## Acute Leukemias

# The prognostic value of Bcl-XL gene expression for remission induction is influenced by cytogenetics in adult acute myeloid leukemia

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*Background and Objectives.* There is growing evidence that altered expression of genes belonging to the BcL-2 family of apoptosis regulators might influence chemotherapy-induced apoptosis in malignant cells and therefore could confer multidrug resistance. So far expression studies of apoptosis-regulating genes on acute myeloid leukemia (AML) have mainly focused on Bcl-2 itself and most of them have not included other factors involved in drug resistance or apoptosis as parameters determining response to chemotherapy, disease progression and survival.

*Design and Methods.* We therefore examined Bcl-2, Bcl-XL and Bax gene expression in 235 adult patients with *de novo* or secondary myeloid leukemia. The expression levels were correlated with established prognostic factors such as age, cytogentic aberrations, mdr1 gene expression and clinical outcome in a multivariate analysis.

*Results.* Bcl-2 and Bcl-XL positive patients had a much lower white blood cell count than negative patients (*p*<0.001 and *p*=0.003, respectively). Bcl-2 expression correlated with FAB subtype M0 (*p*=0.03), Bax with M5b (*p*=0.02) and Bcl-XL with M6 (*p*=0.005). Mdr1 expression was more frequently seen in Bcl-2 and Bcl-XL positive patients (*p*=0.03 and *p*=0.02, respectively). Remarkably Bax was significantly less frequently expressed in *de novo* AML patients with high risk cytogenetics (*p*=0.007). No difference in expression was recognized for Bcl-2 or Bcl-XL when statistical analyses were done for cytogenetic risk groups. However, in the multivariate analysis regarding the group of *de novo* AML patients ≤ 60 years with intermediate risk cytogenetics, Bcl-XL expression was found to be an independent negative prognostic factor for response to induction therapy (*p*=0.04). In contrast, no prognostic impact of Bcl-XL expression on treatment response was seen within the group of patients with high risk cytogenetic findings. Neither Bcl-2 nor Bax nor Bcl-XL expression had a significant influence on overall or disease-free survival.

*Interpretation and Conclusions.* These data indicate that the prognostic value of Bcl-XL gene expression for treatment response in AML patients ≤60 years is dependent on cytogenetics.

Cytotoxic drugs used in the treatment of malignant diseases can damage tumor cells by a variety of mechanisms, such as DNA cleavage, DNA alkylation, inhibition of microtubule aggregation or of nucleotide precursors. Damage of cellular components must be transferred into signals for the apoptotic pathways. However, alterations in the apoptotic threshold of tumor cells may cause cytotoxic drug resistance.[1] The Bcl-2 family of genes are involved in the regulation of the apoptosis process. Enhanced expression of the apoptosis inhibitor Bcl-2 or its homolog Bcl-XL leads to tumor cells having a decreased susceptibility to apoptosis. These proteins prevent proteolytic activation of caspases in response to chemotherapy-induced damage.[2]

On the other hand, other Bcl-2 family members, such as Bax or Bcl-XS, are able to induce apoptosis, so that the ratio of expression of anti-apoptotic and pro-apoptotic members of the Bcl-2 family might determine the apoptotic potential of cancer cells.[1]

Whereas Bcl-2 was initially described as a suppressor of apoptosis in human B-cell follicular lymphomas harboring the t(14;18) translocation,[3,4] high expression levels of the Bcl-2 gene and other Bcl-2 family members were seen in many other hematologic malignancies, including acute myeloid leukemia (AML).[1]

A number of studies have been performed in which the expression of Bcl-2 has been examined in correlation to treatment outcome in AML,[5-13] but none of

them included determination of the individual expression levels of further members of the Bcl-2 family. Whereas Kaufmann et al.[14] examined expression levels of Bcl-2, Bcl-XL, Mcl-1 and Bax in 54 AML patients at diagnosis and at relapse, so far only Stoetzer et al.[15] have correlated Bcl-2, Bcl-XL, Bax expression and apoptosis with prognosis in 14 AML patients. However, many other established prognostic factors, such as age at diagnosis, mdr1 resistance gene expression[16,17] and cytogenetics,[18] were shown for AML.

