# Gene expression profiles and risk stratification in childhood acute lymphoblastic leukemia

[haematologica]
2004;89:801-808

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A B S T R A C T

**Background and Objectives.** Childhood acute lymphoblastic leukemia (ALL) is a heterogeneous disease. There are several distinct genetic subtypes, characterized by typical changes in gene expression pattern. In addition to cytogenetic markers, the *in vivo* response to treatment is an emerging prognostic marker for risk stratification. However, it has not yet been reported whether gene expression profiles can predict risk group stratification already at the time of diagnosis.

**Design and Methods.** We analyzed bone marrow samples of 31 ALL patients to identify changes in gene expression that are associated with the current risk assignment, irrespective of the genetic subtype. Gene expression profiles were established using oligonucleotide microarrays.

**Results.** Considering all low- and high-risk patients, no gene was capable of predicting the risk assignment already at time of diagnosis. However, screening for risk group associated genes using more homogeneous subsets of patients revealed 106 discriminatory probe sets. The prognostic significance of these probe sets was subsequently determined for the entire series of patients. Using the selected subgroups as the training set and the remaining samples as an independent test set, logistic regression using 3 predictor variables could accurately predict current risk assignment for 10 out of 12 patients.

**Interpretation and Conclusions.** Gene expression profiles established from a cytogenetically heterogeneous study group are not, as yet, sufficiently accurate to be used prognostically in a clinical setting. Additional risk-associated gene expression analyses need to be performed in more homogeneous sets of patients.

Key words: childhood acute lymphoblastic leukemia, gene expression, microarray, risk stratification.

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Acute lymphoblastic leukemia (ALL) in children is a heterogeneous disease with a varied response to treatment. Despite overall survival rates approaching 75-80%, a significant fraction of patients still cannot be cured.[1] Because of this heterogeneity, accurate assignment of patients to different risk groups at the earliest time possible is important in order to select the best strategy for each individual.

Current risk stratification into standard- (low-), intermediate- or high-risk groups is based on molecular/cytogenetic markers (*BCR-ABL* and *MLL-AF4* rearrangements) and the *in vivo* response to treatment. Chromosomal aberrations frequently involve non-random chromosomal translocations that produce novel gene fusions or lead to inappropriate expression of oncogenes.[2,3] These genetic alterations have a clear impact on the patient's prognosis as exemplified by the association of Philadelphia chromosome positive ALL with a poor outcome.[4,5] Because of the significance of cytogenetic abnormalities such as t(9;22), t(1;19), t(12;21), and rearrangement of the MLL gene on chromosome 11q23, great efforts have been made recently to establish gene expression profiles that discriminate among these subtypes.[6-11] Intriguingly, distinct expression profiles that can accurately predict cytogenetic subgroups have indeed been identified in these studies.

A patient's initial response to treatment, which can be monitored with molecular biology techniques, is one of the strongest indicators of subsequent prognosis.[12-14] Polymerase chain reaction (PCR)-based analysis of minimal residual disease (MRD) can detect residual leukemic cells during induction therapy down to a level of one leukemic cell in $10^5$ normal cells. Given the

high prevalence in B-cell precursor and T-cell leukemias, clonal T-cell receptor and/or immunoglobulin chain rearrangements are the most suitable targets for this type of analysis.[15,16] Unfortunately, there is a considerable lag period before these results have therapeutic consequences and they do not allow immediate risk stratification at the time of diagnosis.

Hence, we examined whether gene expression profiles of leukemic bone marrow samples at diagnosis could accurately predict subsequent risk group assignment. It was not our intention to identify genes predicting the overall (long-term) outcome. In this study we used oligonucleotide microarrays to evaluate bone marrow aspirates from a total of 31 children.

