Microsatellite DNA Alterations of Gastrointestinal Stromal Tumors Are Predictive for Outcome

Paulus Schurr,1 Stefan Wolter,1 Jussuf Kaifi,1 Uta Reichelt,2 Helge Kleinhans,1 Robin Wachowiak,1 Emre Yekebas,1 Tim Strate,1 Viacheslav Kalinin,1 Ronald Simon,2 Guido Sauter,2 Hansjoerg Schaefer,2 and Jakob Izbicki1

Abstract

Purpose: In gastrointestinal stromal tumors (GIST), loss of heterozygosity (LOH) on chromosome 22 and its presumptive biological function has been described. The prognostic value of these and other DNA regions for patient survival remains unclear.

Experimental Design: Sixty patients who underwent surgery at our institution between 1992 and 2003 for GIST were histopathologically reclassified by immunohistochemistry and the GIST consensus group criteria 2001. Twenty-one microsatellite loci on chromosomes 3, 9, 13, 17, 18, and 22 were screened for alterations in tumor and healthy DNA. Survival was calculated by Kaplan-Meier plots.

Results: Eleven (18.3%) of 60 patients showed metastases at presentation. Thirteen (21.7%) of 60 were high-risk GISTs. LOH was found in all tumors. Twenty-eight (46.7%) of 60 showed more than two LOH in 21 microsatellite marker sites. The frequency of single marker LOH varied from 1.7% to 28.3% among tumors. Frequent LOH was found on chromosomes 22 and 17. The correlation of LOH positivity and the consensus scoring was significant ($P = 0.005$, $\chi^2$ test). After a median observation time of 33.3 months (95% confidence interval, 23.9-42.6), overall survival was best for patients with tumors of very low, low, and intermediate risks with only 6 of 36 death events, whereas 14 of 24 high-risk and metastasized patients had died ($P < 0.001$, log-rank test). Likewise, LOH significantly predicted survival ($P = 0.013$) and the effect was particularly detrimental for LOH on chromosome 17 ($P < 0.001$).

Conclusions: LOH is a useful phenomenon for the prognosis of GIST. Rather than chromosome 22 markers, chromosome 17 markers independently predict survival.

Gastrointestinal stromal tumors (GIST) are genetically unstable (1–4). Generally, instability exists at two distinct levels, at the nucleotide level, which results in base substitutions, deletions, and insertions of a few nucleotides, and at the chromosomal level, resulting in loss or gain of whole chromosomes or large portions thereof (5). In some tumors, both types of alterations may coexist. It is now widely accepted that, in GISTs, DNA alterations play a major role in pathogenesis and disease progression (6). Recently, molecular resistance mechanisms of GIST toward imatinib (Gleevec, Novartis, Basel, Switzerland) therapy are being described. Point mutations in the KIT or PDGFRA gene play a major role in tumor growth and imatinib resistance (7).

Chromosomal losses also play an important role in GIST (6). In particular, losses of fragments of chromosome 22 have been linked to aggressive behavior of GIST (8). From studies on other tumor types, it is also known that loss of heterozygosity (LOH) at other chromosomes often has clinical importance. This particularly applies for chromosomes 3, 9, 13, 17, and 18 perhaps because of the location at several key regulators of the cell cycle (p16, p53, RB, and DPC4; refs. 9–11).

To study the prognostic importance of chromosomal imbalances in these LOH sites in GIST, we analyzed 60 well-characterized GISTs for LOH at chromosomes 3, 9, 13, 17, 18, and 22. Our data pinpoint toward a significant and independent prognostic role of 17p13 losses in GIST. This effect is commonly attributed in the literature to p53, which is located on 17p13 (9, 12). Recently, in GISTs, a variable p53 expression could be shown by immunohistochemistry and was independently prognostic for survival (13). LOH analyses are easily done in clinical routine and can be done from the blood as a tumor marker (12).