Recently, Kornblau et al.[19] found that the prognostic impact of Bcl-2 varies with cytogenetic findings in AML patients. High levels of Bcl-2 correlated with an adverse outcome when associated with favorable and intermediate cytogenetics, but low levels predicted an adverse outcome within the group with unfavorable cytogenetics.

We, therefore, looked for the expression of anti-apoptotic genes, including Bcl-2 and Bcl-XL, and the pro-apoptotic gene Bax in a series of 235 adult AML patients at diagnosis and compared expression of Bcl-2, Bcl-XL and Bax, age, cytogenetic findings and mdr1 gene expression with treatment outcome and survival.

## Design and Methods

### Patients

Two hundred and thirty-five patients with prior untreated *de novo* or secondary AML treated according to the previously published protocol of the German SHG AML 96 treatment trial were included in this study.[20] All patients ≤ 60 years old received uniform double induction therapy containing cytosine arabinoside, etoposide, mitoxantrone and amsacrine. Patients > 60 years old were treated with two courses of daunorubicin and cytosine arabinoside.

Cytogenetic risk stratification was done in patients ≤ 60 years old in accordance with previously published data.[18,21] Cytogenetic risk groups were defined as follows: *high risk*: -5/del(5q), -7/del(7q), hypodiploid karyotypes (not included 45,X,-Y or –X), inv(3q), abnl12p, abnl11q,+11, +13, +21, +22, t(6;9); t(9;22); t(9;11); t(3;3), multiple aberrations; *intermediate risk*: patients without low risk or high risk constellation; *low risk*: t(8;21) and t(8;21) combined with other aberrations.

Post-remission therapy was priority-based in the different cytogenetic risk groups. Whereas AML patients ≤ 60 years old with favorable cytogenetics received chemotherapy only, patients with intermediate risk cytogenetics underwent either related allogeneic peripheral blood stem cell transplantation (PBSCT) as first, autologous PBSCT as second or standard chemotherapy as third priority post-remission treatment. In addition, for patients with unfavorable cytogenetics or secondary AML there was the option of unrelated allogeneic PBSCT, if no related donor could be found.

Complete remission (CR) was defined as the presence of <5% blast cells in a standardized bone marrow puncture after double induction therapy. According to CALGB criteria only patients with a fully regenerated peripheral blood count were considered to be in CR.

In order to determine a threshold of gene expression of apoptosis-regulating genes we compared the results of the AML patients with those of a control group of 20 healthy peripheral blood stem cell donors.

This study was approved by the Ethics Committee of the University of Dresden, Germany. Each patient gave written informed consent.

### Morphologic, flow cytometric and cytogenetic evaluation

Routine analyses were done on fresh material from the patients.

For flow-cytometric detection of CD34 positive cells the monoclonal antibody QBEnd1D (Coulter-Immunotech Diagnostics, Hamburg, Germany) was used according to previously published protocols.[22]

Cytogenetic analyses were performed on metaphases from direct preparations, as well as from 24h and 48h cultures of patients' samples. Routine preparation and G-banding staining techniques were used.

### Preparation of samples for Bcl-2 and mdr1 mRNA expression

Bone marrow or peripheral blood samples were taken at the time of diagnosis. The blast cells within the mononuclear cell fraction were separated by density gradient centrifugation and cryopreserved in liquid nitrogen prior to analysis. Cells were thawed according to routine protocols and patients' probes containing less than 80% of myeloblasts, as well as the probes of the control group, were subjected to CD3 depletion using CD3 coated beads following the recommendations of the manufacturer (Dynal, Hamburg, Germany). CD3 positive cells were eliminated with a sensitivity of 98%. mRNA extraction and cDNA synthesis were done as described previously.[23]