## Design and Methods

### *Patients and RNA preparation*

A total of 31 children with ALL were included in the study. Assignment to risk groups and treatment was carried out according to the ALL-Berlin-Frankfurt-Münster (BFM) 2000 protocol. In the ALL-BFM 2000 protocol, risk-adapted treatment stratification (standard-, intermediate-, or high-risk) is achieved using cytogenetic markers (t(9;22), t(4;11)) or their molecular counterparts (*BCR-ABL* and *MLL-AF4*) and the *in vivo* response to treatment. Response is assessed cytomorphologically by the initial cytoreduction (blast reduction in peripheral blood after 7 days of treatment with prednisone and one application of intrathecal methotrexate; blast clearance from bone marrow after induction therapy on treatment-day 33), or molecularly by measurement of MRD on treatment-day 33 and after induction consolidation at week 12. Further characteristics of the patients are given in Table 1 and Figure 1. Mononuclear cells (MNC) were obtained from heparinized bone marrow (BM) at diagnosis and stored frozen at –70°C within 24 h. Patients were enrolled into the study if they had >75% leukemic blasts in the BM. Total RNA was prepared from each sample by extraction using a combined protocol of TRIZOL (Invitrogen, Paisley, UK) and the RNeasy Mini kit (Qiagen, Hilden, Germany). After lysis of the MNC in TRIZOL, addition of chloroform, and centrifugation for 15 minutes at 4°C, the upper phase was mixed with one volume of 70% ethanol and applied to an RNeasy spin column, continuing with the RNeasy Mini kit protocol step 5 as described by the manufacturer. DNase digestion was performed on column. Total RNA was quantified and finally validated for integrity using the Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA).

### *Array hybridization and scanning*

All experiments described in this study were performed with the Affymetrix HG-U133A GeneChip™, containing 22,283 probe sets. Each GeneChip™ was hybridized using targets synthesized from 100 ng starting material (total RNA). Target synthesis, hybridization, staining, and washing were performed using standard protocols as recommended by the manufacturer (Affymetrix, Santa Clara, CA, USA). Because of the limited amount of RNA starting material, we used the *small sample labeling protocol, version II* (available from Affymetrix website, *http://www.affymetrix.com*). Stained chips were scanned on a Gene Array Scanner (Agilent, Palo Alto, CA, USA), and data files were processed by GeneChip™ software (Affymetrix, Santa Clara, CA, USA), to make background and scaling corrections. All array data are available from the ArrayExpress database (available at URL *http://www.ebi.ac.uk/arrayexpress*).

### *Quantitative reverse transcription polymerase chain reaction (RT-PCR)*

We used quantitative RT-PCR to independently determine the level of gene expression for 6 genes (cyclin H, ribosomal protein large P0, ribosomal protein L34, ribosomal protein S19, neuritin 1, and transcription factor-like 5) in eight randomly chosen samples. cDNA was generated from 500 ng of total RNA by using the first strand step of the SuperScriptII kit (Invitrogen, UK). PCR was carried out with the ABI7700 (Applied Biosystems, Foster City, CA, USA) using commercially available target probes and mastermix (Applied Biosystems, USA): CCNH-Hs00236923_m1 (_at), RPLP0-Hs99999902_m1 (s_at), RPL34-Hs00241560_m1 (_at), RPS19-Hs00357218_q1 (x_at), NRN1-Hs00213192_m1 (_at), and TCFL5-Hs00232-4444_m1 (_at). We calculated the $C_T$ values of each gene with the *ABI sequence detection system 1.9* program. (Applied Biosystems, USA) and normalized them to the level of β2-microglobulin (B2M-Hs99999907_m1). We obtained correlation coefficients of 0.46, 0.94, 0.98, and 0.98 when correlating the TaqMan results to the expression of the four specific (_at) probe sets of the GeneChip, and 0.88, resp. –0.01, when correlating the real-time RT-PCR results to the array data of the possibly unspecific (s_at, resp. x_at) probe sets (*see Supplemental Figure A from URL http://www.kispi.unizh.ch/onkologie*).

### *Statistical analysis*

Clustering, supervised testing for differential expression and classification were done on base 10 log-transformed expression data, using the statistical software bundle R.[17] Prior to an average-linkage hierarchical clustering of all 31 samples based on Euclidean distances, we performed unsupervised gene filtering.[18] By requiring a variation coefficient (SD/mean) of at least 0.3 across samples and a minimal expression of

