarray Suite\textsuperscript{®} (MAS) 5.0 software and log\textsubscript{2}-transformed. To analyze the expression of \textit{OPAL1}, we used the U133A probe set 202808_at (COALL), which covers the same DNA sequence (99.8% sequence identity) as the Affymetrix U95Av2 GeneChip\textsuperscript{®} probe set 38652_at (St. Jude) the latter also used by Mosquera-Caro \textit{et al}.\textsuperscript{8,12} More information on these probe sets and its target sequences is available at Affymetrix\textsuperscript{®} NetAffx Analysis Center http://www.affymetrix.com/analysis/index.affx. \textit{OPAL1} expression determined with both
arrays U133A and U95Av2 was highly correlated and available for a subset of St. Jude patients ($r_s=0.58$, $P<0.0001$, $N=92$).

Statistics

The duration of disease-free survival (DFS) was defined as the time from diagnosis until the date of leukemia relapse (event), the last follow-up or secondary events other than relapse (censored). DFS curves were calculated according to the Kaplan-Meier method or a modification thereof in the presence of competing events. Because no cut-off for OPAL1 expression was provided by Mosquera-Caro et al., we performed survival analyses in two different ways; OPAL1 expression was treated either as a continuous variable or as a categorical variable (OPAL1 expression was divided into 3 equal sized groups by the 33rd and 67th percentile of expression (i.e., low [bottom third], intermediate [intermediate third] and high [top third]). The predictive value of OPAL1 expression in three groups (2 degrees of freedom [d.f.]) and of OPAL1 expression as a continuous variable was analyzed by log-rank test and by a Cox proportional hazard regression model (adjusted for competing events in all analyses of the St. Jude cohort). The association of OPAL1 expression with DFS considering other known prognostic factors was assessed in univariate and in multivariate analyses. The model for multivariate analysis included conventional risk factors (i.e., WBC, age, immunophenotype and genetic abnormalities). Differences in OPAL1 expression between ALL subgroups were tested using the Mann-Whitney U test. Spearman’s correlation test was used to compare the expression of OPAL1 by microarray with the expression data obtained by RTQ-PCR and to relate OPAL1 expression to in vitro drug resistance. All statistical tests were performed at a two-tailed significance level of 0.05. When applicable, Bonferroni correction was applied to correct for multiple comparisons.
RESULTS

The association of OPAL1 expression with prognostic features in ALL was tested in two cohorts of children with ALL. High OPAL1 expression was consistently observed in TEL-AML1-positive ALL in both patient cohorts (Figure 1 and Table 1; 2.8-fold, \(P<0.0001\)). There was no further evidence of a significant relation between OPAL1 expression and any other prognostic features in COALL patients (Table 1A), whereas low OPAL1 expression was related to age >10 years and high OPAL1 expression was related to a hyperdiploid karyotype among St. Jude patients (Table 1B). The frequency distribution of these risk categories between the two cohorts was comparable, except for WBC count where we observed a trend towards higher counts in the COALL cohort (\(P=0.004\), Two-sample Kolmogorov-Smirnov test). The likely reason for these differences between the St. Jude and COALL patients is that patients with higher WBC counts were more likely to have enough cells for \textit{in vitro} drug resistance testing.

Because drug resistance is a major cause of treatment failure, we investigated in the COALL cohort whether OPAL1 expression was related to \textit{in vitro} drug resistance for any of four drugs that form an integral component of contemporary chemotherapeutic protocols for children with ALL. No correlation was observed between \textit{in vitro} drug resistance and OPAL1 expression for prednisolone (\(r_s<0.001\), \(P=0.99\)), L-asparaginase (\(r_s=-0.02\), \(P=0.81\)) and daunorubicin (\(r_s=0.05\), \(P=0.54\)). By contrast, vincristine (VCR) resistance showed a significant positive correlation with OPAL1 expression (\(r_s=0.34\), \(P<0.0001\)) which is opposite of what would be expected if high OPAL1 expression is related to a good prognosis, as previously reported.\(^{12}\) Nevertheless, this observation is concordant with a 3.6-fold increased VCR-resistance among TEL-AML1-positive ALL compared to non-TEL-AML1 B-lineage ALL (\(P<0.0001\); median LC\(_{50}=0.697\) μg/ml and 0.193 μg/ml, respectively).