### Reverse transcription-polymerase chain reaction

The polymerase chain reaction (PCR) was performed in a final volume of 50µL containing 1× reaction buffer (Perkin Elmer-Applied Biosystems, Weiterstadt, Germany), 2.0 mM $MgCl_2$, 15 pmol of each primer, 200 µM of each dNTP (Pharmacia, Freiburg, Germany)

and 1.5U AmpliTaqGold-Polymerase (PE-Applied Biosystems, Weiterstadt, Germany). GAPDH was used as an internal control. GAPDH primers were obtained from Clontech, Heidelberg, Germany. Amplification conditions were 31 cycles with 94°C for 45s, 62°C for 45s and 72°C for 90s.

Mdr1 primer and reaction conditions were as described elsewhere.[17]

The primers for detecting apoptosis-regulating gene expression were designed using "DNASIS" (Hitachi, Yokohama, Japan) and "Primer Detective" software (Clontech, Mannheim, Germany). Amplification products were controlled by specific restriction enzymes.

For detection of Bcl-2 family mRNA expression, the following primers were used:

Bcl-2: sense 5`AAA ACC TGG ATG TCA CTG GC; anti sense 5`GTT TCC TGC TTT CTT GGT GG (412 bp);

Bcl-XL: sense 5`CAT GGC AGC AGT AAA GCA AGC G, antisense 5`AGG CTC TAG GTG GTC ATT CAG G (295 bp);

Bax: sense 5`TTT TCT GAC GGC AAC TTC AAC TGG; antisense 5`CCA CAA AGA TGG TCA CGG (235 bp).

The PCR conditions fo determining the expression of these three genes were 96°C for 15s, 55°C for 30s and 72°C for 75s. We used 29 cycles for Bcl-2 and Bcl-XL, and 31 cycles for Bax.

All primer pairs were tested with cycle kinetic analysis in order to ensure amplification in the exponential range of PCR.

All PCR results represent the mean value of at least two independent experiments. The cell line U937 served as a positive control for Bcl-2 and Bcl-XL gene expression, and the cell line MCF7 as a positive control for Bax gene expression. GAPDH and other PCR products of interest were mixed, resolved after ethanol precipitation and loaded on an 8% polyacrylamide gel. The major advantage of this step is a volume reduction which allows gel electrophoresis of more than two different fragments to be performed within one lane. Ratios of signal intensities between different products remain unaltered and reproducibility could be markedly improved by this approach. After staining with ethidium bromide densitometric evaluation was done using the BioDoc II video documentation system and ScanPack™ 3.0 software (Biometra, Göttingen, Germany).

For semi-quantification, the area under the curve was evaluated for each amplified gene product and amounts of expression were referred to the observed GAPDH signal.

As normal hematopoetic cells, other than T-cells which were depleted, display distinct levels of Bcl-2 family and mdr1 gene expression, the expression levels of AML patients were compared with the expression levels in healthy peripheral stem cell donors. Gene expression levels exceeding the mean levels of this control group were regarded as enhanced expression levels. The probes for the control group were handled in exactly the same way as those for the patients.

Bcl-2/Bax and Bcl-XL/Bax expression signals were evaluated to test the prognostic influence of apoptosis ratios.

### Statistical analysis

Basic statistical data such as mean values, standard deviations and frequencies were obtained using the SPSS software package. Differences in gene expression levels between the analyzed subgroups and univariate analyses of the correlation between experimental findings and response to induction therapy were evaluated by Fisher's exact test. Multivariate analyses of the correlation between experimental parameters and therapy response were done by stepwise logistic regression.

Overall and disease-free survival analyses were performed using the Kaplan-Meier method and survival curves were compared using the log-rank test.

## Results

### Bcl-2, Bcl-XL and Bax mRNA expression in myeloid blasts of AML patients at diagnosis

Enhanced BcL-2 gene expression was found in 30 (13%) of the 235 AML patients, enhanced BcL-XL in 90 (38%) and enhanced Bax in 175 (74%). A typical PCR gel is shown in Figure 1. In 34 patients we compared the Bax, Bcl-XL and Bcl-2 protein expression detected by Western-blot analysis with the corresponding mRNA levels obtained by RT-PCR and found a strong correlation for Bax, whereas the correlations for Bcl-XL and Bcl-2 were weak (data not shown).

