# Gene-expression profiles and their association with drug resistance in adult acute myeloid leukemia

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Background and Objectives. From 20-50% of patients with acute myeloid leukemia (AML) are primarily resistant to induction chemotherapy. It has previously been shown that resistance to the first cycle of induction chemotherapy is an independent prognostic factor. We investigated whether resistance to chemotherapy be represented by gene-expression profiles, and which genes are associated with resistance.

Design and Methods. cDNA microarrays containing ~41,000 features were used to compare the gene-expression profile of AML blasts between 33 patients with good or poor response to induction chemotherapy. Data generated by cDNA-arrays were confirmed by quantitative reverse transcription polymerase chain reaction.

Results. Using significance analysis of microarrays, we identified a characteristic gene-expression profile which distinguished AML samples from patients with good or poor responses. In hierarchical clustering analysis poor responders clustered together with normal CD34$^+$ cells. Moreover, 13/40 (32.5%) genes highly expressed in poor responders are also overexpressed in hematopoietic stem/progenitor cells. Prediction analysis using 10-fold cross-validation revealed an 80% overall accuracy. Using the treatment–response signature to predict the outcome in an independent test set of 104 AML patients, samples were separated into two subgroups with significantly inferior response rate (43.5% vs. 66.7%, $p$=0.04), significantly shorter event-free and overall survival ($p$=0.01 and $p$=0.03, respectively) in the poor-response compared to in the good-response signature group. In multivariate analysis, the treatment-response signature was an independent prognostic factor (hazard ratio, 2.1, 95% confidence interval 1.2 to 3.6, $p$=0.006).

Interpretation and Conclusions. Resistance to chemotherapy in AML can be identified by gene-expression profiling before treatment and seems to be mediated by a transcriptional program active in hematopoietic stem/progenitor cells.

Key words: acute myeloid leukemia, drug resistance, gene expression profiling, prognostic markers, hematopoietic stem cells.

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In acute myeloid leukemia (AML) resistance to induction chemotherapy occurs in 20-50% of patients,[1-5] and most of the remaining will relapse and die of their disease. Therefore, accurate prediction of a patient's individual risk is required to determine the appropriate treatment. Age, white blood cell count, presence or absence of an antecedent hematologic disorder, and above all cytogenetic findings have been identified as prognostic factors.[6] In addition, we[1] and others[5,7] have identified response to the first cycle of induction chemotherapy, as assessed on day 15, as an independent prognostic factor for achievement of complete remission after the second cycle of induction treatment and for the patient's survival.

The introduction of microarray technology[8] has made it possible to simultaneously quantify expression of thousands of genes within well-defined cell populations. This method has been used to examine gene-expression profiles of malignant cells, and recent studies have identified signatures characteristic of various hematologic and non-hematologic tumors.[9-11] It has been shown for acute lymphoblastic leukemia that gene-expression profiles can be used to predict treatment response.[12-15] So far, gene-expression profiling in adult AML has focused on the identification of known classes[16] and new prognostic subgroups based on overall survival.[17,18] However, overall outcome is influenced by many factors which may not be directly reflected in gene-expression profiles of leukemic blasts at diagnosis, such as treatment delay due to infection or availability of an HLA-matched bone marrow donor. In contrast, drug resistance in AML, as assessed on day 15,

is mainly influenced by biological processes. Thus, we hypothesized that this clinically most relevant phenomenon could be well represented and reproducibly detectable by the gene-expression profile present at diagnosis in AML samples. The purpose of the present study was to identify genes predictive of *in vivo* drug resistance. We further evaluated whether gene-expression signatures characteristic for treatment response reliably define prognostic subgroups of AML. DNA microarrays were used to systematically explore the molecular variation underlying the different treatment response kinetics in AML.

