Brief report

High levels of *BAX*, low levels of *MRP-1*, and high platelets are independent predictors of response to imatinib in myeloid blast crisis of CML

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Imatinib induces remissions in approximately 30% of patients with chronic myeloid leukemia (CML) in myeloid blast crisis (M-BC). Because most patients eventually relapse, allogeneic stem cell transplantation (SCT) in remission offers the best chance for cure. Remission induction with imatinib alone would seem ideal because it is less toxic than conventional chemotherapy. Conversely, patients unlikely to respond may benefit from combination therapy up front. To identify prognostic factors, we studied the mRNA expression of genes related to drug resistance and apoptosis in leukemic cells from patients with M-BC and their in vitro sensitivity to imatinib, and analyzed the results with other baseline factors for their impact on response. We show that high levels of *BAX*, low levels of *MRP-1*, and a high platelet count are independently predictive of response to imatinib. Combined into a score, these parameters may be clinically useful for risk-adapted patient stratification. (Blood. 2003;101:2152-2155)

Introduction

Chronic myeloid leukemia (CML) in myeloid blast crisis (M-BC) is probably incurable by chemotherapy.[1,2] The results of stem cell transplantation (SCT) are poor if performed in frank blast crisis[3] but can be improved after induction of a second chronic phase.[4] Imatinib induces sustained hematologic remissions (> 4 weeks) in approximately 30% of patients with CML in M-BC.[5] Compared with conventional chemotherapy, imatinib is less toxic.[5] Thus, remission induction with imatinib alone prior to SCT would be desirable. By contrast, patients unresponsive to single-agent imatinib may benefit from combination treatment up front. To identify factors predictive of response, we studied the in vitro sensitivity to imatinib as well as the expression of apoptosis-related and drug resistance–related genes by quantitative reverse transcription–polymerase chain reaction (RT-PCR) in $CD34^+$ cells from patients with CML in M-BC prior to imatinib therapy. In the past, this panel of genes had been used successfully for identification of prognostic factors in acute myeloid leukemia (AML)[6] and soft tissue sarcoma.[7] Together with clinical baseline parameters, the results were analyzed in a multivariate model for their impact on response to imatinib.

Study design

Patients

Forty-six patients with CML in M-BC (defined as at least 30% of blasts in the peripheral blood [PB] or bone marrow [BM]) were studied after informed consent had been obtained. Thirty-eight of these patients were treated at various European centers within a phase 2 clinical trial,[5] and 8 within the subsequent "expanded access" protocol at the University of Leipzig. All except one patient were started on 600 mg imatinib daily. The diagnosis of myeloid phenotype was based on fluorescence-activated cell sorting (FACS) positivity for myeloperoxidase and FACS negativity for CD79a. The blasts were found to be $CD34^+$ in all cases. Twenty-five patients (54.3%) were women and 21 (45.7%) were men, with a median age of 57 years (range, 24-74 years). Response criteria were defined as previously published.[5] Twelve normal BM samples served as controls to define the normal range of gene expression.

Progenitor cell selection and in vitro sensitivity assays

BM or PB $CD34^+$ cells were selected on MiniMACS columns (Miltenyi Biotech, Bergisch-Gladbach, Germany). Triplicate cultures were plated at 10^4 cells/mL in methylcellulose (MethoCult H4230; Stem Cell Technologies, Vancouver, BC, Canada) in the presence of 50 ng/mL interleukin 3 (IL-3), 200 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), 200 ng/L granulocyte colony-stimulating factor (G-CSF), 200 ng/mL FLT-3 ligand, and graded concentrations of imatinib (0, 0.1, 0.5, 1, 5, 10, 50 μM). Colonies of granulocyte-macrophage colony-forming units (CFU-GMs) were counted after 7 and 14 days. Only samples with an average of at least 25 colonies/dish in the control were included in the analysis.

Expression analysis of candidate genes

Total RNA was extracted from 5×10^4 to 5×10^6 $CD34^+$ cells with RNAeasy columns (Qiagen, Hilden, Germany) and reversely transcribed into cDNA with random hexamers.[8] The expression of *MRP-1*, *MDR-1*,

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Table 1. Expression of candidate genes in CD34+ cells isolated from healthy individuals and patients with CML in M-BC

