HLA-G expression is associated with an unfavorable outcome and immunodeficiency in chronic lymphocytic leukemia

Short title: HLA-G expression in chronic lymphocytic leukemia

Section: Neoplasia

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ABSTRACT

The human leukocyte antigen (HLA)-G molecule exhibits limited tissue distribution and exerts multiple immunoregulatory functions. Recent studies indicate an ectopic up-regulation in tumor cells which may favor their escape from anti-tumor immune responses. The role of HLA-G in B-cell chronic lymphocytic leukemia (B-CLL) has not been defined. HLA-G expression was studied retrospectively in circulating B-CLL cells from 47 patients by flow cytometry using the MEM/G9 monoclonal antibody. The proportion of leukemic cells expressing HLA-G varied from 1% to 54%. Patients with ≤23% HLA-G positive cells (according to ROC-analysis; designated as HLA-G negative group) had a significantly longer progression-free survival (PFS) time than patients with >23% positive cells (median PFS: 120 versus 23 months, p=0.0001). In multivariate analysis HLA-G expression (hazard ratio 4.8; p=0.002) was an even better independent prognostic factor than the ZAP-70 or CD38 status. Humoral and cellular immunosuppression was significantly more prominent in HLA-G positive as compared to HLA-G negative patients group.

In B-CLL the level of HLA-G expression is correlated with the degree of immunosuppression and prognosis. HLA-G may contribute to the impairment of immune responses against tumor cells and infections. Thus, these findings need to be confirmed in a prospective study.

Key words: Chronic lymphocytic leukemia, HLA-G, prognostic factor
INTRODUCTION

B cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia in Western countries. It is characterized by an accumulation of long-lived, functionally inactive, mature-appearing neoplastic B lymphocytes. B-CLL has a variable clinical course. Some patients have an excellent prognosis never requiring treatment, whereas in others survival is short despite early initiation of therapy. During the last few years there has been a continuous effort to identify novel prognostic factors in B-CLL which may help define patient subgroups who may benefit from early therapeutic intervention.

Markers of poor prognosis in both early and advanced B-CLL include absence of mutations in the variable region of the immunoglobulin (Ig) heavy chain gene (IgVH gene), deletion of chromosome 11q23, loss or mutation of the p53 gene as well as CD38 or ZAP-70 positivity. In multivariate analyses, IgVH gene mutational status has been identified as the best predictor of clinical outcome: Patients whose CLL cells have unmutated IgVH genes have a significantly worse prognosis than those whose IgVH genes have undergone somatic mutation.

Classical MHC class I antigens are highly polymorphic molecules which mediate the presentation of peptides to T lymphocytes. They are ubiquitously expressed and play an important role in the recognition of tumor cells and their products by the immune system. Human leukocyte antigen (HLA)-G is a non-classical MHC class I antigen with very little sequence variability. It is not expressed in normal tissues except in trophoblasts from early gestation placentas. HLA-G exerts multiple immunoregulatory functions such as inhibition of natural killer (NK) or T cell mediated
cytolysis, induction of T cell apoptosis or inhibition of transendothelial NK cell migration\textsuperscript{13-14}. Since the net result of these effects is immunosuppression, HLA-G expression in tumor cells may favor their escape from antitumor immune responses thus allowing tumor progression\textsuperscript{15}.

Ectopic HLA-G expression has been demonstrated in various types of tumors including B cell and T cell lymphomas, melanoma as well as lung, kidney, bladder, breast, malignant ascites and colorectal carcinoma\textsuperscript{16-22}. In cutaneous B cell lymphomas and lung cancer HLA-G expression is paralleled by expression of interleukin-10 (IL-10) which has multiple inhibitory effects on the immune system\textsuperscript{23}. It has been hypothesized that IL-10 is also responsible for the up-regulation of HLA-G in tumor cells\textsuperscript{22; 24}.

