The GNAS1 T393C Polymorphism Is Associated with Disease Progression and Survival in Chronic Lymphocytic Leukemia

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Abstract Purpose: B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of monoclonal mature B cells. The G protein Gα subunit has been linked to proapoptotic processes in cancer cell lines. The TT genotype of the GNAS1 T393C polymorphism is associated with increased Gα transcript levels and a more favorable clinical course in different solid cancers.

Experimental Design: We retrospectively genotyped 144 patients with B-CLL to examine a potential association between T393C genotypes with progression-free survival (time from diagnosis to initiation of chemotherapy) and overall survival.

Results: The C-allele frequency in the patient group was 0.57 and not significantly different from that of healthy blood donors. Median progression-free survival was significantly different between genotypes (TT 130 months; TC 100 months; CC 31 months; P = 0.0066). Multivariable analysis showed that besides of ZAP-70 (P = 0.005) and Binet stage (P < 0.001), the T393C polymorphism was an independent prognostic factor for progression-free survival [hazard ratio (HR) CC versus TT 2.7; P = 0.010]. In Binet A stages, ZAP-70– positive patients with CC genotypes had a HR of 4.4 to receive first therapy compared with ZAP-70– negative patients with T-alleles (P = 0.0001). Regarding overall survival, CC genotypes (median overall survival, 197 months) were at highest risk for death compared with T-alleles (median overall survival, 310 months) in both univariate (HR, 4.8; P < 0.0001) and multivariable analysis (HR, 5.6; P = 0.002).

Conclusions: Here, we show that the GNAS1 T393C status is a novel independent prognostic marker in patients with B-CLL. These results could help to define patients who could benefit from an early individualized therapy.

B-cell chronic lymphocytic leukemia (B-CLL) accounts for 25% of all leukemias and is the most common form of lymphoid malignancy in Western countries (1). CLL is a clinically heterogeneous disease originating from B lymphocytes that may differ in activation, maturation state, or cellular subgroup. Alterations in apoptosis and cell cycle regulation have been described in CLL, resulting in an accumulation and proliferation of leukemic cells (2, 3). The staging system devised by Binet (4) is useful for predicting survival and treatment requirements in patients with CLL. However, these staging systems are often of limited prognostic value in early stages of the disease, which applies for most of the patients at first diagnosis. Therefore, the identification of additional prognostic markers helped to define patient subgroups with favorable versus poor clinical outcome in CLL (5–7). For example, the presence or absence of somatic mutations in the immunoglobulin heavy chain variable region (IgVH) of B-CLL cells has been described as one of the most powerful prognostic factors, as B-CLL cases with mutated IgVH genes exhibit a favorable clinical course, whereas B-CLL patients with unmutated IgVH genes are characterized by a poor outcome in terms of reduced survival and response to chemotherapy (6).

Recently, it has been shown that expression analysis of a single gene, the protein tyrosine kinase ZAP-70, could correctly predict the IgVH mutation status in a certain subset of patients and, therefore, was supposed to serve as a surrogate for IgVH mutation status (8, 9). However, up to 25% of patients may have a discordant IgVH and ZAP-70 status (10). There is also growing evidence suggesting that genetic host factors could influence the clinical course of CLL (11). We have recently shown that genotypes of the single nucleotide polymorphism (SNP) T393C in the gene GNAS1, encoding the ubiquitously expressed Gα subunit of heterotrimeric G proteins, predict the clinical outcome of patients with urothelial carcinoma (12), sporadic colorectal cancer (13), and renal cell carcinoma (14). Patients with TT genotypes showed a prolonged survival compared with patients with TC and CC genotypes with a gene-dose effect. We also have shown that Gα mRNA expression is increased in TT genotypes, not only in bladder cancer but also in heart and fat cell specimens (12) possibly.
due to altered mRNA stability associated with different genotypes (13). Studies in human B-precursor cells as well as in other cell types have shown that activation of the Gαs pathway is associated with increased apoptosis (15–18). Hence, it is tempting to hypothesize that increased Gαs expression with concomitantly enhanced apoptosis may be related to better survival in cancer patients with GNAS1 TT genotypes and this could be a general phenomenon in different cancers (12–14).