**Table 1. Patients' characteristics and risk group assignment.**

| *Patient* | *Sex* | *Age* | *Immunophenotype* | *Cytogenetics*[a] | *Leukocytes* $\times 10^9$/L | *Blasts % (BM)* | *Prednisone Response*[b] | *Risk group*[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 6 y 1 mo. | B precursor | *E2A-PBX1* | 66.5 | 83 | good | SR |
| 2 | F | 6 y | B precursor | *TEL-AML1* | 13.9 | 75 | good | SR |
| 3 | F | 1 y 1 mo. | B precursor | normal | 10.2 | 97 | good | SR |
| 4 | M | 4 y 1 mo. | B precursor | *TEL-AML1* | 3.0 | 89 | good | SR |
| 5 | M | 3 y 6 mo. | B precursor | *TEL-AML1* | 7.6 | 88 | good | SR |
| 6 | M | 6 y 10 mo. | B precursor | *TEL-AML1* | 2.9 | 98 | good | SR |
| 7 | F | 6 y 8 mo. | B precursor | normal | 45.9 | 98 | good | SR |
| 8 | M | 2 y 3 mo. | B precursor | *TEL-AML1* | 42.2 | 98 | good | SR |
| 9 | F | 4 y 4 mo. | T cell | normal | 101.0 | 95 | good | SR |
| 10 | M | 12 y 8 mo. | B precursor | *E2A-PBX1* | 18.3 | >80 | good | SR |
| 11 | M | 3 y 5 mo. | B precursor | normal | 56.7 | >80 | good | SR |
| 12 | F | 3 y 7 mo. | B precursor | *E2A-PBX1* | 20.4 | >80 | good | SR |
| 13 | M | 12 y 2 mo. | B precursor | *E2A-PBX1* | 447.0 | >80 | good | SR |
| 14 | M | 5 y 7 mo. | B precursor | *E2A-PBX1* | 17.1 | 96 | good | IR |
| 15 | M | 2 y 11 mo. | B precursor | *TEL-AML1* | 8.8 | 90 | good | IR |
| 16 | F | 7 y 7 mo. | B precursor | hyper>50 | 8.8 | 99 | good | IR |
| 17 | M | 6 y 9 mo. | B precursor | hyper>50 | 5.3 | 99 | good | IR |
| 18 | F | 12 y | B precursor | normal | 46.6 | 92 | good | IR |
| 19 | F | 9 y 9 mo. | T cell | normal | 56.1 | 87 | good | IR |
| 20 | M | 1 y 8 mo. | B precursor | *TEL-AML1* | 144.8 | 93 | good | IR |
| 21 | F | 7 y 3 mo. | B precursor | normal | 493.3 | 97 | good | IR |
| 22 | M | 7 y | B precursor | *BCR-ABL* | 24.4 | 92 | good | HR |
| 23 | M | 2 y 11 mo. | B precursor | *TEL-AML1* | 14.6 | 96 | poor | HR |
| 24 | M | 6 y 4 mo. | B precursor | normal | 7.0 | 95 | good | HR |
| 25 | F | 2 y 3 mo. | B precursor | hyper>50 | 26.9 | 98 | poor | HR |
| 26 | F | 8 y 1 mo. | B precursor | hyper>50 | 15.7 | 89 | good | HR |
| 27 | M | 3 y 5 mo. | B precursor | normal | 35.9 | >80 | good | HR |
| 28 | M | 1 y 1 mo. | B precursor | normal | 91.8 | >80 | good | HR |
| 29 | M | 3 y 9 mo. | B precursor | normal | 77.9 | >80 | poor | HR |
| 30 | F | 12 y 10 mo. | B precursor | normal | 79.6 | >80 | poor | HR |
| 31 | M | 6 y | B precursor | normal | 33.2 | >80 | poor | HR |

[a]*normal indicates DNA index of 1, no TEL-AML1, BCR-ABL, E2A-PBX1, or MLL-AF4 rearrangement;* [b]*as defined by a reduction of leukemic blasts in the peripheral blood to below 1000/mm³ after 7 days of treatment with prednisone;* [c]*SR indicates standard-risk, IR intermediate-risk, and HR high-risk.*



20 absolute units in at least 16 of the 31 samples, we obtained a set of 333 probe sets for the clustering. Supervised testing for differential expression was done by individually applying Welch's two sample t-test for the whole set of 22,283 transcripts. Raw *p* values were computed from a t-distribution with corresponding degrees of freedom; they were then adjusted by the Benjamini-Hochberg false discovery rate.[19] The cut-off for the adjusted *p*-values was set at 5%. The sub-analysis of 5 standard-risk *TEL-AML1* positive samples versus 6 high-risk patients with a DNA index of 1 and without further distinct cytogenetic alterations resulted in a set of 125 probe sets with significant differential expression. Among them, we identified 6 ribosomal proteins with specific probe sets, on which we performed principal component analysis for dimension reduction. The subsequent class prediction was performed with 4 different methods: a support vector machine with radial basis kernel, the 1-nearest neighbor-rule, diagonal linear discriminant analysis and logistic regression.[20]