## Design and Methods

### Patients' characteristics

Of a total of 137 patients 33 were enrolled in the AML-SHG 01/99 multicenter trial and 104 patients were enrolled in multicenter trials of the AML Study Group Ulm, as described previously.[17] Written informed consent was obtained prior to therapy, and the study was approved by the institutional review board of Hannover Medical School. After obtaining peripheral blood or bone marrow samples, patients enrolled in the AML-SHG 01/99 trial received intensive, cytarabine-based induction and consolidation treatment. Induction cycle 1 consisted of cytosine-arabinoside 100 mg/m$^2$ continuous i.v. infusion on days 1-7, idarubicin 12 mg/m$^2$ i.v. infusion on days 2, 4, and 6, and etoposide 100 mg/m$^2$ i.v. infusion on days 3-7. Response to induction cycle 1 was assessed cytomorphologically on day 15 by the initial blast cell reduction in the 33 patients enrolled in the AML-SHG 01/99 trial: good response was defined as no blasts in peripheral blood, <10% blasts in bone marrow, and no extramedullary manifestation, and poor response was defined as residual blasts in peripheral blood or ≥10% blasts in bone marrow or extramedullary manifestation of AML. Conventional cytogenetic banding analysis, fluorescence *in situ* hybridization,[1] polymerase chain reaction,[19] *FLT3* mutation analysis[20] and *MLL*-partial tandem duplication (PTD)[21] analysis were performed as previously described in the central reference laboratory of the AML-SHG Study Group.

Thirty-three diagnostic samples containing more than 70% blasts (median 84% blasts) prior to enrichment with Ficoll density gradient centrifugation were used for gene-expression analysis; 22 were samples from patients who had a good response to induction therapy and 11 were from patients with a poor response. In addition, 104 patients with newly diagnosed AML (FAB M0-2 and M4-5 only) described previously[17] and completely independent of the above mentioned 33 patients were used as a test set for validation. Induction chemotherapy was very similar to the AML-SHG 1/99 protocol, and consisted of two cycles of cytosine arabinoside 100 mg/m$^2$ 24h continuous i.v. infusion on days 1-7, idarubicin 12 mg/m$^2$ i.v. infusion on days 1, 3, and 5, and etoposide 100 mg/m$^2$ 2h i.v. infusion on days 1-3 or a reduced dose of these drugs for elderly patients. CD34$^+$ cells with purity above 97% from two healthy donors (Cytonet, Hannover, Germany) were used for comparative gene-expression analysis.

### RNA extraction and cDNA microarray methods

Total RNA from stored, frozen, Ficoll-separated mononuclear AML-cell pellets was isolated using Trizol reagent (Invitrogen, Paisley, UK) and subsequently purified by a Qiagen RNeasy column (Qiagen, Hilden, Germany). RNA was then linearly amplified using the MessageAmp aRNA Kit [Ambion (Europe), Huntingdon, Cambridgeshire, UK]. Total and amplified RNA was quantified and validated for integrity using the Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). The reference RNA used for all arrays was Universal Human Reference RNA (Stratagene Europe, Amsterdam, The Netherlands), linearly amplified using the same method. We hybridized Cy5-dUTP-labeled sample RNA and Cy3-dUTP-labeled reference RNA (fluorescent dyes from Amersham Pharmacia Biotech Europe, Freiburg, Germany) comparatively to cDNA microarrays that contain 41421 features representing 27285 unique putative genes (Stanford Functional Genomics Facility, Stanford, CA, USA). Detailed protocols are available at *http://brownlab.stanford.edu/protocols.html* and *http://www.microarray.org/doc/protocol/Corning_Slides_Postprocess_and_Prehyb.doc*. The fluorescence intensities of Cy5 and Cy3 were measured using a Genepix 4000B scanner (Axon Instruments, Foster City, CA, USA). Fluorescence ratios (sample value to reference value) were determined using GenePix Pro 4.1 software (Axon Instruments). Areas of the microarrays with obvious blemishes were manually deleted from subsequent analyses.

### Real-time quantitative reverse transcriptase polymerase chain reacton (RT-PCR)

In order to confirm the array results, quantitative RT-PCR was performed on selected genes using samples of all patients evaluable for the specific genes and enrolled in the AML-SHG trial 1/99. We used random hexamer priming and MuLV reverse transcriptase (Fermentas, Hanover, MD, USA) to generate cDNA. Real-time RT-PCR was carried out on a LightCycler (Roche Diagnostics, Mannheim, Germany) using the QuantiTect SYBR Green PCR kit as described in the manufacturer's instructions (Qiagen, Hilden, Germany). The following primers were used