The aim of the present study was to accumulate further support for the hypothesis that the T393C polymorphism–related altered expression of Gαs is not only associated with outcome in patients with solid tumors, but may represent a more general feature with the capacity to predict the clinical course in other hematologic malignancies, too. Moreover, we investigated whether the T393C SNP represents an independent prognostic factor in relation to well-established prognostic factors (Binet stage, genetic aberrations, ZAP-70, and IgVH). To this end, we investigated a potential association between genotypes of the T393C polymorphism and clinical outcome in a cohort of patients suffering from CLL.

Materials and Methods

Patients. One hundred forty-four Caucasian patients with CLL were enrolled in this retrospective study between August 2001 and April 2006. Several anthropometric and molecular variables had been collected at the time of diagnosis, including age, gender, Binet stage, thymidine kinase, and cytogenetics. In each patient, morphologic diagnosis of B-CLL was confirmed by flow cytometry revealing a typical CD19+, CD20+, CD5+, CD23+, and Ig light chain (κ or λ light chain) restricted immunophenotype. ZAP-70 and CD38 expression was assessed by flow cytometry as described (19). CLL was considered CD38-positive when >20% of the gated population (CD19+/CD5+) expressed it, and it was regarded ZAP-70 positive when at least 20% of the gated cells (CD19+ B cells) expressed it. Whole peripheral blood samples were usually obtained during routine follow-up visits to our institutions with all patients giving informed consent according to institutional guidelines. Indications for treatment were based on standard criteria (20). In Table 1, the clinical and laboratory data are shown.

Blood donors. The control group consisted of 255 age- and sex-matched healthy Caucasian individuals (161 males and 94 females; mean age 56.7 ± 4.4 years) who were randomly recruited at the local Institute of Transfusion Medicine, University Hospital of Essen. Details of this control group have been published previously (21).

Determination of the GNAS1 T393C genotypes. Genomic DNA was extracted using the QIAamp blood kit (Qiagen, Hilden, Germany). The T393C polymorphism was identified by PCR using the following primers: forward primer 5'-CTCTCAATGTCATGTGGTCA-3' and reverse primer 5'-TAAGGCCACAAAATGCGGAT-3'. After denaturation at 94°C, 35 cycles of DNA amplification were done using Taq PCR Mastermix (Eppendorf, Hamburg, Germany) at 94°C for 45 seconds, 58°C for 40 seconds, and 72°C for 45 seconds. The 345 bp PCR products were digested using the restriction enzyme FokI and analyzed on a 2% agarose gel. The unrestricted product (345 bp) represents the TT genotype; the completely restricted product (259 and 86 bp) represents the CC genotype.

Detection of genomic aberrations by fluorescence in situ hybridization. To detect prognostically relevant anomalies of chromosomal regions 11q, 13q, 17p, and of chromosome 12, the following fluorescence-labeled DNA probes were used in interphase cytogenetic analyses: LSI ATM (11q23), LSI D13S319 (13q14), LSI p53 (17p13.1), and cep12 (centromere 12; all probes purchased from Abbot Vysis, Stuttgart, Germany). Sample preparation, fluorescence in situ hybridization, and counterstaining with 4,6-diamidino-2-phenylindole dihydrochloride were done as reported (22).