## Results

### *Visualization of gene expression profiles by unsupervised hierarchical clustering*

In order to establish gene expression profiles, we analyzed bone marrow aspirates obtained at the time of diagnosis from 31 children who were assigned to risk classes according to the ALL-BFM 2000 protocol. The series comprised 13 patients assigned to the standard-risk group, 8 patients to the intermediate group, and 10 patients to the high-risk group (Table 1). In the initial analysis of the gene expression data set, we used an unsupervised hierarchical clustering algorithm to arrange all 31 samples according to the similarity in their expression patterns. This analysis grouped the leukemia samples according to genetic and immuno-

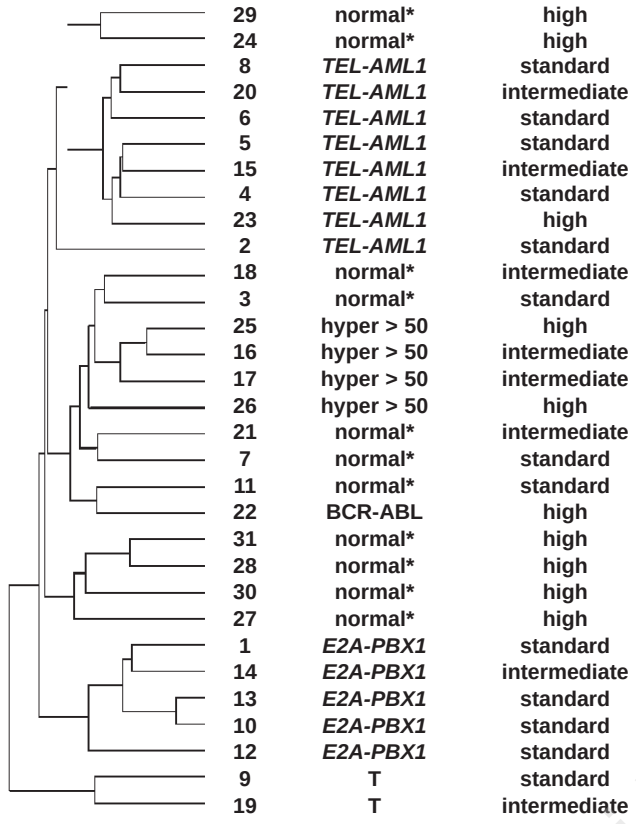


**Figure 1. Unsupervised hierarchical clustering of gene expression data from bone marrow samples of 31 children with acute lymphoblastic leukemia. Cluster dendrogram based on 333 filtered probe sets (see methods for filter criteria). The upper main branch includes precursor B cell leukemias, whereas the lowest branch identifies two T-cell leukemias. The left column shows the patient number, cytogenetic characterization is listed in the middle column, risk group assignment is presented in the right column. The dendrogram did not change substantially when more (or even all) probe sets were used (results not shown). *normal indicates DNA index of 1 and no *TEL-AML1*, *BCR-ABL*, *E2A-PBX1* or *MLL-AF4* rearrangement.**

logical subtypes present in our series, namely 8 patients with TEL-AML1 rearrangement, 5 patients with E2A-PBX1, 4 patients with hyperdiploid (>50) chromosomes, and 2 with T-ALL (Figure 1). The two patients with T-cell leukemia formed a separate branch whereas samples with none of the above defined karyotypic abnormalities were distributed over the dendrogram. Interestingly, the t(1;19) was previously known to be present in only two patients. However, unsupervised clustering grouped three more patients (# 10, 12 and and 13) into the same branch. These were then analyzed in a retrospective manner for the *E2A-PBX1* translocation by specific, quantitative PCR and indeed found to be positive.[21]

To assess the quality of our data further and to verify the reproducibility of recently published data, we identified those genes that are most strongly associated with the TEL-AML1 translocation (*see Supplemental Table A at http://www.kispi.unizh.ch/onkologie*). Using both the HG-U133A and B GeneChips, Ross *et al.* published a gene list of classifiers of the top 100 $\chi^2$ probe sets selected for TEL-AML1. Using Welch's two sample t-test, we identified 48% of these probe sets with adjusted *p*-values below 5% within our data from HG-U133A only (*see Supplemental Table B from URL http://www.kispi.unizh.ch/onkologie*). Hence, despite variation in the series of patients, labeling and statistical processing, a high number of genes were found to be identical in the published data and in our current study.