Neither age, disease status or CD34 expression was found to have a significant impact on the expression of apoptosis-regulating genes (see Table 1). Blast cells from patients with FAB subtype M0 more frequently expressed Bcl-2 than those from patients with subtype M2 ($p$=0.03), all probes of patients with FAB subtype M6 expressed Bcl-XL ($p$=0.005) and all probes of patients with FAB subtype M5b had enhanced Bax expression ($p$=0.02) (see Table 1).

Patients with enhanced Bcl-2 or Bcl-XL expression had much lower peripheral white blood cell counts (WBC) than patients without. The median WBC in patients with Bcl-2 over-expressing blasts was 5.8 Gpt/L and in patients with Bcl-XL over-expressing
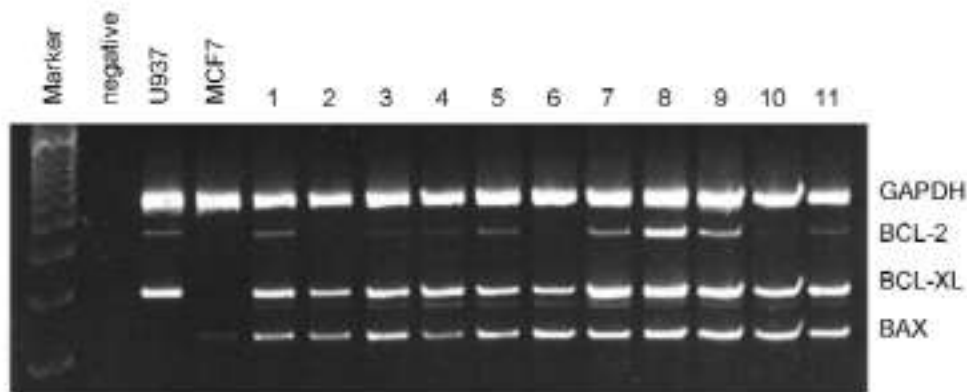
**Figure 1. Polyacrylamide gel electrophoresis of GAPDH (600 bp), Bcl-2 (412 bp), Bcl-XL (295 bp) and Bax (235 bp) RT-PCR reaction products of 11 AML patients (numbered 1-11) at diagnosis. Lane 1 shows a 100 bp molecular weight marker, lane 2 a negative control, lane 3 the cell line U937 serving as a positive control for Bcl-2 and Bcl-XL and lane 4 the cell line MCF7 serving as the positive control for Bax.**

**Table 1. Frequencies of enhanced Bcl-2, Bcl-XL and Bax mRNA expression in 235 AML patients divided by disease status, age, FAB subtype, CD34 expression, white blood cell count (WBC) and multi drug resistance gene (mdr1) expression. Bcl-2, Bcl-XL and Bax mRNA positivity was defined as expression levels exceeding the levels determined in cells from healthy stem cell donors.**