IgVH gene characterization. VH gene rearrangements were amplified from genomic DNA, isolated from peripheral blood mononuclear cells, by VH gene family-specific primers binding to the framework regions I combined with primer mixes for the JH gene segments, as previously described (23). In some instances, e.g., when the framework region 1 PCR failed, primers binding to the leader regions of VH genes were used with the JH primer mixes (24). VH rearrangements were amplified for a total of 29 cycles. PCR products were gel purified and directly sequenced with Big Dye Terminator Cycle Sequencing Kit V3.1 (AppliedB GmbH, Darmstadt, Germany) on an ABI 3130 sequencer. Sequence analysis was done with the IMGT

**Table 1. Clinical and laboratory data at diagnosis in patients with GNAS1 T393C polymorphism**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients</th>
<th>CC</th>
<th>TC</th>
<th>TT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients (%)</td>
<td>144</td>
<td>45 (31.3)</td>
<td>72 (50.0)</td>
<td>27 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Median age at diagnosis (y)</td>
<td>60</td>
<td>63</td>
<td>59</td>
<td>60</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>97 (57%)*</td>
<td>29 (64%)</td>
<td>54 (75%)</td>
<td>13 (48%)</td>
<td>NS</td>
</tr>
<tr>
<td>Binet stage at diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>105 (73%)</td>
<td>33 (73%)</td>
<td>51 (71%)</td>
<td>21 (78%)</td>
<td>NS</td>
</tr>
<tr>
<td>B</td>
<td>28 (19%)</td>
<td>7 (16%)</td>
<td>15 (21%)</td>
<td>6 (22%)</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>11 (8%)</td>
<td>5 (11%)</td>
<td>6 (8%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>CD38+ leukemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 136</td>
<td>55 (38%)</td>
<td>22 (49%)</td>
<td>24 (33%)</td>
<td>9 (33%)</td>
<td>NS</td>
</tr>
<tr>
<td>ZAP-70+ leukemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 81</td>
<td>65 (48%)</td>
<td>24 (57%)</td>
<td>33 (48%)</td>
<td>8 (32%)</td>
<td>NS</td>
</tr>
<tr>
<td>Thymidine kinase (IU/L; n = 81)</td>
<td>18.2 ± 2.9'</td>
<td>17.8 ± 5.7</td>
<td>19.6 ± 4.0</td>
<td>14.7 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Genomic aberrations (n = 98)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion 11</td>
<td>10 (10%)</td>
<td>7 (25%)</td>
<td>1 (2%)</td>
<td>2 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Deletion 17</td>
<td>7 (7%)</td>
<td>2 (7%)</td>
<td>3 (6%)</td>
<td>2 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>11 (11%)</td>
<td>2 (7%)</td>
<td>7 (13%)</td>
<td>2 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Normal</td>
<td>25 (18%)</td>
<td>7 (16%)</td>
<td>13 (18%)</td>
<td>5 (19%)</td>
<td>NS</td>
</tr>
<tr>
<td>Deletion 13</td>
<td>56 (57%)</td>
<td>15 (54%)</td>
<td>31 (57%)</td>
<td>10 (63%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mutated IgVH gene (n = 74)</td>
<td>40 (54%)</td>
<td>10 (25%)</td>
<td>22 (55%)</td>
<td>8 (20%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

*Percentage of total number of patients.

Mean ± SE.
database.\textsuperscript{5} VH gene sequences close to the threshold of the hyper-mutation status of CLL patients (being 2% difference between the particular VH gene rearrangement and its closest germ line counter-part) were confirmed by Blast database search (25).\textsuperscript{1}

\textbf{Statistical analysis.} The clinical outcomes analyzed in this study were time to first therapy and overall survival. The time to first therapy was calculated from the date of first diagnosis until initiation of first therapy. Overall survival was calculated from the date of first diagnosis until death. Kaplan-Meier plots and the log-rank test for trend were used to evaluate the relationship between T393C genotypes from the date of the primary diagnosis to the date of initiation of first therapy. To produce multivariable models of clinical follow up, variables previously described as predictors of prognosis of CLL and those which were significant by univariate analysis, including genotypes of the T393C polymorphism, were assessed for hazard ratios (HR), 95\% confidence interval (95\% CI), and \( P \) values using the backward Cox proportional hazard model (26). Nonsignificant variables were stepwise removed from the model. Data from 136 patients were used for multivariable comparisons (ZAP-70 expression was not available for eight patients). Comparison of clinical and laboratory variables between genotypes was done using Mann-Whitney \( U \)-test for continuous variables and the \( \chi^2 \) test for categorical data. Differences were regarded significant at \( P < 0.05 \). All statistical analysis was done using SPSS 11.0 (SPSS, Chicago, IL). Continuous variables are given as means \( \pm \) SE.