### *Expression patterns associated with risk groups*

Our next goal was to identify genes whose expression levels best discriminate between patients in the standard risk group as compared to those in the high-risk group. We first used supervised testing to search for genes that discriminated the 13 standard-risk patients from the 10 high-risk ones. However, no genes were found to have a significantly differential expression at the false discovery rate (FDR)-adjusted 5%-level.

Thus, we focused on two cytogenetically more homogeneous subgroups for screening risk-group discriminatory genes, namely the 5 standard-risk *TEL-AML1* positive samples as well as the 6 high-risk samples with a DNA index of 1 and without further distinct cytogenetic alterations. Supervised testing for differential expression using the t-test and *p*-values adjusted by the FDR then revealed 125 probe sets with significant expression differences ($p < 0.05$) (*Figure 2, and see Supplemental Table C from URL http://www.kispi.unizh.ch/onkologie*). We excluded that this comparison is looking at the cytogenetic subtypes instead of the overall risk group association, since 106 of the 125

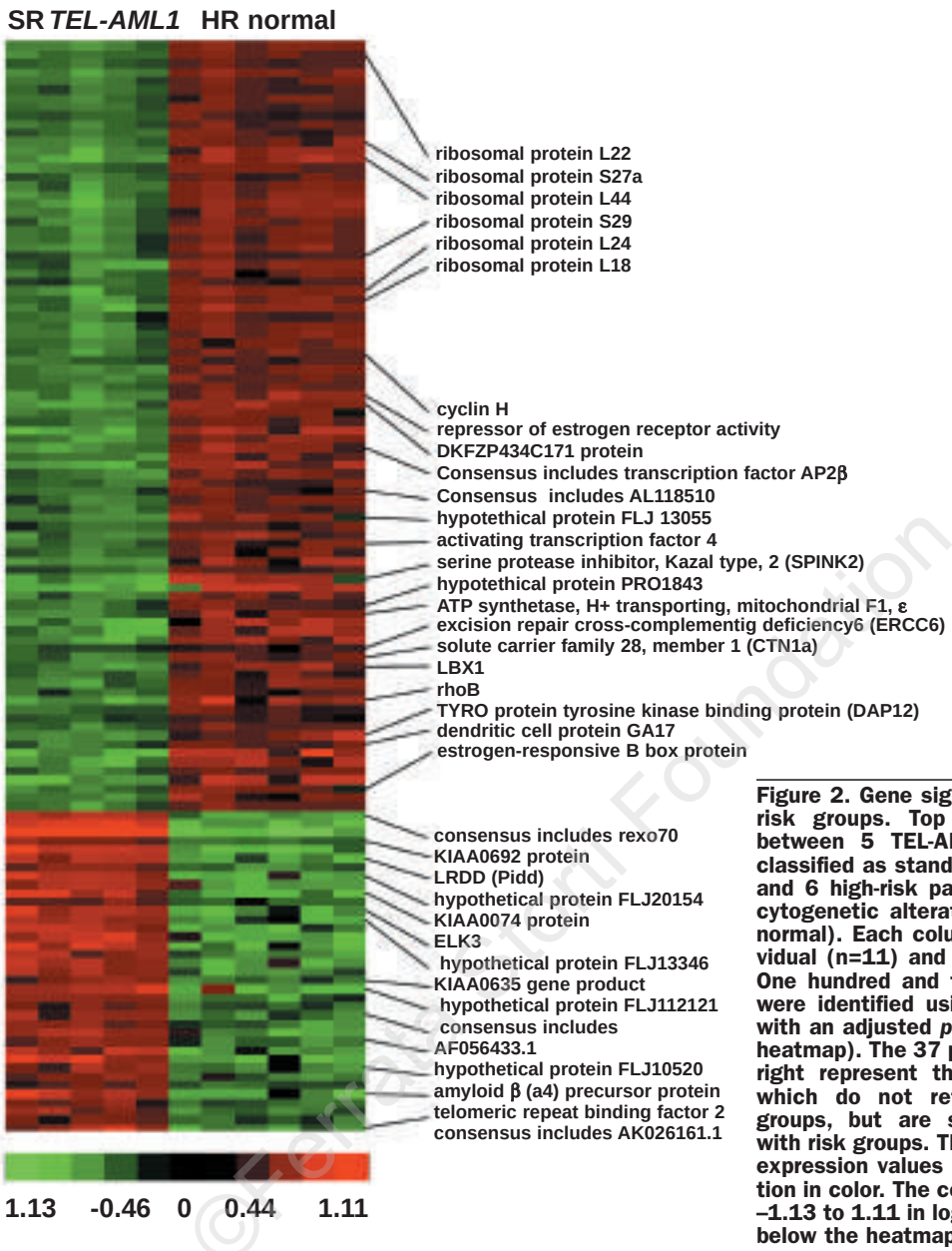


**Figure 2. Gene signature associated with risk groups. Top discriminating genes between 5 TEL-AML1 positive patients classified as standard-risk (SR *TEL-AML1*) and 6 high-risk patients without distinct cytogenetic alterations (indicated as HR normal). Each column represents an individual (n=11) and each row a probe set. One hundred and twenty five probe sets were identified using the t-test statistic with an adjusted *p* value below 0.05 (left heatmap). The 37 probe sets listed on the right represent the specific probe sets which do not reflect the cytogenetic groups, but are specifically associated with risk groups. The variation of absolute expression values is displayed as a variation in color. The color scale extends from –1.13 to 1.11 in log10 space as indicated below the heatmap.**



probe sets did not coincide with the genes discriminating all *TEL-AML1* positive patients (n=8) from all samples without distinct cytogenetic alterations (n=13) (*see Supplemental Table D from URL http://www.kispi.unizh.ch/onkologie; 19 probe sets also appearing in Supplemental Table C are marked by an asterix*). About 75% of the selected probe sets showed higher expression in high-risk samples. Remarkably, these included a large number of genes coding for several ribosomal proteins.