The relation between OPAL1 expression and treatment outcome was subsequently investigated in both cohorts. Of the 180 patients who were part of the COALL cohort, 42 had disease-related events and 6 had competing events (2 secondary malignancies and 4 deaths in remission). Of the 257 patients included in the St. Jude study Total Therapy 13, 42 had
disease-related events and 20 had competing events (16 secondary malignancies and 4
deaths in remission). \(^7\) In the COALL cohort, \(\text{OPAL1}\) expression was significantly
associated with disease-free survival (DFS) when the patient population was divided into 3
equally sized groups based on the individual rank in \(\text{OPAL1}\) expression (2 d.f., \(P=0.01\);
Figure 2A) but not when \(\text{OPAL1}\) expression was treated as a continuous variable (\(P=0.45\)),
or when the top 33% of patients with high expression were compared with the bottom 33% of
patients with low expression (\(P=0.28, \text{Table 2}\)). In contrast, as reported in a preliminary
analysis of the St. Jude cohort by others, \(^{11-13}\) \(\text{OPAL1}\) expression was significantly associated
with DFS among the St. Jude cohort, whether patients were divided into three equally sized
groups (2 d.f. \(P=0.002\); Figure 2B), \(\text{OPAL1}\) expression was treated as a continuous variable
(\(P<0.0001\)), or the top third of patients with high expression were compared with the bottom
third with low expression (\(P=0.01, \text{Table 2}\)).

The clinical value of \(\text{OPAL1}\) expression was further studied within major
prognostically important ALL subtypes (Table 2). In both cohorts the opposite (although not
statistically significant in St. Jude patients) correlation was observed in T-ALL, i.e. a low
\(\text{OPAL1}\) expression correlated with a favorable DFS (Table 2). This result differs from our
results for the total group of ALL patients (Figure 2, Table 2) as well as those obtained for the
initial POG cohort \(^{11-13}\) where high \(\text{OPAL1}\) expression was found to be a favorable outcome
predictor in T-ALL patients.

Among B-lineage ALL cases, higher expression of \(\text{OPAL1}\) was only significantly
related to a favorable prognosis in the St. Jude cohort, both as a continuous variable
(\(P=0.0001\)) and when expression was applied as a categorical variable for the top third
(\(P=0.02; \text{Table 2}\)).

Among \(\text{TEL-AML1}\)-positive B-lineage ALL cases treated on the COALL protocols,
\(\text{OPAL1}\) expression was not significantly associated with DFS (Table 2A; \(P=0.61, P>0.89\)).
When all \(\text{TEL-AML1}\)-positive patients treated on the St. Jude protocols (Total Therapy 13A
and 13B) were analyzed, the association between high \(\text{OPAL1}\) expression and higher DFS
was only significant when the expression was analyzed as a continuous variable (\(P<0.0001\)).
Interestingly, *OPAL1* expression treated as a continuous variable had no prognostic significance among the 36 *TEL-AML1*-positive patients treated in study 13A (*P*=0.32), but was significant among the 34 *TEL-AML1*-positive patients treated in study 13B (*P*=0.0002). Notably, because of different criteria for assigning patients to the low-risk versus high-risk treatment in protocols Total 13A versus Total 13B, 97% (35 out of 36) of patients with *TEL-AML1*-positive ALL enrolled on the Total 13A, compared to only 29% (10 out of 34 patients) on Total 13B were treated according to the high-risk arm of the respective protocols, suggesting that the prognostic impact of *OPAL1* expression may be treatment dependent. When the outcome analyses was limited to the St. Jude high-risk protocols, *OPAL1* expression was not significantly related to DFS (*P*=0.05). In contrast, among patients treated on the Total 13B low-risk protocol (N=70), *OPAL1* expression was significantly related to DFS in the univariate analysis (*P*=0.021, [HR=0.54]), and when other prognostic factors were considered, (i.e., age, WBC, hyperdiploid karyotype, *TEL-AML1* gene fusion; *P*=0.017, [HR=0.28]). No association of *OPAL1* expression and DFS was detected within patients treated according to the COALL high-risk and low-risk protocol. It should be noted that *TEL-AML1* status was not a criteria for treatment stratification in COALL protocols.