\section*{Results}

\textbf{T393C genotype distributions and correlation with clinical and laboratory data.} Demographic characteristics and clinical and laboratory data at diagnosis in the whole case group and by genotype are displayed in Table 1. The median age was 60.0 years (range, 27-98 years) and median follow-up time to first therapy was 81 months (range, 1-303 months). The frequency of the \( C \) allele (\( f_C \)) in the patient group was 0.57 and the distribution was compatible with the Hardy-Weinberg equilibrium.

To investigate whether the T393C polymorphism is predictive for an increased risk to develop CLL, we compared genotypes and allele frequencies with those from 255 age-and sex-matched healthy white blood donors (CC, \( n = 68 \); TC, \( n = 125 \); TT, \( n = 62 \)). Genotype distribution as well as \( C \)-allele frequency (\( f_C = 0.51 \)) was not significantly different from that of the patient group, which argues against an association of T393C genotypes with an increased susceptibility for CLL.

We found no genotype association with age at first diagnosis. Moreover, genotypes were not significantly associated with different Binet stages, CD38-positive and CD38-negative patients, ZAP-70-positive or ZAP-70-negative disease, genomic aberrations, and IgV\textsubscript{H} status (Table 1).

Within the median follow up of 81 months, 58 patients (40.1\%) required first chemotherapy based on National Cancer Institute working group criteria (20). We used Kaplan-Meier plots to calculate genotype-associated differences in disease progression as indicated by different treatment-free intervals and observed a significant genotype-dependent, treatment-free interval with an apparent gene-dose effect (Fig. 1; \( P = 0.0066 \)). GNAS1 393C homozygous patients (\( n = 27 \)) displayed a higher risk for disease progression (median 31 months) than T393 homozygous patients (\( n = 27 \); median 130 months), with heterozygous patients being at intermediate risk (\( n = 72 \); median 100 months; CC versus TT: HR, 2.7; 95\% CI, 1.2-4.6; \( P = 0.010 \); CC versus TT: HR, 1.7; 95\% CI, 1.0-3.0; \( P = 0.041 \)). Proportions of 5-year treatment-free intervals were 73.0\% for TT, 63.3\% for TC, and 33.2\% for CC genotypes, respectively (Fig. 1).

\textbf{Multivariable Cox proportional analysis for independent prognostic factors.} To investigate whether genotypes of the T393C polymorphism are an independent risk factor for clinical outcome in patients with CLL, multivariable analysis was done based on two different Cox proportional hazard models (Table 2). Both models consisted of all available prognostic factors except of “genomic aberrations” and IgV\textsubscript{H} status because of missing data in a substantial proportion of patients.

Multivariable analysis revealed that, in addition to Binet stage and ZAP-70 expression, the T393C genotype status was an independent factor predicting disease progression (Table 2). CD38 status was removed from the model due to a significant interaction with ZAP-70 (\( P < 0.001 \)). The risk for 393CC patients to receive first therapy was thrice higher compared with TT homozygous patients (95\% CI, 1.3-7.8; \( P = 0.010 \)). As our CLL sample consisted of 27 patients with 393TT genotype and 11 patients with stage Binet C only, we dichotomized variables with T393C genotypes dividing them into T+ alleles (TT + TC) and T– genotypes (CC) and Binet stages divided into A versus B + C (Model B). Using this model, the significant and independent risk factors for disease progression could be defined as follows: Binet stage B + C, ZAP-70 positive, and GNAS1 393T– (Table 2).