### *Risk group prediction by principal component analysis*

To test whether the expression of ribosomal proteins can predict risk class assignment, the gene expression data from all 31 children were analyzed. In a first step, the 11 samples of the two cytogenetically homogeneous subgroups were projected into the space of the first two principal components (PC) of the 6 ribosomal proteins (with specific probe sets; _at) identified as differentially expressed in the subanalysis (S27a, S29, L18, L22, L24, L44; Figure 2). This showed that the risk class assignment was clearly dependent on the ribosomal expression. The first two PC accurately discriminated the two subgroups (*see Supplemental Figure B from URL http://www.kispi.unizh.ch/onkologie*). The predominant effect in separating these groups came from the first PC, which summarized 93.40% of the total variation, whereas the second PC was of minor importance, adding only a further 2.85% of the total

**Table 2. Classification errors using different statistical methods. Numbers depict misclassified samples from 12 test samples.**

| *Probe sets* | *SVM*[a] | *NNR*[b] | *DLDA*[c] | *LR*[d] |
|---|---|---|---|---|
| 125 selected probe sets | 8 | 7 | 7 | 7 |
| ribosomal (1.PC)[e] | 6 | 6 | 7 | 7 |
| cyclin H | 3 | 3 | 3 | 3 |
| LRDD/Pidd | 4 | 4 | 4 | 4 |
| 1.PC & cyclin H | 4 | 6 | 3 | 4 |
| 1.PC & LRDD/Pidd | 4 | 6 | 4 | 4 |
| LRDD/Pidd & cyclin H | 2 | 2 | 3 | 3 |
| 1.PC & cyclin H & LRDD/Pidd | 3 | 3 | 3 | 2 |

*[a]support vector machine; [b]1-nearest-neighbor rule; [c]diagonal linear discriminant analysis; [d]logistic regression; [e]first principal component (PC) of the 6 specific ribosomal probe sets.*

variation. Next, we used this subanalysis group (n=11) as training data and predicted the remaining standard-risk (n=8) and high-risk (n=4) samples with 4 different classifiers, each based on the first two PC as the input. While the training data were perfectly separable, we observed between 5 and 7 misclassifications among the 12 samples of the cytogenetically less homogeneous test set. The two-dimensional scatterplot in Supplemental Figure B (*available from URL http://www.kispi. unizh.ch/onkologie*) shows the distribution of all 31 samples, also including those from the intermediate-risk patients.