| | | Bcl-2 | | | Bcl-XL | | | Bax | | |
| | | negative | positive | p | negative | positive | p | Negative | positive | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Status | de novo (n=193) | 169 (88%) | 24 (12%) | | 116 (60%) | 77 (40%) | | 51 (26%) | 142 (74%) | |
| | secondary (n=42) | 36 (86%) | 6 (14%) | n.s. | 29 (69%) | 13 (31%) | n.s. | 9 (21%) | 33 (79%) | n.s. |
| Age | <= 60 years (n=143) | 125 (87%) | 18 (13%) | | 94 (66%) | 49 (34%) | | 34 (24%) | 109 (76%) | |
| | > 60 years (n=92) | 80 (87%) | 12 (13%) | n.s. | 51 (55%) | 41 (45%) | n.s. | 26 (28%) | 66 (72%) | n.s. |
| FAB subtype# | M0 (n=11) | 7 (64%) | 4 (36%) | p=0.03* | 5 (46%) | 6 (54%) | n.s. | 5 (46%) | 6 (54%) | n.s. |
| | M1 (n=52) | 42 (81%) | 10 (19%) | n.s. | 28 (54%) | 24 (46%) | n.s. | 13 (25%) | 39 (75%) | n.s. |
| | M2 (n=73) | 66 (90%) | 7 (10%) | | 50 (69%) | 23 (31%) | | 22 (30%) | 51 (70%) | |
| | M4 (n=33) | 30 (91%) | 3 (9%) | n.s. | 18 (55%) | 15 (45%) | n.s. | 8 (24%) | 25 (76%) | n.s. |
| | M4eo (n=9) | 8 (89%) | 1 (11%) | n.s. | 8 (89%) | 1 (11%) | n.s | 4 (44%) | 5 (56%) | n.s. |
| | M5a (n=36) | 34 (94%) | 2 (6%) | n.s. | 24 (67%) | 12 (33%) | n.s. | 6 (17%) | 30 (83%) | n.s. |
| | M5b (n=12) | 12 (100%) | 0 (0%) | n.s. | 8 (67%) | 4 (33%) | n.s. | 0 (0%) | 12 (100%) | p=0.02* |
| | M6 (n=5) | 4 (80%) | 1 (20%) | n.s. | 0 (0%) | 5 (100%) | p=0.005* | 0(0%) | 5 (100%) | n.s. |
| | M7 (n=3) | 2 (67%) | 1 (33%) | n.s. | 3 (100%) | 0 (0%) | n.s. | 2 (67%) | 1 (33%) | n.s. |
| WBC | median Gpt/L | 29.6 | 5.8 | p<0.001* | 30.3 | 18.8 | p=0.003* | 26.8 | 26.7 | n.s. |
| CD34 | positive (n=118) | 101 (86%) | 17 (14%) | | 70 (59%) | 48 (41%) | | 28 (24%) | 90 (76%) | |
| | negative (n=97) | 88 (91%) | 9 (9%) | n.s. | 63 (65%) | 34 (35%) | n.s. | 28 (29%) | 68 (71%) | n.s. |
| mdr1 | positive (n=54) | 42 (78%) | 12 (22%) | | 26 (48%) | 28 (52%) | | 8 (15%) | 46 (85%) | |
| | negative (n=168) | 150 (89%) | 18 (11%) | p=0.03* | 111 (66%) | 57 (34%) | p=0.02* | 41 (24%) | 127 (76%) | n.s. |

# *Expression frequencies of FAB subtypes were compared to the frequencies of FAB subtype M2.  *Statistical analysis was done by Fisher's exact test. Significance of differences in gene expression according to disease status, age, FAB subtype, CD34 and mdr1 expression and WBC; n.s., not significant.*

**Table 2. Bcl-2, Bcl-XL and Bax mRNA expression in blasts from patients ≤ 60 years old with _de novo_ AML assigned to risk groups determined by cytogenetic findings. Cytogenetic data were available for all 117 _de novo_ patients. Bcl-2, Bcl-XL and Bax mRNA positivity was defined as expression levels exceeding the levels determined in cells from healthy stem cell donors.**

| Cytogenetic risk | Bcl-2 | | | Bcl-XL | | | Bax | | |
|---|---|---|---|---|---|---|---|---|---|
| | negative | positive | p | negative | positive | p | Negative | positive | p |
| low (n=10) | 8 (80%) | 2 (20%) | n.s. | 8 (80%) | 2 (20%) | n.s. | 3 (30%) | 7 (70%) | n.s. |
| intermediate (n=84) | 75 (89%) | 9 (11%) | | 50 (60%) | 34 (40%) | | 16 (19%) | 68 (81%) | |
| high (n=23) | 20 (87%) | 3 (13%) | n.s. | 18 (78%) | 5 (22%) | n.s. | 11 (48%) | 12 (52%) | p=0.007 |

_Statistical analysis was done by comparing gene expression levels determined in the intermediate risk group with either the low or high risk group by Fisher`s exact test . n.s., not significant._

blasts 18.8 Gpt/L compared to 29.6 ($p$=<0.001) and 30.3 Gpt/L ($p$=0.003), respectively in patients without enhanced expression of the blasts (see Table 1).