Most importantly, when known risk factors (i.e. age, WBC, immunophenotype and genetic subtypes) were included in a multiple regression model, *OPAL1* expression was no longer predictive of prognosis in ALL in the COALL or in the St. Jude study groups (*P*>0.25, continuous variable; Table 3; *P*>0.10, categorical variable, data not shown).
DISCUSSION

Recently, we identified expression signatures associated with cellular drug resistance and outcome in ALL. In our prior study, OPAL1 was not among the top 124 most discriminating genes for cellular drug resistance that were also associated with treatment outcome. This per se does not exclude a predictive role for OPAL1 in childhood ALL, as this gene may be significant at a lower level than the cut-off P-values used for the construction of our resistance signature models, or may be directly related to treatment outcome as the selection of genes in our earlier study was focused on in vitro drug resistance profiles and not directly on outcome. Therefore, we analyzed the expression patterns of OPAL1 in leukemic cells at initial diagnosis of ALL of two independent groups of 180 COALL and 257 St. Jude patients and assessed the relation between the expression of this gene to age, WBC, gender, immunophenotype, genetic subtype, in vitro drug resistance and clinical outcome. The COALL and St. Jude protocols represent two independent protocols, which use similar chemotherapeutic agents.

We observed a 2.8-fold higher OPAL1 expression in children with TEL-AML1-positive B-lineage ALL in both patient cohorts. This is consistent with the higher OPAL1 expression levels observed in TEL-AML1-positive cases as initially reported by our group and by Mosquera-Caro et al. OPAL1 expression was elevated in hyperdiploid B-lineage ALL samples in the St. Jude, but not in the COALL cohort. This is in disagreement with the previous POG report, which describes a consistently higher expression of OPAL1 in hyperdiploid ALL. In the St. Jude patient group, higher levels of OPAL1 expression were found in hyperdiploid ALL, and lower levels of OPAL1 expression were found in patients older than 10 years. With the exception of TEL-AML1-positive ALL, OPAL1 expression was not associated with any other prognostic factor in COALL patients. However, interpretation of associations in some subgroups may be difficult due to limited sample size. Taken together, with the exception of TEL-AML1-positive ALL these data suggest that, in contrast to the observation made by Mosquera-Caro et al., high OPAL1 expression was not consistently related to ALL subgroups with a favorable prognosis in these two cohorts.
In vitro sensitivity to several drugs is related to favorable outcome.\textsuperscript{3,19,28,29} Based on the previously observed relation between high OPAL\textsubscript{1} expression and favorable prognosis\textsuperscript{12,13} we tested the relation between high OPAL\textsubscript{1} expression and in vitro drug sensitivity. However, in the present study we observed no relation between a high OPAL\textsubscript{1} expression and sensitivity to prednisolone, L-asparaginase and daunorubicin and only a weak positive correlation with vincristine resistance, which is in opposite direction of what would be expected if high OPAL\textsubscript{1} expression is related to a good prognosis. This indicates that OPAL\textsubscript{1} may not be a major determinant of cellular drug sensitivity.