To improve the classification, we tested several combinations of additional genes from the list of 125 risk group discriminatory probe sets (Table 2). In this way we excluded: genes that were associated specifically with the TEL-AML1 translocation (*see supplemental Table D from URL http://www.kispi.unizh.ch/onkologie*) and genes that were represented by possibly non-specific probe sets. From the remaining genes, cyclin H and LRDD/Pidd were the two statistically best discriminating genes between all standard- (n=13) and all high-risk (n=10) patients ($p$=0.205, resp. 0.297). Hence, cyclin H, an important enzyme in cell cycle control, and LRDD/Pidd, a gene involved in apoptosis, were tested for their predictive value as supplement to the first ribosomal PC.[22-24] Using four different classification methods (namely a support vector machine, the 1-nearest-neighbor rule, diagonal linear discriminant analysis and logistic regression), we found at best only 2 samples that were misclassified among the 12 samples tested, i.e. ten samples were assigned to the correct risk group currently used in the clinic, already at the time of diagnosis (Figure 3). This corresponds to a specificity of 83.3%. We also obtained only 2 misclassifications if cyclin H and LRDD/Pidd were tested alone. In contrast, a prediction using all 125 probe sets yielded at least 7 misclassifications and therefore was much worse than using the selected probe sets described above.

## Discussion

Genome-wide expression patterns are able to identify immunological subgroups (precursor-B ALL versus T-cell leukemia) and cytogenetic abnormalities such as *TEL-AML1*, *E2A-PBX1*, *BCR-ABL*, hyperdiploid karyotype with >50 chromosomes, and MLL gene rearrangements in childhood ALL with high accuracy.[6-11] In contrast, no studies have been carried out that describe gene expression signatures related to the initial risk group

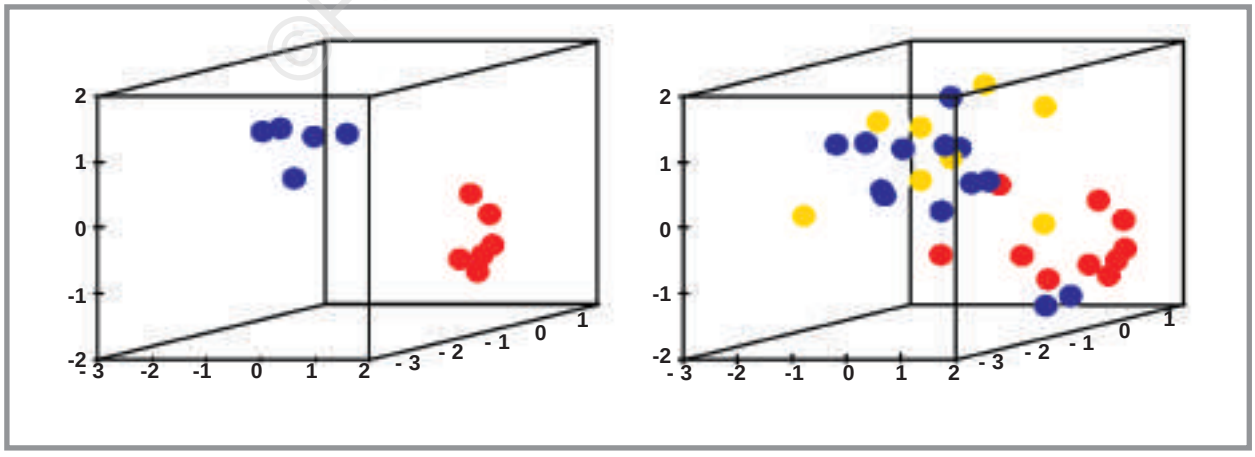


**Figure 3. Risk group prediction by selected signature genes. Three-dimensional scatterplots with three predictor variables; the first PC of 6 ribosomal proteins (S27a, S29, L18, L22, L24, L44; x-axis), cyclin H (y-axis) and LRDD/Pidd (z-axis). The left panel shows standard-risk *TEL-AML1* positive samples (n=5, blue) and high-risk patients without distinct cytogenetic alterations (n=6, red). The right panel shows all 31 samples; blue: standard-risk; yellow: intermediate-risk; red: high-risk.**

stratification. Based on strict quality parameters, we established expression profiles of 31 patients (*see Supplemental Table E from URL http://www.kispi.unizh.ch/onkologie*). Despite the rather small study group, all distinct subgroups of ALL occuring in children older than one year (except *MLL* rearrangements which are rare in this group of patients) are represented in our series of patients. Analyzing the microarray data of the entire study group, no gene could be identified that was capable of discriminating clearly between all standard- and all high-risk patients. This is most likely due to the cytogenetic heterogeneity in the two risk groups. In addition, a variety of other risk-related factors, such as sex, age, and leukocyte count, might have prevented a distinct signature from being identified. As a third aspect, we only analyzed expression data established with the HG-U133A GeneChip™; other more complex microarrays would, perhaps, reveal different results.