to measure RNA abundance (5` to 3`): MN1 forward primer: GACGACGACAAGACGTTGG, *MN1* reverse primer: GACAGACAGGCACTGCAAG; *FHL1* forward primer: CCAACACCTGTGTGGAATG, *FHL1* reverse primer: GAGTCCTCCCGAGTGGTG; *FLJ14054* forward primer: ATGGTGTTCAGAGGGACCAG, *FLJ14054* reverse primer: ATGGGCAAAGCTACCCTCTT. Primers for *MN1* and *FHL1* are intron-spanning and do not function on genomic DNA. *FLJ14054* contains only one exon. Therefore, we used RNA treated with 1 unit DNase 1/µg RNA according to the manufacturer's instruction (Promega, Mannheim, Germany). The expression of *ABL* was used as an endogenous control.[22] The reactions were carried out in triplicate under the following conditions: 95°C for 15 min, then 45 cycles of 94°C for 15 sec, 53°C (*MN1, FHL1*) or 54°C (*FLJ14054*) for 25 sec and 72°C for 15 sec. Melting curve analyses were performed to verify the amplification specificity. First, quantitative PCR reactions were carried out on a dilution series of a reference cDNA to obtain standard curves for the target genes and *ABL*. Based on these curves, the relative concentrations were calculated using the second derivative maximum method and LightCycler Relative Quantification Software.

### Data normalization and statistical analysis

Fisher's exact test, Kaplan-Meier survival analysis, and the log-rank test were performed with SPSS 11.5 software or the statistical software bundle R (vers. 2.0.0). $p$ values that were <0.05 in two-tailed tests were considered statistically significant.

Fluorescence intensity values of each array were normalized using a scaling factor to set the mean sample/reference ratio to one. Only spots with a regression correlation >0.6 were used to calculate the scaling factor. For all subsequent analyses, $\log_2$ of the normalized sample/reference ratios were used. We defined well-measured clones as clones with a ratio of signal intensity to background noise of more than three for either the Cy5-labeled AML sample or the Cy3-labeled reference sample, in at least 70 percent of the AML samples hybridized. We defined clones that were highly variably expressed as clones whose expression was higher or lower by a factor of at least four than the average expression of all AML samples in at least one sample. In our 33 test samples, 2869 clones were both well measured and highly variably expressed. Each clone meeting the filtering criteria was mean-centered prior to subsequent analysis. Data normalization and clone filtering were performed with the Stanford Microarray Database software tools. The primary data and the image files are publicly available through the Stanford Microarray Database[23] (*http://genome5-www.stanford.edu*). For unsupervised data analysis we used either all well-measured and highly variably expressed clones or only the top 102 discriminating genes for average-linkage hierarchical clustering[24] on Pearson's correlation dissimilarities. Supervised data analyses were performed with significance analysis of microarrays (SAM)[25] for the whole set of 2869 well-measured clones. Supervised prediction analysis was performed with the method of nearest shrunken centroids (prediction analysis of microarrays, PAM)[26] for the top differentially expressed genes using the pamr library of R software. To get a rough estimate of the errors of prediction, we used 10-fold cross-validation, in which 90% of the samples were used to construct the predictor and 10% of the samples were used to evaluate the test error. The errors of the 10-fold cross-validation were used to compute the overall error rate. Cross-validation was repeated 20 times with similar results to rule out extreme error rates due to random selection of the cross-validation groups. We used the cross-validated predictor to assign response labels to an independent test set of 104 AML patients treated with protocols of the AML Study Group Ulm. Samples which were assigned a poor-response signature were compared with samples assigned a good-response signature for differences in overall outcome by Kaplan-Meier analysis, log-rank test, and multivariate proportional-hazard analysis.

## Results

### Patients' characteristics

Overall, 137 patients were included in our study: 22 patients with good response and 11 patients with poor response to the first induction course were enrolled in the AML-SHG 01/99 multicenter trial, and 104 patients were enrolled in multicenter trials of the AML Study Group Ulm.[17] Patients were newly diagnosed with *de novo* or secondary AML and had FAB subtypes M0-2 or M4-5. The characteristics of the patients enrolled in the AML-SHG 1/99 trial were well balanced between the two groups for sex, age, FAB subtype, initial white cell count, *FLT-3* mutations ($p$=0.2, Fisher's exact test) and *MLL* mutations ($p$=1, Fisher's exact test) (Table 1). Adverse risk cytogenetics were only found in the poor response group. The characteristics of the patients enrolled in the AML Study Group Ulm have been described previously.[17]

### Unsupervised hierarchical clustering

First, gene expression data of 33 patients enrolled in the AML-SHG 01/99 trial were analyzed by hierarchical clustering. Only well measured and highly