Bcl-2 and Bcl-XL gene expression correlated significantly with mdr1 gene expression: 22% of the patients' probes without enhanced Bcl-2 expression were positive for mdr1 compared to 40% of those with enhanced Bcl-2 expression ($p$=0.03).

The ratios for Bcl-XL were similar, with 19% mdr1 positive blast probes in the group without enhanced Bcl-XL expression, but 33% in the group with enhanced Bcl-XL expression ($p$=0.02). However, the pro-apoptotic Bax gene did not correlate with mdr1 expression (see Table 1).

### Bcl-2, Bcl-XL and Bax gene expression and treatment outcome within cytogenetic risk groups of de novo AML patients ≤60 years old

Cytogenetic analyses were performed in all 117 _de novo_ AML patients ≤ 60 years old.

Additionally, all patients were screened for AML1/ETO and CBF-β/MYH11 gene rearrangements by nested PCR. Ten patients were positive for AML1/ETO and 7 for CBF-β/MYH11 (data not shown).

Of the ten patients with cytogenetic low risk disease, two over-expressed Bcl-2, two Bcl-XL and seven Bax (see Table 2). Furthermore, in the high risk cytogenetics group the anti-apoptotic genes, Bcl-2 or Bcl-XL, were not expressed more frequently than in the intermediate risk group. The pro-apoptotic gene, Bax, was remarkably less frequently expressed in the high risk group (52%) than in the intermediate risk group (81%) ($p$=0.007) (see Table 2).

Expression levels of Bcl-2, Bcl-XL and Bax were comparable in the different post-remission therapy groups, i.e. conventional chemotherapy, autologous PBSCT, related and unrelated allogeneic PBSCT (data

not shown).

Overall remission rates were 100% for cytogenetic low risk, 68% for intermediate risk and 52% for high risk patients.

Within the young AML patients with intermediate risk cytogenetics, 40/50 (80%) of patients without enhanced Bcl-XL expression reached CR criteria. Only 17/34 (50%) patients with enhanced Bcl-XL expression had a CR ($p$=0.004) (see Table 3).

Neither Bcl-2, Bcl-XL or Bax was of prognostic value for predicting CR in the patient group with cytogenetic high risk aberrations (see Table 3).

Multivariate analysis was performed including WBC, FAB subtype, cytogenetic aberrations, CD34, mdr1 and Bcl-2 family gene expression.

Mdr1 expression proved to be the strongest inde-

**Table 3. Numbers of _de novo_ AML patients ≤ 60 years old (n =117) in complete remission within different cytogenetic risk groups subdivided according to Bcl-2, Bcl-XL or Bax gene expression.**

| Cytogenetic risk group | Bcl-2 | | Bcl-XL | | Bax | |
|---|---|---|---|---|---|---|
| _All_ | — pt. | + pt. | — pt. | + pt. | — pt. | + pt. |
| 10/10 (100%) | 8/8 | 2/2 | 8/8 | 2/2 | 3/3 | 7/7 |
| 57/84 (68%) | 51/75 | 6/9 | 40/50 | 17/34 <br> p=0.004 | 14/16 | 43/68 |
| high <br> 12/23 (52%) | 10/20 | 2/3 | 8/10 | 4/5 | 7/11 | 5/12 |

_p-level indicates significance in the CR rate in the univariate analysis._
_— pt. = negative patients; + pt. = positive patients._