Nevertheless, when we screened for risk-related genes by focusing on more homogeneous subgroups, we identified a combination of single genes whose expression is associated with either a standard- or a high-risk classification with an accuracy of over 80%. Although these genes were initially identified in association with *TEL-AML1* samples, their expression pattern was correlated with risk group independently of the cytogenetic subtype. This finding is further supported by analysis of *TEL-AML1* specific signatures defined in two recent studies with a very large group of patients.[10,11] None of our final classifier genes can be found in the *TEL-AML1* characterizing gene lists.

Using different statistical methods, the genes identified, namely genes for 6 ribosomal proteins, cyclin H, and LRDD/Pidd, correctly predicted risk group assignment for 10 out of 12 cytogenetically heterogeneous samples. The 2 misclassified samples were from standard-risk patients but, according to the expression of our predictor genes, were apparently associated with a high risk. One of these samples (# 12) was inconspicuous with a translocation t(1;19). In contrast, it is interesting to note that the other sample (# 11), clustered in the unsupervised hierarchical analysis, most closely to the one sample expressing *BCR-ABL*, which by this fact had been assigned to the high-risk group. Hence, our analysis suggests that the risk group assignment and further course of disease evolution of patient #11 should be monitored particularly closely during following treatment periods. Unsurprisingly, we were not able to define a distinct expression profile or to subclassify the patients in the intermediate-risk group further, since the intermediate group comprises all samples not classified in either the standard- or the high-risk group, and is very heterogeneous. Nevertheless, most samples from this group were placed closer to those from standard-risk patients. Expression profiles, as a single platform, will probably not be able to refine their classification significantly.

Differential expression of ribosomal genes in cancer has been discussed in several studies.[25] Upregulation of transcripts for ribosomal proteins has been shown in several malignancies, including carcinoma of the colon, rectum, prostate, and esophagus.[26-28] Contrariwise, some authors have described that ribosomal proteins are downregulated in more aggressive subtypes of cancer compared to more favorable subtypes; this effect has been reported for carcinoma of the ovary, breast cancer and chronic lymphocytic leukemia.[29-31] In our study we observed upregulation of the expression of ribosomal proteins in childhood ALL patients with an unfavorable outcome. In addition, two single genes were found to be associated with risk group: cyclin H and LRDD/Pidd. Cyclin H was upregulated in the high-risk samples and, as part of the general transcription factor TFIIH complex, is clearly involved in cell cycle regulation.[23] The other gene, LRDD/Pidd, was expressed at higher levels in standard-risk patients; its significance is less well understood. Its murine homolog, Pidd, can be regulated by p53 and seems to be able to promote apoptosis.[24] However, functional studies are needed to define the role of these genes in childhood ALL.

Our results provide an initial assessment of risk-associated gene expression in childhood ALL. Considering all low- and high-risk patients, no gene was capable of predicting the risk assignment already at diagnosis. Nevertheless, we report a classifier of 3 predictor variables that could predict current risk assignment with an accuracy rate of more than 80% in an independent test set. Despite these findings, our results suggest that gene expression profiling cannot predict final risk stratification accurately and independently from various other risk-related factors. Further investigations aimed at identifying a signature with a stronger predictive value, by using a larger, homogeneous group of patients, seem to be warranted.

*OT did the microarray analyses, contributed to the analysis of the data and wrote the manuscript. MD analyzed and interpreted data and contributed to writing manuscript. BWS and FKN co-ordinated the study, the sample and data collection, and contributed to the writing. PB contributed to the data analysis and interpretation and to writing the manuscript. GC, MS and MSc helped with sample processing, experimental work and writing the manuscript. All investigators contributed to and reviewed the report.*

*We thank the Functional Genomics Center of the University and ETH, Zurich, for providing access to the Affymetrix work station and in particular A. Patrignani for excellent technical support. We are also very grateful to David Betts and Rahel Schaub for excellent diagnostic analyses within the ALL-BFM 2000 trial. The authors reported no potential conflicts of interest.*

*This work was supported by a grant from the Krebsliga of the Kanton Zurich and the "Schweizer Forschungsstiftung für Kind und Krebs".*

*Manuscript received December 15, 2003. Accepted April 23, 2004.*

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