When we analyzed genomic aberrations (98 patients) stratified into the groups “low risk” (deletion 13q), “intermediate risk” (normal and trisomy 12), and “poor risk” (deletion 17p and del 11q) as prognostic factors, we could show that del17p and del11q patients were at 2-fold risk (95 CI, 1.0-4.0; \( P = 0.048 \)) to receive first therapy compared with del13q patients. When genomic aberrations were included into the
multivariable analysis, the T393C genotype remained a significant independent prognostic marker for disease progression (HR, 2.9 for CC versus TT; P = 0.037).

To investigate whether genotypes of the T393C polymorphism are associated with the immunoglobulin heavy-chain gene mutation status, which is regarded to be one of the best prognostic markers for progression-free survival, we did a sequence analysis of rearranged IgVH genes in 74 cell samples from patients with CLL. Cells of 40 patients (54%) had mutated IgVH genes and a significantly longer median time to initial therapy (119 months) than patients with CLL cells that expressed an unmutated IgVH gene (31 months, P < 0.001), which is comparable with data from the literature (6, 10, 27, 28). Moreover, we could confirm a significant association between the presence of unmutated IgVH genes and positive ZAP-70 status (10) with discordant results in 27% of patients (P = 0.001). We found no significant association of genotypes of the T393C polymorphism with IgVH gene status (Table 1). Moreover, Cox proportional hazards analysis of the IgVH mutation status and the T393C polymorphism as predictors of the time from diagnosis to initial therapy in 74 patients revealed that the T393C status was independently associated with outcome (CC versus TT HR, 2.3; 95% CI, 1.1-4.5; P = 0.0778).

Combined ZAP70, Binet, and T393C status: association with disease progression. Based on the results from the multivariable Cox regression model from patients with all data available, we defined four hypothetical risk groups to potentially improve the combined prognostic information: Group I (n = 43) consisted of Binet A and ZAP-70-negative patients with T+ alleles (no risk factor). Group II (n = 53) consisted of patients with any one risk factor (Binet B/C or ZAP-70 positive or T– genotype), group III (n = 32) of patients with any two, and group IV (n = 8) of patients with all three risk factors present (P < 0.0001). A total of 101 patients with Binet stage A were stratified for the following risk factors: T+ alleles and ZAP-70 negative (n = 43), T– genotype or ZAP-70 positive (n = 42), and T– and ZAP-70 positive (n = 16). Statistical analysis was done using the log-rank test for trend.

### Table 2. Multivariable Cox regression analysis model for treatment-free survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease stage at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>101</td>
<td>1.00*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>4.0 (2.2-7.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>2.6 (1.1-6.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>ZAP-70 status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>70</td>
<td>1.00*</td>
<td>0.005</td>
</tr>
<tr>
<td>Pos</td>
<td>65</td>
<td>2.1 (1.3-3.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>T393C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>24</td>
<td>1.00*</td>
<td>0.005</td>
</tr>
<tr>
<td>TC</td>
<td>69</td>
<td>1.8 (0.8-4.4)</td>
<td>0.167</td>
</tr>
<tr>
<td>CC</td>
<td>42</td>
<td>3.2 (1.3-7.8)</td>
<td>0.010</td>
</tr>
<tr>
<td>Model B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease stage at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>101</td>
<td>1.00*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B+C</td>
<td>34</td>
<td>3.4 (2.0-5.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ZAP-70 status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>70</td>
<td>1.00*</td>
<td>0.006</td>
</tr>
<tr>
<td>Pos</td>
<td>65</td>
<td>2.1 (1.2-3.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>T393C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T+</td>
<td>93</td>
<td>1.00*</td>
<td>0.013</td>
</tr>
<tr>
<td>T–</td>
<td>42</td>
<td>1.9 (1.1-3.3)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

NOTE: Nonsignificant variables (age, gender, and CD38 status) were stepwise removed from the model. The cutoff levels used in the analysis were as follows: age, 60 years; ZAP-70 or CD38 status, 20% positive cells. Model B consists of dichotomous variables.