**Table 4. Correlation with response to induction therapy in *de novo* AML patients ≤ 60 years: multivariate analysis.**

| | Stepwise forward logistic regression analyses | | |
|---|---|---|---|
| | *All de novo* (n=117 with 79 CR/ 38NR) | *Intermediate cytogenetic risk* (n=84 with 57 CR/ 27 NR) | *High cytogenetic risk* (n=23 with 12 CR/ 11 NR) |
| WBC | *p*=0.95 | *p*=0.94 | *p*=0.99 |
| FAB subtype | *p*=0.27 | *p*=0.34 | *p*=0.08 |
| Cytogenetics | *p*=0.09 | Not applicable | Not applicable |
| CD 34 expression | *p*=0.46 | *p*=0.11 | *p*=0.09 |
| mdr1 expression | *p*=0.03 | *p*=0.02 | *p*=0.04 |
| Bcl-2 expression | *p*=0.84 | *p*=0.67 | *p*=0.90 |
| BCl-XL expression | *p*=0.06 | *p*=0.04 | *p*=0.55 |
| Bax expression | *p*=0.15 | *p*=0.36 | *p*=0.98 |

*CR, complete remission; NR, no complete remission; WBC, white blood cell count in Gpt/L. CD34 expression, positive cases reflect more than 20% positivity on the blast cells; mdr1, multi drug resistance gene. mdr1, Bcl-2, Bcl-XL and Bax mRNA positivity was defined as expression levels exceeding the levels determined in cells from healthy stem cell donors.*

pendent negative predictor (*p*=0.03) of treatment response for all *de novo* AML patients ≤ 60 years old. The next best predictors were enhanced Bcl-XL expression (*p*=0.06) and cytogenetics (*p*=0.09) (see Table 4).

Whereas mdr1 expression retained independent significance for both the intermediate (*p*=0.02) and the high risk cytogenetic group (*p*=0.04), Bcl-XL expression was a significant independent prognostic marker only for the intermediate cytogenetic risk group (*p*=0.04) and was not an independent prognostic factor for the high risk group (*p*=0.55) (see Table 4).

Bcl-2, Bcl-XL and Bax gene expression did not have a significant impact on overall survival or disease-free survival in either the high risk group or the intermediate risk one (data not shown).

## Discussion

In this survey we found a significant impact of Bcl-XL mRNA expression on treatment outcome in AML patients. This was most prominent in the group of patients ≤ 60 years old with intermediate risk cytogenetics. Those patients with enhanced BCL-XL expression in their blasts displayed a CR rate that was as low as the CR rate of patients with cytogenetic high risk karyotypes. In contrast, Bcl-XL gene expression had no influence on CR within this group of patients.

Furthermore, Bcl-XL and Bcl-2 expression correlated with a lower WBC, which might reflect the anti-apoptotic and possibly anti-proliferative stage of

these patients. As described before[8,24] we also found that AML patients with the immature FAB subtype M0 expressed Bcl-2 more frequently.

So far only Stoetzer *et al.*[15] has examined just 14 AML patients for Bcl-2, Bcl-XL and Bax protein expression and correlated the results to treatment outcome. However, valid statistical data could not be obtained. Pallis *et al.*[25] demonstrated that Bcl-XL is heterogeneously expressed in myeloblastic leukemia cells. Their cohort of patients was again too small to investigate whether expression levels might have an influence on treatment outcome, but showed that high Bcl-XL expression correlated with p-glycoprotein (mdr1) expression.

Our study substantiated this finding that Bcl-XL correlates with mdr1 expression in AML blasts. However, enhanced Bcl-XL expression was found to be an independent prognostic factor for therapy response, especially in AML patients with intermediate cytogenetic risk. This promotes the idea that different resistance pathways, e.g. classical multidrug resistance on the one hand and anti-apoptotic pathways on the other, may independently confer drug resistance in AML.