**Table 1.** Baseline characteristics of the patients.

| Characteristic | Response to first induction treatment good (n= 22) | poor (n=11) | p value |
|---|---|---|---|
| Age | | | |
| mean - yr. | 43.8 | 46.6 | 0.6 |
| range - yr. | 18-58 | 22-60 | |
| Sex | | | |
| male - no. (%) | 15 (68) | 8 (72.7) | |
| female - no. (%) | 7 (32) | 3 (27.3) | 0.8 |
| Karyotype | | | |
| favorable - no. (%) | 1 (4.5) | 2 (18.2) | |
| intermediate - no. (%) | 21 (95.5) | 6 (54.5) | |
| adverse - no. (%) | – | 3 (27.3) | 0.01 |
| FAB-Subtype | | | |
| M0-2 - no. (%) | 12 (54) | 5 (45) | |
| M4-5 - no. (%) | 10 (46) | 6 (55) | 0.6 |
| Type of Specimen | | | |
| bone Marrow - no. (%) | 14 (63.6) | 7 (63.6) | |
| peripheral Blood - no. (%) | 8 (36.4) | 4 (36.4) | 1 |
| % Blasts | | | |
| median | 82.5 | 84 | |
| mean | 81.6 | 83 | 0.7 |
| range | 70-100 | 70-91 | |
| Development of AML | | | |
| *de novo* - no. (%) | 19 (86.5) | 10 (90.9) | |
| post MDS - no. (%) | 1 (4.5) | 1 (9.1) | |
| secondary - no. (%) | 2 (9) | – | 0.5 |
| White cell count ($\times 10^9$/L) | | | |
| median | 18.8 | 9.6 | |
| mean | 34.6 | 32.3 | 0.9 |
| range | 0.9 - 181 | 0.7 - 142 | |
| ECOG Performance status | | | |
| 0 - no. (%) | 5 (22.7) | 1 (9.1) | |
| 1 - no. (%) | 15 (68.3) | 8 (72.7) | |
| 2 - no. (%) | 2 (9) | 2 (18.2) | 0.5 |
| *FLT3* mutation – no. (%) | 7 (32) | 1 (9.1) | 0.2 |
| *MLL* PTD – no. (%) | 4 (12) | 2 (18) | 1 |

variably expressed clones were considered. Although potentially resulting in the loss of some information, strict selection of clones will decrease the possibility that clustering is mainly influenced by clones with only little difference in expression. The samples were separated into two main branches (*Supplementary Figure 1, online version only*). No statistically significant differences were observed for response to induction treatment, age at diagnosis, initial white cell count, karyotype or overall survival, although there was a trend to more myelomonocytic leukemias in cluster 2 (*Supplementary Table 1, online version only*).

### *Identification of genes associated with poor induction response using supervised analysis methods*

Significance analysis of microarrays was used to identify genes associated with good and poor induction response by comparing 22 patients with good responses to induction against 11 patients with poor response. We selected the top 102 discriminating clones ($\delta$=0.16, 1000 permutations, fold change 2) for further analysis. Of these, 40 were overexpressed in patients with a poor response and 62 were overexpressed in patients with a good response to induction treatment. With a stricter significance threshold expected to produce fewer than 16.8% false positives, SAM identified only 11 clones (indicated by * in Figure 1) ($\delta$=0.28) that distinguished poor from good treatment response. We then applied hierarchical cluster analysis to the top 102 discriminating clones and included the expression profiles of $CD34^+$ cells from two healthy donors. Ten of the 11 poor responders clustered together, whereas 21 of 22 good responders clustered into two separate additional clusters, indicating genetic influences other than those which determine response to treatment. The one poor response sample that clustered together with good response samples was from a patient diagnosed as having FAB M4 by morphology and expression of lysozyme and myeloperoxidase. At day 15 this patient had >10% persisting blasts with megakaryoblast morphology as in FAB M7. In this case, induction chemotherapy may have selected a chemoresistant clone which might have been dominated initially by a chemosensitive cell population. Strikingly, normal $CD34^+$ cells, which contain hematopoietic progenitor cells, clustered within the group of poor response samples (Figure 1).

To determine whether genes which are overexpressed in the poor-response group play a role in normal hematopoiesis, and if so, at which stage of differentiation, we compared our list of 102 differentially expressed clones with published expression profiles of hematopoietic stem and progenitor cells (Table 2). Wagner *et al.*[27] specifically compared the slow-dividing with the fast-dividing fraction of human $CD34^+CD38^-$ progenitor cells. Of the 40 genes associated with poor induction response, six were overexpressed in the slow-dividing fraction, which is supposed to contain self-renewing cells: *MN1*, *FHL1*, *CD34, RBPMS, P2RY5*, and the gene coding for the hypothetical protein *FLJ14054*. Of these, the expression of *FHL1* and *FLJ14054* is more than four-fold higher in the slow-dividing fraction. The expression of only 36 of approximately 17,000 overexpressed genes is more than four-fold higher in the slow-dividing fraction than in the fast-dividing fraction. Thus, the finding of *FHL1* and *FLJ14054* among our 40 genes represents a highly significant enrichment