In an effort to gain insight into the role of HLA-G in B-CLL we measured its expression in circulating leukemic cells and correlated our findings with a variety of clinical and laboratory variables.
MATERIALS AND METHODS

Between June 2002 and January 2004, 47 Caucasian patients with B-CLL were enrolled into this retrospective study and analyzed for biological and clinical characteristics including age, gender, Binet stage, treatment history, time from diagnosis to first treatment, white blood cell count, total T cell count and T cell subpopulations, hemoglobin concentration, platelet count, serum activities of lactate dehydrogenase and thymidine kinase as well as serum concentrations of β2-microglobulin and immunoglobulins. In each patient morphologic diagnosis of B-CLL was confirmed by flow cytometry revealing a typical CD19+ CD20+ CD5+ CD23+ Ig light chain (κ or λ) restricted immunophenotype. ZAP-70 and CD38 expression was determined by flow cytometry as described 8; 25. Blood samples were obtained during routine follow-up visits to our institution with all patients giving informed consent according to institutional guidelines. Indications for treatment were based on standard criteria.

Flow cytometry

Peripheral blood mononuclear cells were separated from freshly drawn anticoagulated blood by Ficoll-Paque™ (Pharmacia, Uppsala, Sweden) density gradient centrifugation. B cells were enriched by CD19 microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. To block unspecific binding the enriched B cells were incubated with 10% human serum in phosphate buffer saline (PBS, pH 7.4) for one hour at 4 °C. HLA-G expression on CD5 positive B cells was evaluated by dual-color immunofluorescence staining. To this end 1x10⁶ B cells were incubated in 200 µl PBS at room temperature for 30 min in the dark with 15 µl (1 mg/ml) fluorescein isothiocyanate (FITC) conjugated anti-
human HLA-G-specific monoclonal antibody MEM/G9 (IgG1; Serotec, Oxford, UK) and 20 µl phycoerythrin (PE) conjugated anti-human CD5-specific monoclonal antibody L17F12 (Becton-Dickinson, San Jose, CA, USA). After two washing steps with PBS HLA-G expression was quantified by flow cytometry (FACS Calibur, Becton Dickinson, Heidelberg, Germany). Isotype matched FITC and PE labeled mouse IgG served as a negative control. Data were acquired and analyzed using CellQuest software (Becton Dickinson).

**Soluble HLA-G (sHLA-G) and IL-10 levels**

Levels of β2-microglobulin associated sHLA-G molecules (G1 and G5 isoforms) in plasma samples were determined by ELISA as described previously. IL-10 plasma levels were measured using a commercially available kit according to the manufacturer's instructions (OptEIA™ human IL-10 set, Pharmingen, San Diego, CA, USA).

**Statistical analysis**

The impact of HLA-G expression on progression-free survival was assessed by performing “Receiver Operating Characteristics” (ROC) curve analysis. Progression-free survival times were measured from the time of diagnosis to first therapy, plotted according to the Kaplan-Meier method and compared using the log-rank test. Comparison of clinical or laboratory parameters between patient subgroups was performed using the non-parametric Mann-Whitney-U test for continuous variables and the χ² test for categorial data. Differences were regarded significant at p<0.05. To identify prognostic factors for the duration of progression-free survival multivariate Cox regression analysis was performed. The results were analyzed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA).
RESULTS

Characteristics of B-CLL patients

The cohort of patients analyzed was characteristic of B-CLL, with a median age of 61 years, male and stage A predominance, and about half the patients displaying adverse prognostic factors such as high expression of CD38 or ZAP-70 (Table 1).

HLA-G expression and prognosis

The proportion of CLL cells expressing HLA-G molecules varied from 1% to 54% (Figures 1 and 2). In an effort to assess the impact of HLA-G expression on the course of the disease, the percentage of cells expressing the molecule was correlated with the patients’ progression-free survival time using ROC curve analysis. HLA-G expression was significantly correlated with survival, best separation of the curves being achieved at a cut-off level of 23% positive leukemic cells. Thirty-seven patients had leukemias with fewer than 23% HLA-G positive cells (designated as the HLA-G negative group). This group had a significantly longer progression-free survival time than the HLA-G positive group (Figure 3). With a median follow up of 34 months (range: 0 – 281), 23 patients (49%) required first line chemotherapy. In the HLA-G positive group treatment became necessary in all patients while in the HLA-G negative group this was only the case in 13 patients (35%; p=0.0003).