More data regarding Bcl-XL expression and treatment outcome in leukemia exist for acute lymphocytic leukemia, in which Bcl-XL expression was found only in 6 cases of 47 children with ALL. Once again, with such a small number of patients, no impact on prognosis could be demonstrated.[26]

In contrast to the obvious influence of Bcl-XL expression on treatment response, survival was not significantly different between patients with enhanced Bcl-XL expression in their blasts and those without. It is believed that the prognostic influence of the anti-apoptotic genes is more reliable than that of expression of pro-apoptotic genes such as Bax. Kornblau *et al.*[27] reported that Bcl-2/Bax ratio was strongly prognostic in AML patients; nevertheless Bcl-2 expression as a single factor had the same prognostic value.[19] This might be due to a lack of influence of Bax expression as a single factor in their study. In accordance, Bax expression had no prognostic value in our study. Furthermore, we found that apoptotic ratios such as Bcl-XL/Bax did not change the results.

We did not find that Bcl-2 expression had a significant impact on CR or survival, which is in agreement with recently published data from Filipits *et al.*[7] who performed multivariate analyses of different resistance factors in adult AML. In their survey, including resistance proteins such as p-glycoprotein (mdr1) and lung resistance protein (LRP), Bcl-2, clinical parameters and cytogenetics, Bcl-2 expression did not influence remission rate or survival. Even in the univari-

ate model Bcl-2 did not predict lower survival rates. Similar findings concerning adult AML were published by Wuchter et al.[8] There was no prognostic relevance of Bcl-2 expression on remission rates in the multi-variate analysis. Furthermore, Bcl-2 could not be correlated with poor treatment response or decreased survival in children with AML.[11] However, using uni-variate analyses other groups showed that Bcl-2-expressing adult AML patients had a poorer treatment outcome.[5,6,12] These different findings might reflect on the one hand that the series varied considerably between 20 and 131 AML patients examined and on the other hand that different detection techniques were used, i.e. protein-based procedures, such as Western-blot, immunocytochemistry and flow-cytometry or mRNA-based PCR procedures. The results of these techniques are difficult to compare. So far one survey has tried to compare Bcl-2 protein expression detected by flow-cytometry with mRNA-expression detected by RT-PCR. In a small number of patients they got very similar results, like ours, as they likewise found discrepancies between mRNA and protein levels in various patients.[13] Thus it must be kept in mind that our results are true for mRNA expression as a prognostic marker, but they might not be linearly transferable to the protein level.

Furthermore, treatment response and survival might be influenced by a combination of factors, e.g. by clinical or cytogenetic parameters.

In a previously published study the impact of Bcl-2 expression on survival was completely different in the cytogenetic low/intermediate risk group from that in the high risk group.[19] Whereas Bcl-2 was an unfavorable prognostic factor in the low cytogenetic risk group it was a favorable factor in the high risk group. The authors stated that expression analysis of apoptosis-regulating genes might not reflect the true apoptotic potential of leukemic cells, which might be influenced by chromosomal aberrations. In our survey Bcl-XL expression was correlated with a bad treatment response in patients with intermediate risk cytogenetics. In high risk patients, who generally respond poorly to treatment, response rates were not further reduced by enhanced Bcl-XL expression. The modus of influence between apoptosis-regulating genes and cytogenetics, has not yet been understood, but it seems that cytogenetic aberrations cause or alter a lot of other mechanisms that reduce the ability of chemotherapy to induce apoptosis in leukemic cells. These mechanisms, such as mdr1 expression, ras mutations or PKC-expression may interfere with each other in cytogenetic high risk patients.[20,27]

However, for the first time we have given evidence that Bcl-XL is a prognostic marker for treatment response in AML patients with intermediate risk cytogenetics but not in patients with high risk karyotypes. The prognostic value of Bcl-XL expression might, therefore, be dependent on cytogenetic findings. Further prospective studies are needed to confirm these results.

### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

### Potential implications for clinical practice

*De novo* AML patients ≤ 60 years old with intermediate risk cytogenetics and enhanced Bcl-XL gene expression at diagnosis should be considered at high risk of induction treatment failure. This should lead to a refined risk assessment in AML and might have implications for further risk-adapted treatment strategies in AML.

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