*Reference.

Consistent with previous studies, the combination of Binet stage and ZAP-70 status was associated with outcome in this study. The number of risk factors present (P < 0.0001) with median treatment-free intervals of >303, 116, 21, and 21 months, respectively. Pairwise comparisons between risk groups revealed statistically significant differences (P < 0.001) between all groups except of comparison of groups I and II (P = 0.177) and III and IV (P = 0.155; Fig. 2A). Thus, a HR of 4.7 (95% CI, 2.8-7.9; P < 0.0001) could be calculated for risk groups III and IV versus I and II. We subsequently defined these above-mentioned risk strata exclusively for those patients in stage A according to Binet, because this group may, in the future, benefit from more specific and individually tailored treatment decisions (n = 101). Figure 2B clearly shows that this risk stratification provides significantly more information.
about progression-free survival in Binet A patients ($P = 0.0014$). Median treatment-free intervals were >303, 303, and 31 months, respectively, and pairwise comparisons revealed the biggest difference between $T^-$ and ZAP-70–positive patients versus $T^+$ and ZAP-70–negative patients (HR, 4.4; 95% CI, 2.8-27.8; $P = 0.0001$) followed by $T^-$ and ZAP-70–positive versus $T^-$ or ZAP-70–positive patients (HR, 2.8; 95% CI, 1.5-10.4; $P = 0.006$).

**T393C polymorphism and overall survival.** Twenty-two of the 144 patients had died during the total follow-up time. The estimated median survival time of the entire group was 310 months (95% CI, 165-454). The estimated median survival times from the date of first diagnosis for 393T– allele carriers were 310 and 197 months for 393T– genotypes (Fig. 3A), resulting in a HR for 393T– genotypes of 4.8 (95% CI, 2.9-20.2; $P < 0.0001$) for death. Multivariable Cox regression analysis revealed that besides ZAP-70 status (HR, 3.5; 95% CI, 1.1-10.4; $P = 0.029$), the 393T– genotype was the only independent risk factor for death (HR, 5.6; 95% CI, 1.9-16.4; $P = 0.002$), whereas Binet stage was removed from the model eventually due to the limited number of patients who had died during follow-up. When we applied the aforementioned hypothetical risk groups to calculate survival (see above), we observed significant differences dependent on the presence of these respective risk factors (Fig. 3B; $P = 0.0003$). The estimated median survival times for the presence of risk factors were as follows: no risk factor, >330 months; any one risk factor, 310 months; any two risk factors, 146 months; any three risk factors, 92 months, respectively. Pairwise comparisons between risk groups revealed significant differences between all groups except of comparisons of group I and II ($P = 0.219$) and III and IV ($P = 0.256$; Fig. 2A). A HR for death of 6.3 (95% CI, 2.2-17.8; $P < 0.01$) could be calculated for risk groups III and IV versus I and II. This model, therefore, suggests that patients with any two risks factors (CC genotype, ZAP-70 positive, and Binet B or C) have a hazard of dying that is 6.3 times higher compared with that of patients with only one risk factor.

**Discussion**

The last decade has shown the gradual transition from the use of single factors as prognostic markers in patients with B-CLL, such as Binet stage, to the application of molecular and genetic markers. These markers will eventually enhance our ability to stratify patients into more refined risk categories, ultimately better predicting the clinical course and survival of the individual patient, especially in Binet stage A (2). A variety of markers has been examined and some have shown enough promise to justify their future application as prognostic tools in clinical practice (5–10, 28). However, quantification of the expression of biomarkers that is done by flow cytometry requires stringent standardization of flow cytometry protocols (29, 30). Moreover, the predictive cutoff levels for, e.g., CD38 in terms of risk stratification remains a matter of debate. Although some groups favor a threshold of 20% CD38-positive cells (31, 32), others use 30% or 7% (7, 28, 33). From our own experience, we have obtained reproducible results with a threshold of 20% (19) and, therefore, preferred to use this value for the present study.