	Healthy individuals (HP)			M-BC			HP versus M-BC
Variable	N	Median	Range	N	Median	Range	*P*
BAD	12	5.7	3.0-11.7	37	4.4	0.6-39.0	.209
BAX	12	5.8	2.3-9.2	37	10.4	0.9-81.4	.015
BCL-2	12	7.4	2.3-15.0	37	4.2	0.4-101.2	.710
BCL-XL	12	4.6	1.3-8.5	36	10.7	3.3-144.5	<.0005
MDR-1	12	0.007	0.004-0.03	37	3.8	0.02-94.2	<.0005
MDM-2	12	20.1	8.0-84.2	36	1.8	0.1-1600	.001
MRP	12	15.5	4.8-28.6	37	28.1	3.2-3586	.003
Survivin	12	19.1	8.7-38.8	37	57.6	0.04-400	.027

Table 2. Baseline factors tested for correlation with response to imatinib

Factor/category	No. of patients	No. of responses (%)	Univariate	Multivariate	Odds ratio	Score
Pretreatment						
No	21	8 (38)	.944	—	—	—
Yes	23	9 (39)				
Time diagnosis to BC*						
Less than 2 y	19	10 (53)	.139	—	—	—
2 y or older	26	8 (31)				
Age*						
Younger than 60 y	33	14 (42)	.582	—	—	—
60 y or older	12	4 (33)				
Sex						
Male	20	6 (30)	.221	—	—	—
Female	25	12 (48)				
Weight*						
Less than 70 kg	21	9 (43)	.714	—	—	—
70 kg or greater	24	9 (38)				
Hepatomegaly						
No	20	9 (45)	.540	—	—	—
Yes	25	9 (36)				
Splenomegaly						
No	19	7 (37)	.712	—	—	—
Yes	26	11 (42)				
Hemoglobin level*						
Less than 100 g/L	20	6 (30)	.179	—	—	—
100 g/L or greater	24	12 (50)				
WBC count*						
Less than 50×10^9/L	32	13 (41)	.893	—	—	—
50×10^9/L or greater	13	5 (38)				
Platelet count*						
Less than 150×10^9/L	27	6 (22)	.003†	.015	21.6	1
150×10^9/L or greater	18	12 (67)			1.0	0
Basophils in PB*						
Less than 10%	35	11 (31)	.053†	NS	—	—
10% or greater	9	6 (67)				
Blasts in PB*						
Less than 50%	28	15 (54)	.007†	NS	—	—
50% or greater	16	2 (13)				
Blasts in BM*						
Less than 50%	25	11 (44)	.573	—	—	—
50% or greater	17	6 (35)				
Clonal evolution						
No	22	10 (45)	.465	—	—	—
Yes	23	8 (35)				

WBC indicates white blood cell; NS, not significant; —, not significant in univariate analysis.

*These parameters were also analyzed as continuous variables, without significant association with response.

†Parameters that were significant at $P < .1$ in univariate analysis were included in the multivariate model.

MDM-2, *BAX*, *BAD*, *survivin*, *BCL-2*, *BCL-X_L*, and *BCR-ABL* mRNA was measured by high-throughput *Taq*man-PCR, normalized for *GAPDH*, and expressed as transcripts/amol *GAPDH*. Samples with low expression of *GAPDH* (< 0.01 amol/μL cDNA) were excluded. Details of the assays and their validation were recently published.[6,7]

Statistical analysis

The impact of clinical baseline parameters, in vitro response, and the level of expression of the various candidate genes on response to imatinib were analyzed in a logistic regression model, where the expression levels of candidate genes were introduced as categorical variables (above and below median). Only parameters with $P < .1$ in univariate analysis were included in the multivariate model and introduced according to the method "Wald forward." Comparison of gene expression between M-BC and normal BM was by Mann-Whitney *U* test. All calculations were done with the SPSS software package.

Results and discussion

Using the criteria published by Sawyers et al,[5] there were 6 complete (13%) and 6 partial responses (13%), whereas 5 patients (11%) returned to chronic phase (overall response rate, 37%). Because the percentage of blasts was very variable, $CD34^+$ cells were selected to obtain a more homogenous population of cells. As a rule, the purity of the $CD34^+$ selections was more than 90%. Nonetheless, the expression levels of the various mRNAs studied were highly variable (Table 1), likely reflecting the biologic heterogeneity of M-BC. In contrast, a much more even distribution was observed in normal bone marrow $CD34^+$ cells. Highly significant differences between normal cells and M-BC were observed in the case of *BCL-X_L*, *MDR-1*, *MDM-2*, *MRP*, and *survivin*.