We found no evidence of an association between low OPAL\textsubscript{1} expression and worse outcome in T-lineage and TEL-AML\textsubscript{1}-negative B-lineage ALL patients treated according COALL and St. Jude protocols. In fact, in our univariate analysis of patients with T-lineage ALL, a worse outcome was consistently associated with high OPAL\textsubscript{1} expression. This contradicts the results reported by Mosquera-Caro et al. The significant association between high OPAL\textsubscript{1} expression and favorable DFS in TEL-AML\textsubscript{1}-positive ALL patients treated at St. Jude may depend on the risk-group stratification applied to these patients. OPAL\textsubscript{1} expression was only significant within TEL-AML\textsubscript{1}-positive ALL patients treated on the Total 13B protocol, where the majority of patients (24 out of 34) were stratified in the low risk treatment arm. In contrast, OPAL\textsubscript{1} expression had no predictive value in Total 13A treated patients, where the majority (35 out of 36) of TEL-AML\textsubscript{1}-positive patients were treated according to a high risk protocol.

These data indicate that OPAL\textsubscript{1} expression may be prognostic in patients with TEL-AML\textsubscript{1}-positive ALL treated with reduced-intensity chemotherapy. The same remission induction and re-induction treatment was used in all St. Jude protocols, but the main difference was the reduced number of antileukemic agents (4 versus 7 [8]) used in the continuation phase of the low-risk protocol (i.e., mercaptopurine, methotrexate, prednisolone, vincristine) compared to the high-risk protocol (i.e., mercaptopurine, methotrexate, dexamethasone [or prednisone], vincristine, etoposide, cyclophosphamide, cytarabine, [and for protocol 13A: L-asparaginase]).
No association of DFS and \textit{OPAL1} expression in patients treated on either low- or high-risk protocols was found for the COALL group. Overall both COALL protocols use the same medications for the treatment of low- and high-risk patients, but treatment for low-risk patients was reduced by one or two doses per drug. Furthermore, in comparison with the 13B low-risk regimen, all COALL patients had an additional intensification phase (6 to 7 antileukemic agents for 4 to 8 weeks). This again points to a relation of \textit{OPAL1} expression and DFS only if patients are treated with lower intensity chemotherapy, (i.e. fewer antileukemic agents) such as the low risk arm of St. Jude protocol 13B. Importantly, in both COALL and St. Jude cohorts, the relationship of \textit{OPAL1} expression with DFS was not independent of known risk factors (i.e., age, WBC and ALL subtype) in a multivariate analysis. In addition, increased \textit{OPAL1} expression was not independently associated with \textit{in vitro} drug sensitivity of COALL-treated children with ALL (MTT data were not available for St. Jude patients).

In conclusion, previously reported prognostic properties of \textit{OPAL1} expression in childhood ALL may be related to the relatively modest intensity of treatment given to the patients in whom this observation was previously made and may not be significant when other known risk factors are included or when more-intensive chemotherapy is given. The present data thus indicate that \textit{OPAL1} expression is not universally predictive of treatment outcome in childhood ALL, particularly in the context of contemporary treatment protocols.

\textbf{ACKNOWLEDGMENTS}

We thank Drs. Cheng Cheng, Wenjian Yang and Renée X. de Menezes for helpful discussion and Susan C.J.M. Peters for her technical assistance. We are indebted to all clinical staff at St. Jude and the COALL centers of all who cared for these patients and to the patients and parents for their participation in these studies. Supported by grants from the Pediatric Oncology Foundation Rotterdam, the Nijbakker-Morra Foundation, and the René Vogels Stipendium 2002, The Netherlands, the Elterninitiative Kinderkrebsklinik Duesseldorf,
Germany and supported in part by grants from the National Institutes of Health and the American Lebanese Syrian Associated Charities.
REFERENCES


22. Stams WAG, Den Boer ML, Beverloo HB, et al. Sensitivity to L-asparaginase is not associated with expression levels of asparagine synthetase in t(12;21)+ pediatric ALL. Blood. 2003;101:2743-2747


Legends of figures and tables

Figure 1. **OPAL1** expression in different ALL subtypes.