Of the known prognostic factors, only thymidine kinase was unevenly distributed between the HLA-G negative and HLA-G positive groups (Table 1). In order to test the prognostic factors for interdependency, HLA-G status, age (cut-off level: 60 years), Binet stage (A versus B versus C), β2-microglobulin (cut-off level: 3.5 mg/l), CD38 status and ZAP-70 status (cut-off level: 20% positive cells) were subjected to
multivariate Cox regression analysis. In addition to stage, \( \beta_2 \)-microglobulin and ZAP-70 expression, HLA-G status was identified as an independent factor predicting disease progression (Table 2). The prognostic value of HLA-G expression in the B-CLL patients studied was even better than the value of the established markers like ZAP-70 and CD38 status. The risk for HLA-G positive patients receiving a first line therapy is nearly 5 times higher compared with HLA-G negative patients (Hazard ratio 4.8, \( p=0.002 \)). Only the initial staging according to Binet, known as one of the best prognostic markers, showed a higher hazard ratio (8.6, \( p=0.0001 \)).

**HLA-G expression and immunodeficiency**

B-CLL is characterized by humoral immunodeficiency, and a substantial proportion of patients also develop abnormalities in the T cell compartment. Table 3 shows that HLA-G status was correlated with both humoral and cellular immunosuppression. In comparison to the HLA-G negative group patients with HLA-G positive leukemias had significantly lower levels of IgG, total T cells and CD4+ T cells. In addition, plasma levels of IL-10 and soluble HLA-G tended to be higher in patients with HLA-G positive leukemias than in patients with HLA-G negative leukemias.
DISCUSSION

Expression of HLA-G has been suggested to contribute to immune evasion of tumor cells. This view has recently been strengthened by data demonstrating cell surface expression of HLA-G in melanoma\textsuperscript{18, 29, 30}, renal cell carcinoma\textsuperscript{17, 31}, cutaneous lymphoma\textsuperscript{32} and glioblastoma\textsuperscript{33} to be associated with inhibitory effects on diverse types of immune effector cells.

Data regarding HLA-G expression in B-CLL are limited and controversial. While Amiot and coworkers\textsuperscript{34} found transcription of the HLA-G gene in three of 6 cases by reverse transcriptase polymerase chain reaction, Polokova and coworkers\textsuperscript{35} and Mizuno and coworkers\textsuperscript{36} were unable to detect the HLA-G antigen by flow cytometry in eight or two samples of B-CLL, respectively. In our study, the HLA-G antigen was readily demonstrable in a variable proportion of tumor cells in all samples analyzed. Apart from problems related to small sample numbers, these differences are likely to be explained by differences in the properties of the monoclonal antibodies used to detect the HLA-G antigen. While the above investigators used indirect immunofluorescence employing the 87G antibody\textsuperscript{36}, our analysis involved direct immunofluorescence using the MEM/G9 antibody. This may be a more sensitive approach to visualize the HLA-G antigen. Furthermore, we measured the HLA-G expression in CD19 enriched B-CLL cells.

Using ROC curve analysis we were able to show that the degree of HLA-G expression was correlated with the propensity of the disease to progress. Leukemias with a proportion of 23% HLA-G positive cells or more had a more aggressive course than leukemias with fewer positive cells. Most other prognostic factors, such as the
Binet stage, β2-microglobulin or the CD38 or ZAP-70 expression status, were equally distributed between the two groups. Multivariate analysis revealed the HLA-G status to possess independent prognostic value even better than the prognostic value of the established prognostic markers like ZAP-70 and CD38 status. The risk for HLA-G positive patients receiving a first line therapy was nearly 5 times higher in our study group compared with HLA-G negative patients (Table 2). To our knowledge this is the first report that describes an association of HLA-G antigen expression and the course of the disease in B-CLL. If our findings are confirmed in an unrelated cohort of patients, the expression of HLA-G will further refine our ability to identify prognostic subgroups in B-CLL.