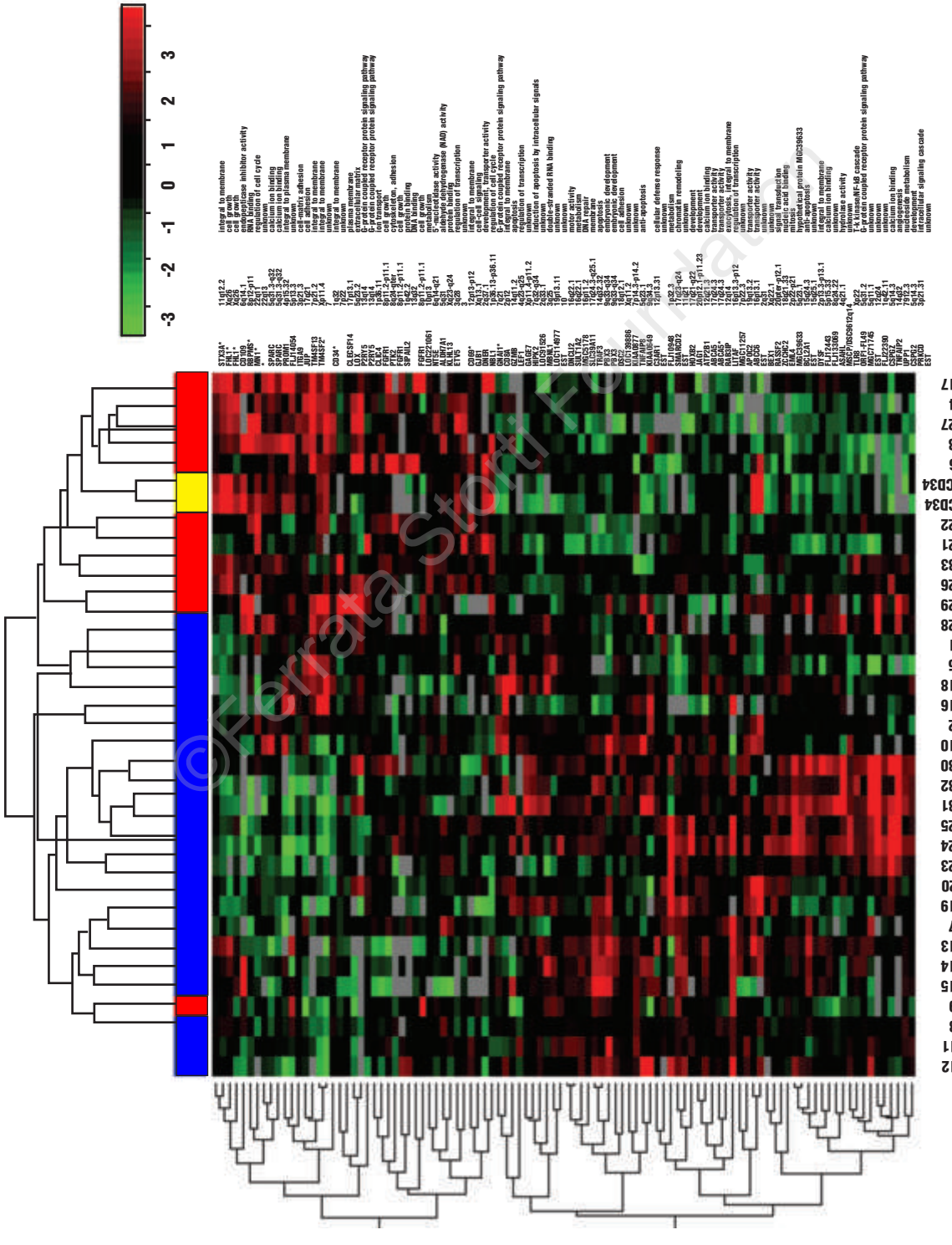




Figure 1. Hierarchical cluster analysis of good and poor response patients and normal CD34+ cells. Two-way (genes against samples) hierarchical cluster analysis of good response patients (blue bar), poor response patients (red bar) and CD34+-cell samples from two healthy donors (yellow bar) and the top 102 clones discriminating good and poor response samples identified by SAM. Mean-centered ratios of gene expression are depicted by a log-transformed pseudocolor scale. Gray areas indicate poorly measured clones. Gene ontology annotation and chromosomal location are shown for each clone. *indicates significantly differentially expressed clones at a false discovery rate (FDR) of 16.8%.