**OPAL1** expression was compared in a total of 180 children with ALL treated according to COALL protocols (A), and in a total of 257 children with ALL treated according to St. Jude protocols (B). **OPAL1** expression is shown in log2-transformed scaled arbitrary units (AU), the medians (horizontal lines), the 25th and 75th percentiles (boxes), the ranges (bars) and the outliers (open circles) are shown. **Indicates P<0.0001, determined by the Mann-Whitney U test.

Figure 2. Disease-free survival according to **OPAL1** expression in children with ALL.

**OPAL1** expression was not associated with disease-free survival among 180 children with newly diagnosed ALL treated on COALL 92/97 (A), but an association was observed among 257 newly diagnosed children with ALL treated on St. Jude Total 13 protocols (B).

Table 1. **OPAL1** expression in prognostic subgroups of ALL at diagnosis

**OPAL1** expression was compared in prognostic ALL subgroups defined by age, white blood cell count (WBC), gender, immunophenotype and genetic subtype in children treated on (A) COALL and (B) St. Jude protocols. Indicated are the number of patients (N) for each group, the ratio of **OPAL1** expression in non-reference versus reference (*) group (fold-difference in median scaled **OPAL1** expression) and P-values comparing the non-reference versus reference group determined by the Mann-Whitney U test.

Table 2. Univariate analysis of the prognostic value of **OPAL1** expression in pediatric ALL

Overview of disease-free survival (DFS) analyses for low, intermediate and high **OPAL1** expression in COALL and St. Jude study cohorts in the total group of pediatric ALL patients; within B-lineage and T-lineage ALL patients; and within genetic subgroups of ALL that were associated with **OPAL1** expression in (A), for COALL patients and (B), for St. Jude patients. Reported are 4-year disease-free survival rates, P-values and hazard ratios derived from a Cox univariate model using **OPAL1** expression as a categorical and as a continuous variable.
Table 3. Multivariate analysis of the prognostic value of OPAL1 expression in pediatric ALL

Cox multivariate proportional hazards analysis computed with known prognostic factors (i.e., age, white blood cell count (WBC), immunophenotype and genetic subtype). OPAL1 expression was treated as continuous variable. Hazard ratios (HR), 95% confidence intervals (CI) and P-values are shown.
Figures and tables

Figure 1. OPAL1 expression in different ALL subtypes.

![Box plot of OPAL1 expression in different ALL subtypes](image1)

**A**

- COALL
- B-other
- BCR-ABL
- Hyperdiploid
- MLL
- T-ALL
- TEL-AML1

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-other</td>
<td>53</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>4</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>40</td>
</tr>
<tr>
<td>MLL</td>
<td>4</td>
</tr>
<tr>
<td>T-ALL</td>
<td>35</td>
</tr>
<tr>
<td>TEL-AML1</td>
<td>44</td>
</tr>
</tbody>
</table>

**B**

- St. Jude
- B-other
- BCR-ABL
- Hyperdiploid
- MLL
- T-ALL
- TEL-AML1

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-other</td>
<td>82</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>9</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>50</td>
</tr>
<tr>
<td>MLL</td>
<td>8</td>
</tr>
<tr>
<td>T-ALL</td>
<td>38</td>
</tr>
<tr>
<td>TEL-AML1</td>
<td>70</td>
</tr>
</tbody>
</table>

Figure 1, Holleman et al

Figure 2. Disease-free survival according to OPAL1 expression in children with ALL.