In trophoblasts and monocytes, expression of HLA-G can be induced by IL-10, involving regulatory pathways not shared by other MHC class I genes. IL-10 may act as an autocrine growth factor for B-cell lymphomas. In primary cutaneous lymphomas, its expression is associated with up-regulation of HLA-G lending support to the speculation that IL-10 may control HLA-G expression. In our study, plasma levels of IL-10 tended to be higher in the group of patients with HLA-G positive leukemias than in the group with HLA-G negative leukemias. Thus, it appears possible that IL-10 may also be involved in the control of HLA-G expression in B-CLL.

The group of patients with HLA-G positive B-CLL had more pronounced immunological abnormalities than the group of patients with HLA-G negative leukemias. HLA-G molecules possess potent immunosuppressive properties as exemplified in vitro by the induction of apoptosis in T cells and the inhibition of effector functions in NK and T cells. HLA-G induced immunosuppression may result in inadequate responses of the immune system to tumor cells displaying the
antigen\textsuperscript{39}. In our group of patients HLA-G expression was associated with a more general state of immunosuppression involving both the humoral and the cellular immune systems. In accordance with LeMaoult and coworkers\textsuperscript{40}, who demonstrated that HLA-G\textsubscript{1}-transfected antigen-presenting cells inhibit the proliferation of CD4\textsuperscript{+} T cells and induce CD4\textsuperscript{+} T cell anergy, we could show a significantly decreased T-cell count, especially in CD4\textsuperscript{+} T cells, in the group of patients with HLA-G positive leukemias. Such global immunodeficiency is a characteristic feature of B-CLL predisposing the patients to bacterial and viral infections. To what extent these observations are causally related to HLA-G, IL-10 or other regulators remains to be clarified.

To our knowledge this is the first report demonstrating consistent expression of the HLA-G protein in B-CLL. Unexpectedly, its level of expression was correlated with the degree of immunosuppression accompanying the disease and the prognosis of the patients. The major limitation of our analysis is its retrospective nature. HLA-G and other immunological parameters were determined at variable intervals from diagnosis, and chemotherapy was likely to have had an impact on the results obtained. Therefore, our findings need to be confirmed in a prospective study involving a larger cohort of patients.

Acknowledgments

We are indebted to Brigitte Fischer for generously contributing information on the clinical course and the treatment histories of the study patients. We thank Babette Pohle and Annika Dolar for expert technical assistance with flow cytometry and measuring sHLA-G and IL-10.
Reference List


Table 1  Clinical data and prognostic factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients</th>
<th>&lt; 23% HLA-G positive cells</th>
<th>≥ 23% HLA-G positive cells</th>
<th>p value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>47</td>
<td>37 (79%)</td>
<td>10 (21%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>61 (30 –88)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>60.5 (30 –80)</td>
<td>62.5 (41 -88)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>37 (79%)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>29 (78%)</td>
<td>8 (80%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Binet stage at diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>37 (79%)</td>
<td>30 (81 %)</td>
<td>7 (70 %)</td>
<td>n.s.</td>
</tr>
<tr>
<td>B</td>
<td>6 (13%)</td>
<td>5 (14 %)</td>
<td>1 (10 %)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4 ( 9%)</td>
<td>2 ( 5 %)</td>
<td>2 (20 %)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl) (n=47)</td>
<td>13.05 ± 1.75&lt;sup&gt;3&lt;/sup&gt;</td>
<td>13.35 ± 1.6</td>
<td>11.94 ± 1.87</td>
<td>0.023</td>
</tr>
<tr>
<td>Platelets /nl (n=47)</td>
<td>147 ± 65</td>
<td>153 ± 64</td>
<td>124 ± 68</td>
<td>n.s.</td>
</tr>
<tr>
<td>Leukocytes /nl (n=47)</td>
<td>56.96 ± 64.5</td>
<td>58.5 ± 69.1</td>
<td>51.1 ± 45.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neutrophils / nl (n=46)</td>
<td>4.8 ± 4.3</td>
<td>5.3 ± 4.4</td>
<td>3.0 ± 3.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Lymphocytes / nl (n=46)</td>
<td>50.2 ± 61.0</td>
<td>51.2 ± 65.7</td>
<td>46.6 ± 42.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Monocytes / nl (n=46)</td>
<td>1.4 ± 2.6</td>
<td>1.5 ± 2.9</td>
<td>1.1 ± 1.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>CD38+ leukemia (n=47)</td>
<td>24 (51%)</td>
<td>17 (46%)</td>
<td>7 (70%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ZAP-70+ leukemia (n=42)</td>
<td>19 (45%)</td>
<td>15 (45%)</td>
<td>4 (44%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>β&lt;sub&gt;2&lt;/sub&gt;-microglobulin (mg/l) (n=45)</td>
<td>4.0 ± 2.2</td>
<td>3.8 ± 2.0</td>
<td>5.0 ± 2.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Thymidine kinase (U/l) (n=32)</td>
<td>28.5 ± 34.5</td>
<td>22.3 ± 29.3</td>
<td>55.8 ± 44.8</td>
<td>0.055</td>
</tr>
</tbody>
</table>