**Table 2.** Comparison of drug-resistance-enriched genes with hematopoietic stem cell-enriched genes.

| | *Wagner et al.*[27] | *Georgantas et al.*[45] | *Ivanova et al.*[46] | *Ramalho-Santos et al.*[47] |
|---|---|---|---|---|
| Stem cell population | HCB, SDF of $CD34^+CD38^-$ cells | HBM, $CD34^+CD38^-Lin^-$ vs. $CD34^+CD38^+Lin^+$ | **1.** MFL $AA4.1^+$ $c\text{-}kit^+Sca\text{-}1^+$ **2.** upregulated genes in both MFL and human homologs (HFL, $CD34^+CD38^-Lin^-$) | MBM:Hoechst $33342^{low}$, $Sca1^+c\text{-}Kit^+$ $CD34^-Lin^-$ |
| Overlap of HSC-enriched genes with our drug-resistance -enriched genes[39] | CD34, FHL1, *MN1, RBPMS, P2RY5, FLJ14054* | FHL1, RBPMS, *FLJ14054, GNAI1, NBL1* | **1.** *CD34, FHL1, MN1, RBPMS, P2RY5, STX3A, FGFR1* **2.** *CD34, FHL1, MN1, RBPMS, STX3A, FGFR1* | CD34, FHL1, *FGFR1, LOX, JUP* |

*HSC: hematopoietic stem cell; HCB: human cord blood; HBM: human bone marrow; SDF: slow dividing fraction within $CD34^+CD38^-$ cell population; MFL: murine fetal liver; HFL: human fetal liver; MBM: murine bone marrow.*

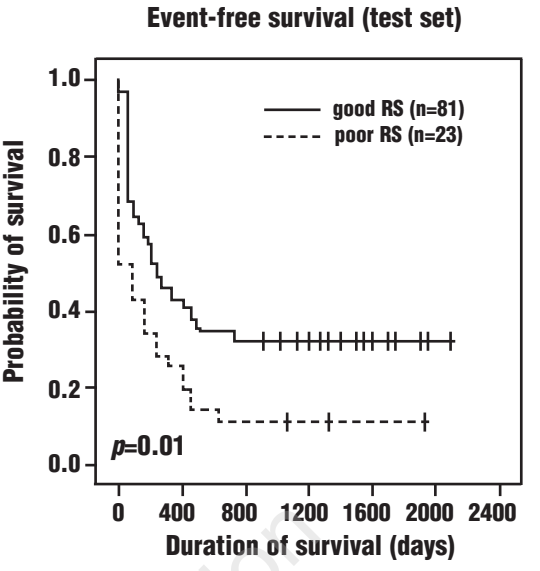


**Figure 2.** Event-free survival in predicted groups. Event-free survival probability by Kaplan-Meier plot for the independent test set (104 patients) assigned to either a good-response signature (RS) or a poor-response signature; there was a significant difference between the groups.

compared to what would be expected in 40 randomly selected genes ($p<0.00001$, $\chi^2$ test). Moreover, similar and additional genes were found to be enriched in hematopoietic stem cells in four other studies, namely *FGFR1, CD109,*[28] *STX3A, GNAI1, NBL1, LOX, JUP* in addition to the above mentioned genes *MN1, FHL1, CD34, RBPMS, P2RY5 and FLJ14054* (Table 2). All together, 13 (32.5%) of the 40 genes associated with a poor response to induction therapy have been found to be overexpressed in hematopoietic stem/progenitor cells with self-renewing capacity. To confirm the microarray data, three of these genes (*MN1, FHL1, FLJ14054*) were analyzed by quantitative RT-PCR in 30 evaluable samples. Data generated by quantitative RT-PCR and cDNA arrays correlated well, with correlation coefficients (RT-PCR data vs. array data) between $R^2=0.78$ (*FLJ14054*) and $R^2=0.89$ (*FHL1*) (*Supplementary Figure 2, online version only*).

