The mutation status of genes encoding the variable region of immunoglobulin heavy chains (IgV\textsubscript{H}) is a strong predictor of disease progression and survival in B-cell chronic lymphocytic leukemia (B-CLL). We investigated whether there is an association between the concentration of both vascular endothelial growth factor and basic fibroblast growth factor and IgV\textsubscript{H} mutation status in 49 untreated B-CLL patients.

Forty-nine patients with never-treated B-CLL, diagnosed according to NCI-WG criteria, and a control group of fifty age-matched healthy blood donors were enrolled. IgV\textsubscript{H} mutation status was determined as described in detail elsewhere.\textsuperscript{11} IgV\textsubscript{H} sequences were aligned to the nearest germline using the Ig BLAST program; IgV\textsubscript{H} genes with less than 98% sequence homology to the corresponding germline were considered mutated. We quantified bFGF and VEGF in EDTA plasma samples (stored at -70°C until the time of analysis) using sandwich ELISA kits (Human bFGF and VEGF Quantikine\textsuperscript{®} Kit, R&D Systems, Minneapolis, MN, USA) according to manufacturer’s instructions. Software Analyse-It (Analyse-It Software Ltd., UK) was used for the statistical analyses. The non-parametric Mann-Whitney U test was used to compare differences between subgroups. The study was conducted according to Helsinki Declaration, approved by local ethics committee and study participants signed a written informed consent form. Twenty-six patients had mutated IgV\textsubscript{H} genes and 23 unmutated ones. The male:female ratio was 12.14 in IgV\textsubscript{H}-mutated subgroup and 16.7 in the unmutated subgroup. The median of the IgV\textsubscript{H}-mutated and unmutated patients was 58.2 and 62.3 years, respectively (95% CI [confidence interval], 57.5-67.1 and 57.8-62.6 years, respectively). According to modified Rai staging, 20, 5 and 1 IgV\textsubscript{H}-mutated patients and 8, 12, and 3 IgV\textsubscript{H}-unmutated patients had low, intermediate and high risk B-CLL, respectively. The concentrations of both VEGF and bFGF in peripheral blood plasma were significantly higher in B-CLL patients than in the control patients (p<0.0001 for both cytokines). The concentration of bFGF was significantly higher in both IgV\textsubscript{H}-mutated subgroups than in controls (p<0.0001) while VEGF was significantly increased only in IgV\textsubscript{H}-mutated patients (p=0.0002); the difference between concentrations in controls and IgV\textsubscript{H}-unmutated patients not being significant (p=0.0788, Table 1). Interestingly, the plasma levels of bFGF were significantly higher in the IgV\textsubscript{H}-mutated group than in the IgV\textsubscript{H}-unmutated subgroup (p=0.0149, Figure 1). On the other hand, VEGF concentrations in the mutated and unmutated subgroups were not significantly different (p=0.146). There was also no difference in VEGF or bFGF between patients with modified Rai low vs. intermediate vs. high risk disease. Likewise, no differ-

### Table 1. Descriptive statistics of bFGF and VEGF levels in B-CLL patients and controls; results of Mann-Whitney tests.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI of Mean</th>
<th>Mann-Whitney test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>bFGF Mutated</td>
<td>26</td>
<td>175.2</td>
<td>212.7</td>
<td>165.2</td>
<td>146.0-279.4</td>
<td>bFGF mutated vs. controls</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>bFGF Unmutated</td>
<td>23</td>
<td>46.3</td>
<td>91.7</td>
<td>98.0</td>
<td>49.3-134.0</td>
<td>bFGF unmutated vs. controls</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>bFGF Controls</td>
<td>50</td>
<td>8.9</td>
<td>11.0</td>
<td>10.3</td>
<td>8.1-13.9</td>
<td>bFGF mutated vs. unmutated</td>
<td>0.0149</td>
</tr>
<tr>
<td>VEGF Mutated</td>
<td>26</td>
<td>104.9</td>
<td>141.7</td>
<td>90.9</td>
<td>105.0-178.4</td>
<td>VEGF mutated vs. controls</td>
<td>0.0002</td>
</tr>
<tr>
<td>VEGF Unmutated</td>
<td>23</td>
<td>80.3</td>
<td>134.4</td>
<td>156.8</td>
<td>66.6-202.2</td>
<td>VEGF unmutated vs. controls</td>
<td>0.0788</td>
</tr>
<tr>
<td>VEGF Controls</td>
<td>50</td>
<td>49.0</td>
<td>68.4</td>
<td>63.6</td>
<td>50.4-86.5</td>
<td>VEGF mutated vs. unmutated</td>
<td>0.146</td>
</tr>
</tbody>
</table>

SD: standard deviation; CI: confidence interval. Concentrations are in pg/mL.
ence in either cytokine was seen between patients divided according to results of fluorescent in situ hybridization studies into a group with favorable cytogenetics (i.e. no abnormality or del 13q, n=30) and one with unfavorable cytogenetics (any other aberrations including del17p, 11q and +12, n=19) [data not shown].

As expected, plasma levels of bFGF and VEGF were significantly higher in the patients with B-CLL than in the controls; this is in agreement with previously published data. Suprisingly, however, the concentration of bFGF was significantly elevated in patients with a favorable prognosis with mutated IgV(H) genes while VEGF was raised in both mutated and unmutated cases. Plasma/serum concentrations of bFGF in CLL are by far highest of all hematologic malignancies and increased bFGF in peripheral blood has been associated with an unfavorable disease course because of its correlation with advanced clinical stage, increased survival of B-CLL cells and enhanced resistance to fludarabine. We hypothesize that our conflicting results could be caused by preferred usage of bFGF signaling by CLL cells in patients with IgV(H) mutations patients due to different gene expression profiles. We cannot exclude bias caused by the relatively small number of samples and patient selection; on the other hand, some of the abovementioned studies investigating the role of bFGF in B-CLL also investigated limited numbers of patients’ samples (3 in Koenig’s article, 36 in Menzel’s study); so all these results should be interpreted with caution. In conclusion, our data suggest a possible association between elevated bFGF and mutated IgV(H) genes in B-CLL. Further investigation of the exact role of bFGF and VEGF signaling in B-CLL in larger series of patients, in particular with respect to modern prognostic factors, is clearly warranted.

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