![Graph of disease-free survival](image2)

**A**

- High OPAL1
- Intermediate OPAL1
- Low OPAL1

Follow-up (years)

- 60
- 58
- 52
- 44
- 36
- 24
- 20
- 10
- 8
- 6
- 4
- 2
- 0

Disease-free survival (%)

- 100
- 80
- 60
- 40
- 20
- 0

3 groups, 2 d.f.: P=0.01

Continuous variable: P=0.45

**B**

- High OPAL1
- Low OPAL1
- Intermediate OPAL1

Follow-up (years)

- 60
- 58
- 52
- 44
- 36
- 24
- 20
- 10
- 8
- 6
- 4
- 2
- 0

Disease-free survival (%)

- 100
- 80
- 60
- 40
- 20
- 0

3 groups, 2 d.f.: P=0.002

Continuous variable: P<0.0001

Figure 2, Holleman et al
Table 1. OPAL1 expression in prognostic subgroups of ALL at diagnosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>A</th>
<th>COALL</th>
<th>B</th>
<th>St. Jude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Ratio</td>
<td>P-value</td>
<td>N</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 years</td>
<td>131</td>
<td>1.00*</td>
<td></td>
<td>181</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>49</td>
<td>0.86</td>
<td>0.39</td>
<td>76</td>
</tr>
</tbody>
</table>

| WBC      |   |       |         |   |       |         |
| <10/nL   | 43 | 1.00* |   | 90 | 1.00* |   |
| 10-49/nL | 67 | 0.91  | 0.97 | 80 | 1.11  | 0.42 |
| 50-100/nL| 28 | 1.04  | 0.93 | 40 | 0.95  | 0.88 |
| >100/nL  | 42 | 1.04  | 0.76 | 47 | 0.85  | 0.023 |

| Gender   |   |       |         |   |       |         |
| Female   | 76 | 1.00* | 0.74 | 95 | 1.00* | 0.53 |
| Male     | 104 | 1.00 |       | 162 | 1.09 |       |

| ALL subtype |   |       |         |   |       |         |
| B-other     | 53 | 1.00* |   | 82 | 1.00* |   |
| BCR-ABL     | 4  | 0.89  | 0.74 | 9  | 1.02  | 0.63 |
| hyperdiploid| 40 | 0.94  | 0.47 | 50 | 1.17  | 0.002** |
| MLL rearranged | 4  | 1.23  | 0.79 | 8  | 1.10  | 0.19 |
| TEL-AML1    | 44 | 2.69  | <0.0001** | 70 | 2.82  | <0.0001** |
| T-lineage   | 35 | 1.13  | 0.32 | 38 | 1.10  | 0.056 |

Button reference group; *cytogenetic analysis revealed more than 50 chromosomes **significant after Bonferroni correction

Table 2. Univariate analysis of the prognostic value of OPAL1 expression in pediatric ALL

<table>
<thead>
<tr>
<th>Variable</th>
<th>A</th>
<th>COALL</th>
<th>B</th>
<th>St. Jude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>4-yr DFS with SE [%]</td>
<td>OPAL1 P-value (HR)</td>
<td>OPAL1 P-value (HR)</td>
</tr>
<tr>
<td>Total group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low#</td>
<td>60</td>
<td>78 ±5 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>60</td>
<td>63 ±7 (0.07) (1.88)</td>
<td>0.45 (0.92)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>60</td>
<td>87 ±5 (0.28) (0.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-lineage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low#</td>
<td>48</td>
<td>79 ±6 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>49</td>
<td>69 ±7 (0.33) (1.49)</td>
<td>0.078 (0.81)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>48</td>
<td>91 ±5 (0.18) (0.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-lineage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low#</td>
<td>12</td>
<td>92 ±8 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>11</td>
<td>50 ±16 (0.06) (7.90)</td>
<td>0.06 (1.9)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>12</td>
<td>42 ±14 (0.03 (9.80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-TEL-AML1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low#</td>
<td>34</td>
<td>76 ±7 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>33</td>
<td>75 ±8 (0.93) (1.04)</td>
<td>0.84 (0.97)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>34</td>
<td>66 ±9 (0.55 (1.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEL-AML1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low#</td>
<td>15</td>
<td>92 ±3 (1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td>14</td>
<td>92 ±7 (0.89 (0.82)</td>
<td>0.61 (0.70)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>100 (0.97 (0.94)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cox univariate analysis adjusted for competing events. OPAL1 expression categorical* or as continuous** variable. ***significant after Bonferroni correction, #This group served as the reference group; HR= hazard ratio, nd= not detected, i.e. no events occurred.