### Prediction of treatment response

We applied PAM to test whether the identified gene expression signature can be used to predict response to induction treatment. PAM is a nearest-centroid classification method with an automated gene selection step integrated into the algorithm. Our analysis was based on 102 differentially expressed genes comparing 22 good response patients with 11 poor response patients enrolled in the AML-SHG 1/99 trial identified by SAM as described above. Ten-fold cross validation was performed on these 33 patients, always using 90% of the patients to build the classifier and 10% of the patients to evaluate the prediction error. On an average of 20 independent analyses, the mean overall error rate was 0.2 using 75 clones for prediction analysis at a threshold of 1.16 (accuracy 80%). We then used this predictor to investigate whether the treatment-response signature had prognostic significance in an unselected population of patients. The 33 patients enrolled in the AML-SHG 1/99 trial were used as a training set and the 104 patients enrolled in trials of the AML Study Group Ulm were used as a test set. When applied to the test set the predictor assigned a good-response signature to 81 test samples and a poor-response signature to 23 test samples (threshold 1.16).

No differences were found in the patients' characteristics between these predicted groups except for a higher median age in the group with a poor-response signature (Table 3). Interestingly, the response rate (complete or partial remission) was significantly lower in the poor-response signature group than in the good-response signature group (43.5% vs. 66.7%, respectively, $p=0.044$, $\chi^2$ test). In addition, both event-free and overall survival of patients with the poor-response signature were significantly shorter than those of the subgroup of patients whose samples had a good-response signature ($p=0.01$ and $p=0.03$ by the log-rank test, respectively) (Figure 2 and 3, respectively, for Kaplan-Meier plots). In multivariate analysis including karyotype (low vs. intermediate vs. high risk), *FLT3* mutation, *MLL*-PTD, and predicted response groups (good vs. poor), only karyotype (hazard ratio (HR): 2.2, 95% confidence inter-

val 1.5 to 3.3, $p<0.001$), *FLT3* mutation status (HR: 1.8, 95% confidence interval 1.06 to 2.9, $p=0.03$), and predicted response group (HR: 2.1, 95% confidence interval 1.2 to 3.6, $p=0.006$) were independent prognostic factors.

## Discussion

We have identified a gene-expression signature which reflects molecular differences between AML cases with good and poor response to induction chemotherapy. The response kinetics of AML blasts during the first two weeks of treatment has prognostic significance, as reported previously.[1,5] We hypothesized that the response kinetics to initial chemotherapy is most likely an intrinsic feature of the gross blast population present at diagnosis. To determine the characteristics of drug resistance, we therefore investigated unselected, enriched AML blast populations at diagnosis. In unsupervised hierarchical clustering analysis patient or disease characteristics did not differ significantly between the two main clusters. However, using discriminating genes between good and poor responders with 10-fold cross-validation, response to induction chemotherapy could be predicted with an accuracy of 80%. Moreover, our predictor divided a large independent data set into two groups with significantly inferior response rate and overall survival in the group assigned a poor-response signature. Our data indicate that resistance to chemotherapy is at least in part an intrinsic feature of AML blasts and can be evaluated prior to treatment. However, further studies are needed to determine whether this information can be applied to clinical practice.

We also found that the signature predictive of drug-resistance is correlated to the gene-expression signature of hematopoietic stem/progenitor cells, especially the self-renewing fraction. Of note, FAB subtypes with more immature morphology (FAB M0, M1) were equally distributed between good and poor response samples. Some reports have previously described CD34 as a marker of prognostic value in AML[29,30] but others have not.[31,32] In a meta-analysis Kanda *et al.*[33] concluded that CD34 has no independent prognostic value in AML. Our findings suggest that CD34 is expressed in AML with poor response and poor outcome but is not sufficient, as a single marker, to predict poor response and poor outcome. Rather, poor response seems to be more consistently defined by expression of additional genes which might play a role in self-renewal, as they are also overexpressed in self-renewing normal hematopoietic stem cells. For example, *FGFR1* is a marker of neural stem cells,[34] and its constitutive activation causes a myeloproliferative syndrome;[35] MN1 is a known fusion partner in AML with t(12;22)(p13;p11). Its sequence is a component of the fusion protein necessary to transform NIH3T3 cells[36] and it has been associated with drug resistance;[37] CD109