<sup>1</sup> Median (range).
<sup>2</sup> Percent of all patients. Because of rounding, percentages do not always add up to 100.
<sup>3</sup> Mean ± standard error of the mean.
<sup>4</sup> Comparison between the HLA-G positive and HLA-G negative subgroups using the chi-square test (Binet stage, CD38+ and ZAP-70+ leukemias) or the Mann-Whitney-U test (all other variables).

In most patients, the laboratory parameters were not determined at diagnosis, but during the course of the disease. Normal ranges: Hemoglobin, 14.0 - 18.0 g/dl (male), 12.0 - 14.0 g/dl (female); platelets, 140 - 440 /nl; leukocytes, 4.0 - 10.0 /nl; neutrophils, 1.5-7.0 /nl; lymphocytes, 1.0 – 4.0 /nl; monocytes, < 1.0 /nl; β<sub>2</sub>-microglobulin, < 3.5 mg/l; thymidine kinase < 7 U/l. CD38+ or ZAP-70+ leukemias were diagnosed when more than 20 % of the leukemic cells expressed the respective marker.
<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Hazard ratio</th>
<th>CI (95%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47</td>
<td>1.02</td>
<td>0.97 - 1.06</td>
<td>0.50</td>
</tr>
<tr>
<td>Disease stage at diagnosis</td>
<td>47</td>
<td>8.66</td>
<td>3.0 - 25.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>45</td>
<td>3.08</td>
<td>1.06 - 8.95</td>
<td>0.039</td>
</tr>
<tr>
<td>ZAP-70 status</td>
<td>42</td>
<td>3.58</td>
<td>1.14 - 11.27</td>
<td>0.029</td>
</tr>
<tr>
<td>CD38 status</td>
<td>47</td>
<td>1.83</td>
<td>0.48 - 6.94</td>
<td>0.37</td>
</tr>
<tr>
<td>HLA-G status</td>
<td>47</td>
<td>4.81</td>
<td>1.74 - 13.29</td>
<td>0.002</td>
</tr>
</tbody>
</table>