Table 2 Holleman et al
Table 3. Multivariate analysis of the prognostic value of OPAL1 expression in pediatric ALL

<table>
<thead>
<tr>
<th>Variable</th>
<th>COALL N</th>
<th>HR</th>
<th>CI</th>
<th>P-value</th>
<th>St. Jude N</th>
<th>HR</th>
<th>CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 years</td>
<td>131</td>
<td>1.0*</td>
<td></td>
<td></td>
<td>181</td>
<td>1.0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>49</td>
<td>1.24</td>
<td>0.64-2.41</td>
<td>0.52</td>
<td>76</td>
<td>1.37</td>
<td>0.67-2.79</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10/nL</td>
<td>43</td>
<td>1.0*</td>
<td></td>
<td></td>
<td>90</td>
<td>1.0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-49/nL</td>
<td>67</td>
<td>0.54</td>
<td>0.19-1.49</td>
<td>0.23</td>
<td>80</td>
<td>0.99</td>
<td>0.42-2.34</td>
<td>0.99</td>
</tr>
<tr>
<td>50-100/nL</td>
<td>28</td>
<td>1.03</td>
<td>0.35-3.02</td>
<td>0.95</td>
<td>40</td>
<td>0.78</td>
<td>0.24-2.46</td>
<td>0.68</td>
</tr>
<tr>
<td>&gt;100/nL</td>
<td>42</td>
<td>1.59</td>
<td>0.58-4.32</td>
<td>0.36</td>
<td>47</td>
<td>1.32</td>
<td>0.52-3.34</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>ALL subtype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-other</td>
<td>53</td>
<td>1.0*</td>
<td></td>
<td></td>
<td>82</td>
<td>1.0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>4</td>
<td>2.32</td>
<td>0.51-10.6</td>
<td>0.28</td>
<td>9</td>
<td>9.09</td>
<td>2.48-33.3</td>
<td>0.0009</td>
</tr>
<tr>
<td>Hyperdiploid*</td>
<td>40</td>
<td>0.15</td>
<td>0.03-0.65</td>
<td>0.01</td>
<td>50</td>
<td>0.84</td>
<td>0.28-2.45</td>
<td>0.74</td>
</tr>
<tr>
<td>MLL rearranged</td>
<td>4</td>
<td>9.51</td>
<td>2.47-36.5</td>
<td>0.001</td>
<td>8</td>
<td>1.97</td>
<td>0.37-10.3</td>
<td>0.42</td>
</tr>
<tr>
<td>TEL-AML1</td>
<td>44</td>
<td>0.18</td>
<td>0.05-0.68</td>
<td>0.01</td>
<td>70</td>
<td>0.93</td>
<td>0.18-4.73</td>
<td>0.93</td>
</tr>
<tr>
<td>T-lineage</td>
<td>35</td>
<td>0.79</td>
<td>0.33-1.93</td>
<td>0.6</td>
<td>38</td>
<td>2.73</td>
<td>1.11-6.71</td>
<td>0.029</td>
</tr>
<tr>
<td>OPAL1 expression</td>
<td>180</td>
<td>1.03</td>
<td>0.77-1.34</td>
<td>0.9</td>
<td>257</td>
<td>0.71</td>
<td>0.4-1.28</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*reference group,* cytogenetic analysis revealed more than 50 chromosomes

Table 3 Holleman et al
Expression of the outcome predictor in acute leukemia 1 (OPAL1) gene is not an independent prognostic factor in patients treated on COALL or St. Jude protocols