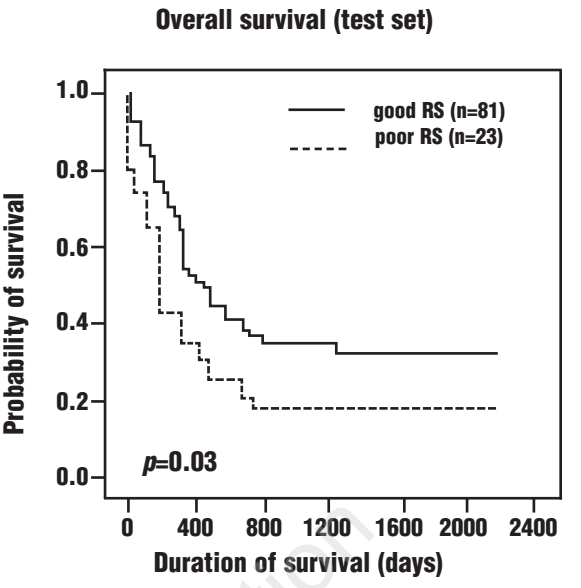


**Figure 3.** Overall survival in predicted groups. Overall survival probability by Kaplan-Meier plot for the independent test set (104 patients) assigned either a good-response signature (RS) or a poor-response signature; there was a significant difference between the groups.

**Table 3.** Characteristics of the test set.

| *Characteristic* | *Assigned response group* *good* *(n=81)* | *poor* *(n=23)* | *p-value* |
|---|---|---|---|
| Age | | | |
| median – yr. | 48.3 | 58.3 | 0.03 |
| range – yr. | 22–75 | 35–73 | |
| Sex | | | |
| male – no. (%) | 45 (55.6) | 14 (60.9) | |
| female – no. (%) | 36 (44.4) | 9 (39.1) | 0.65 |
| Karyotype | | | |
| fav. or intermed. – no.(%) | 78 (96.3) | 21 (91.3) | |
| adverse – no. (%) | 3 (3.7) | 2 (8.7) | 0.32 |
| FAB subtype | | | |
| M0-2 – no. (%) | 30 (37) | 11 (47.8) | |
| M4-5 – no. (%) | 43 (53.1) | 7 (30.4) | |
| n.d. – no. (%) | 8 (9.9) | 5 (21.7) | 0.11 |
| Development of AML | | | |
| *de novo* – no. (%) | 62 (76.5) | 18 (78.3) | |
| secondary – no. (%) | 8 (9.9) | 2 (8.7) | |
| n.d. – no. (%) | 11 (13.6) | 3 (13) | 0.98 |
| *FLT3* mutation – no. (%) | 25 (30.9) | 6 (26.1) | 0.47 |
| *MLL* PTD – no. (%) | 9 (11.1) | 1 (4.3) | 0.33 |

has been used to enrich CD34+ cells with self-renewing capacity.[28] Poor response and poor outcome seem to be characteristics of a molecularly and functionally defined subgroup of AML in which the majority of blasts has retained or regained properties of self-renewal. Typical features of stem cells, such as slow cell cycling,[38] cell adhesion,[27] and expression of drug efflux pumps[39] may explain the poor outcome in this subgroup. Interestingly, in B- and T-cell acute lymphoblastic leukemia, low expression of cell cycle- and proliferation-associated genes in leukemic cells has been associated with treatment resistance.[12,14] Markers previously described to be associated with poor prognosis in AML are in line with our model, e.g. *MDR*,1[27] *EVI*,1[40] integrin α4[41] and *BAALC*,[42] which are also overexpressed in self-renewing hematopoietic cells. Recently, Hope *et al.*[43] described heterogeneity of leukemic stem cells with respect to the self-renewal capacity in AML. Although they provided evidence that only one out of every $2.5\times10^5$ leukemic blast cells is a SCID leukemia-initiating cell,[44] we found a subgroup of AML in which the gross gene-expression signature of unselected leukemic cells showed a gene-expression profile closely related to that of self-renewing hematopoietic cells. Therefore, we propose that AML samples not only consist of a heterogeneous stem-cell compartment but differ in their ability to retain a gene-expression signature similar to self-renewing stem cells in more committed cells or in their ability to expand the stem-cell pool.

In conclusion, we identified a combination of markers, including the hematopoietic progenitor cell marker CD34, the cell growth regulator *FHL1*, the cell cycle regulator *MN1*, the RNA-binding molecule *RBPMS*, the G-protein-coupled receptor *P2RY5*, and the hypothetical protein *FLJ14054* besides others, as marker genes for a stem cell-like poor-risk group in AML. Functional evaluation of these genes may identify new therapeutic targets to improve treatment for AML patients.

*All authors contributed to the conception and writing of the manuscript. MH: author taking primary responsibility for the paper. Tables 1, 3: MH: Table 2: MH, LUW; Figure 1: LUW; Figures 2, 3: MH.*

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