CI: Confidence interval. The cut-off levels used in the analysis were: age, 60 years; disease stage at diagnosis, Binet stage A versus B versus C; β2-microglobulin, 3.5 mg/l; ZAP-70 or CD38 status, 20% positive cells; HLA-G status, 23% positive cells. Because of missing data in a substantial proportion of patients, the prognostic marker thymidine kinase (cf. Table 1) was not included in the analysis.
Table 3  
**Immunological parameters**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients</th>
<th>&lt; 23% HLA-G positive cells</th>
<th>≥ 23% HLA-G positive cells</th>
<th>p value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunglobulin G (g/l) (n=43)</td>
<td>7.08 ± 3.03₁</td>
<td>7.51 ± 2.9</td>
<td>5.47 ± 3.1</td>
<td>0.042</td>
</tr>
<tr>
<td>Immunglobulin A (g/l) (n=43)</td>
<td>0.88 ± 0.7</td>
<td>0.91 ± 0.7</td>
<td>0.77 ± 0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Immunglobulin M (g/l) (n=43)</td>
<td>0.54 ± 0.6</td>
<td>0.54 ± 0.7</td>
<td>0.53 ± 0.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total T cells (per µl) (n=32)</td>
<td>2780 ± 2475</td>
<td>3095 ± 2567</td>
<td>1076 ± 581</td>
<td>0.005</td>
</tr>
<tr>
<td>CD4+ T cells (per µl) (n=32)</td>
<td>1508 ± 1304</td>
<td>1685 ± 1344</td>
<td>556 ± 291</td>
<td>0.016</td>
</tr>
<tr>
<td>CD8+ T cells (per µl) (n=32)</td>
<td>1172 ± 1249</td>
<td>1299 ± 1317</td>
<td>586 ± 351</td>
<td>0.077</td>
</tr>
<tr>
<td>CD4/CD8 ratio (n=32)</td>
<td>1.85 ± 1.6</td>
<td>1.93 ± 1.7</td>
<td>1.4 ± 0.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>IL-10 (pg/ml) (n=47)</td>
<td>5.1 (6.1 – 43.1)²</td>
<td>4.9 (6.1 – 43.1)</td>
<td>10.2 (9.9 – 40.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>sHLA-G (pg/ml) (n=47)</td>
<td>17.3 (0.0 – 82.8)</td>
<td>16.7 (0.0 – 82.8)</td>
<td>26.9 (0.0 – 34.8)</td>
<td>0.043</td>
</tr>
</tbody>
</table>

₁ Mean ± standard error of the mean.  
² Median (range).  
³ Comparison between the HLA-G positive and HLA-G negative subgroups using the Mann-Whitney-U test.

In most patients, the laboratory parameters were not determined at diagnosis, but during the course of the disease. Normal ranges: IgG, 7 – 26 g/l; IgA, 0.7 – 4.0 g/l; IgM, 0.3 – 2 g/l; total T cells, 690 – 2540 /µl; CD4+ T cells, 410 – 1590 /µl; CD8+ T cells, 190 – 1140 /µl; CD4/CD8 ratio, 1.0 – 3.0; IL-10, 20.1 ± 33.2 pg/ml; sHLA-G, 22.9 ± 16.9 ng/ml.
**Figure legends**

**Figure 1. Cell surface expression of HLA-G in two cases of B-CLL.**

B-CLL cells were subjected to dual-color flow cytometry using a CD5-specific antibody and the HLA-G-specific antibody MEM/G9 or the respective isotype control. Panel A: Low HLA-G expression. Panel B: High HLA-G expression.

**Figure 2. Proportion of HLA-G positive leukemic cells in 47 patients with B-CLL.**

HLA-G expression was analyzed by flow cytometry. The dotted vertical line at 23% positive cells separates the patients into a prognostically favorable HLA-G negative and an unfavorable HLA-G positive group (cf. Figure 3).

**Figure 3. Probability of disease progression (interval from diagnosis to initiation of chemotherapy) in 10 patients with HLA-G positive and 37 patients with HLA-G negative B-CLL.**

Kaplan-Meier analysis. The median progression-free survival time was 23 months in HLA-G positive and 120 months in HLA-G negative B-CLL (p=0.0001, log-rank test). All patients with HLA-G positive B-CLL received chemotherapy within 42 months.
Figure 1
Figure 2

Number of patients

% HLA-G positive leukemic cells

n=37

n=10

0-4
4-8
8-12
12-16
16-20
20-23
23-26
26-30
30-34
34-38
38-42
42-46
46-50
50-54
Figure 3

- < 23% HLA-G positive cells (n=37)
- ≥ 23% HLA-G positive cells (n=10)

p=0.0001
HLA-G expression is associated with an unfavorable outcome and immunodeficiency in chronic lymphocytic leukemia

Holger Nuckel, Vera Rebmann, Jan Durig, Ulrich Duhrsen and Hans Grosse-Wilde