For analysis of their impact on response, continuous parameters were introduced as categorical variables (Tables 2-3). The candidate genes were categorized according to expression above and below median (Table 3). In univariate testing, low circulating blasts, basophils, *MRP1*, and high platelets and *BAX* were associated with responsive disease. In multivariate analysis, only high platelets, high *BAX*, and low *MRP* expression retained significance and predicted response to imatinib. Combining the 3 risk factors into a score distinguished between likely responders and likely nonresponders (Table 4).

Bax is a proapoptotic protein and may be important for inducing an apoptotic response to imatinib. Data regarding its role in CML are limited to the notion that its level of expression is not associated with disease stage.[9] Our findings in M-BC of CML are in contrast to those in AML where low levels of *BAX* mRNA are positively correlated with chemosensitivity.[6] This discrepancy may reflect the different natures of the diseases as well as the treatments. The role of *Mrp-1* in CML has also not been studied in any detail. In AML, high *Mrp* activity is associated with a poor response to induction

Table 3. Candidate genes tested for correlation with response

Parameter	No. of patients	No. of responses (%)	Univariate	Multivariate	Odds ratio	Score
BAD, median						
Less than 4.4	19	7 (37)	.638	—	—	—
4.4 or greater	18	8 (44)				
BAX, median						
Less than 10.4	19	5 (26)	.070†	.017	26.1	1
10.4 or greater	18	10 (56)			1.0	0
BCL-2, median						
Less than 4.2	19	8 (42)	.842	—	—	—
4.2 or greater	18	7 (39)				
BCL-XL, median						
Less than 10.7	18	6 (33)	.310	—	—	—
10.7 or greater	18	9 (50)				
MDR-1, median						
Less than 3.8	19	9 (47)	.385	—	—	—
3.8 or greater	18	6 (33)				
MDM-2, median						
Less than 1.8	18	9 (50)	.310	—	—	—
1.8 or greater	18	6 (33)				
MRP, median						
Less than 24.0	16	10 (63)	.018†	.011	1.0	0
24.0 or greater	21	5 (24)			14.4	1
Survivin, median						
Less than 57.6	19	8 (42)	.842	—	—	—
57.6 or greater	18	7 (39)				
BCR-ABL, median						
Less than 70 500	18	9 (50)	.310	—	—	—
70 500 or greater	18	6 (33)				
CFU-GM* day 7, 0.1 μM imatinib						
50% or greater	26	12 (46)	.149	—	—	—
Less than 50%	10	2 (20)				

— indicates parameters that were not significant in univariate analysis.

*Colony survival in percent of control; all other dose/time points were also tested, but also failed to show a correlation with response.

†Parameters that were significant at $P < .1$ in univariate analysis were included in the multivariate model.

Table 4. Response according to the score

Score	N	Response	%
3	8	0/8	0
2	13	2/13	15
1	15	12/15	80
0	1	1/1	100
2 + 3	21	2/21	10*
0 + 1	16	13/16	81*

*P = .00001 by Fisher exact test.

chemotherapy.[10] It is conceivable that, as a transmembrane transporter protein, *Mrp-1* may also confer primary resistance to imatinib. All other genes tested, including the expression levels of *BCR-ABL*, did not influence response in multivariate analysis.

There was no correlation between the in vitro response assessed by progenitor cell assays and the response in vivo. Failure to produce colonies was not more frequent in responders versus nonresponders ($P = .5$, χ^2). Because the cytokine mix used supports not only the growth of blasts but also of mature colonies, it is possible that the assay predominantly favored the proliferation of progenitor cells that did not belong to the blastic cell clone and may as such not reflect the clinical situation.

We also analyzed the impact of all parameters on survival in a Cox regression model. In univariate analysis, previous treatment (other than hydroxyurea) for blast crisis ($P = .058$), platelet counts less than 150×10^9/L ($P < .0005$), circulating blasts more than 50% ($P = .006$) were associated with shorter survival. In multivariate analysis, only low platelets retained significance ($P = .003$). Thus, the factors that determine the initial response to imatinib are, for the most part, distinct from those that determine survival.

Ideally, this score should be confirmed in an independent series of patients. Unfortunately, because our system involves separation of $CD34^+$ cells, no such series of sufficient size is currently available for study. If confirmed, the score may allow identifying those patients who are likely to respond to imatinib alone. In the context of a subsequent SCT, where outcome is much better for patients receiving transplants in remission,[4,11] this would be particularly advantageous; remission could be induced without the toxicity associated with conventional chemotherapy, which might in turn improve performance status at the time of SCT. By contrast, patients unlikely to respond to imatinib alone may benefit by combining imatinib with conventional cytotoxic drugs up front. In vitro studies indicate that this approach may be effective.[12]

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