Genetic host factors can influence the risk of developing B-CLL but also the natural course of this disorder (11, 34). The aim of the present study was, therefore, to investigate whether a genetic host factor, the common T393C polymorphism in the gene GNAS1, may predict treatment-free and overall survival in patients with B-CLL. In addition, we wanted to accumulate further evidence that genotypes of the T393C polymorphism are not only associated with the natural course of solid tumors (12–14), but may represent more general markers with the capacity to predict the clinical course in hematologic malignancies, too.

The results of this study clearly indicate that treatment-free and overall survival was significantly increased in TT genotypes compared with TC or CC genotypes. After having confirmed the prognostic value of the ZAP-70 status (and its interaction with CD38), as well as the stage of disease at time of diagnosis in our cohort, we used a multivariable analysis to investigate whether the T393C polymorphism is an independent prognostic factor for clinical outcome. This is of importance because in our study, CC genotypes tended to be more likely ZAP-70 and CD38 positive (Table 1). We could gather evidence that the T393C polymorphism is indeed an independent prognostic
factor without an interaction with ZAP-70 or CD38 status. CC genotypes showed a 4-fold increased risk to require first therapy compared with TT genotypes (Table 2). This study is, therefore, in line with previous studies showing a prolonged progression-free survival for TT genotypes in patients with different solid tumors (12–14). Moreover, combination with established molecular (ZAP-70) and clinical (Binet stage) risk factors showed that inclusion of the T393C polymorphism provides additional prognostic information concerning treatment-free and overall survival with a gradual worsening of clinical outcome associated with alterations in one or more of these factors. Most importantly, refined risk stratification of CLL patients in stage A according to Binet may, in the future, guide better individualized treatment decisions (Fig. 2B).

The GNAS1 T393C polymorphism is located in exon 5 within a recombination hotspot that is in linkage equilibrium with two haplotype blocks, one located 5′ and one 3′ of the T393C polymorphism (13, 35). Moreover, although synonymous, the T>C substitution at position 393 could be calculated with two haplotype blocks, one located 5′ within a recombination hotspot that is in linkage equilibrium with the other block. The T393C polymorphism shows that genetic-dependent differences in mRNA decay due to change mRNA folding (13). We have, therefore, proposed that genotype-dependent differences in mRNA decay due to altered secondary structure could finally cause differences in Gas mRNA expression, which was confirmed in different tissues (12). In vitro experiments suggest that increased expression of Gas is associated with enhanced apoptosis (16–18). The second messenger cyclic AMP (cAMP), which is generated following activation of Gas, seems to play a major role in this proapoptotic process. An increased concentration of cAMP promotes apoptosis in several cell types, including leukemic cells (15), ovarian cancer cells (36), and lymphoma cells (37).

Because alterations in apoptosis and cell cycle regulation have been described in CLL, interference with signal transduction mediated by the Gas pathway could be involved in this antiapoptotic process.

With regard to the design and application of apoptosis-inducing drugs (38–40), it would be of particular interest to investigate whether patients with specific T393C genotypes would especially profit from such a therapy. This could help to early start therapy in diagnostic groups with a poor prognosis (2).

Both the results of the present study and previous findings (12–14) strongly suggest a significant role of the T393C polymorphism in cancer progression. Nevertheless, it has to be emphasized that, in the present study, a limited number of patients was investigated. Therefore, although our findings again support the concept of a role of GNAS1 genotypes in tumor progression, further independent replication studies are necessary to confirm the general validity of our findings and deduced hypotheses.

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References


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