Materials and Methods

This study was approved by the Ethics Committee of the Chamber of Physicians in Hamburg, Germany. Written informed consent was...
obtained from all patients for use of the resected samples. For this study, 60 patients who were surgically treated at our institution between 1992 and 2004 were chosen retrospectively.

All patients were recruited into the study after reclassification, which, besides histologic criteria, included CD117 (c-kit; rabbit polyclonal; DAKO, Glostrup, Denmark), CD34 (mouse IgG1; Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom), muscle actin (mouse IgG1; clone HHF35; Enzo Diagnostics, Inc., Farmingdale, NY), desmin (clone DE-R-11; DAKO), and S-100 protein (polyclonal; DAKO) immunostaining of paraffin sections. The proliferative index was determined with Ki-67 (MIB-1; IgG1; DAKO). In case of surgery before the year 2000, the diagnoses included various terminologies, such as schwannoma, sarcoma, leiomyoma, leiomyosarcoma, leiomyoblastoma, and malignant peripheral nerve sheath tumor. Moreover, all GISTs were scored according to the consensus group criteria from 2001 and classified as very low, low, intermediate, and high risks (14). In addition to this scoring system, metastatic tumors at presentation were classified as overtly malignant tumors and allocated to a fifth group.

DNA was extracted from snap-frozen tumor tissue samples at −70°C using a standard extraction protocol (Qiagen, Hilden, Germany). Before extraction, seven consecutive slides (two flanking slides of 7 μm and five core slides of 15 μm) were cryosectioned and stained with H&E and the pathologist marked the tumor in the flanking first and last slides so that, by excision of the marked area in the core slides, at least 90% of the tissue contained tumor. Microsatellite analysis. DNA (1 μg) was subjected to PCR using AmpliTaq Gold polymerase. Primer sequences and locations were obtained from the Genome Database (National Center for Biotechnology Information). They were ordered as fluorescent dye-labeled primers from MWG (5¶-labeled polynucleotides containing fluorescein/carboxyfluorescein, rhodamine/6-carboxytetramethylrhodamine, and HEX; Ebersberg, Germany). DNA was subjected to 35 cycles at a denaturing temperature of 95°C for 1 minute followed by various annealing temperatures (52-60°C) for 1 minute depending on the primer sequence and followed by an extension step at 72°C for 5 minutes using a thermocycler by Biometra (Göttingen, Germany). Amplification

Table 1. Sequence of exons 9 and 11, respective 5¶ and 3¶ primers, and the protein sequence

<table>
<thead>
<tr>
<th>Exon 9:</th>
<th>C S A S V L P</th>
</tr>
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<tbody>
<tr>
<td>tcctagagtaagccagggctttttgtttttcctctttctagttctctgcttcttgtactgc</td>
<td></td>
</tr>
<tr>
<td>aggatctcattcggtcccgaaaaaacaaaaagaggaattacaacccaggtatatata</td>
<td></td>
</tr>
<tr>
<td>taccacctctctgtaagacgggataaatgtaaccgtaaatttccattgtgttccatata</td>
<td></td>
</tr>
<tr>
<td>ttcttttttaaatcaggttaagggagttttagctctgtctcaaccagagagatggt</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>ccagaggtctctaatgactgagacaataaatatttatattataaaggtgatctatatttttcctcttcgggttccagagattactgtccgtttattaataaattttccactagataaaagggtaagkgpmyevqwwkvveeingnn</td>
</tr>
<tr>
<td>cccccacagaaaccctgtatgagtaagcaggtgaaaagttggtgtagagagataaatgaaacaaggggtgtctttgggtacataacttcgtacacgctccccaacaactctcctatattaccttttgg</td>
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</tr>
<tr>
<td>attatgtttaacatagcccaacacactctctttatgatcaaaatggagtttccccagaaataataaatagtctatctggttggttgtagaggaataactagtgtggttttaccctcaagagtcttt</td>
<td></td>
</tr>
<tr>
<td>R L S F acagggctagtttttgtaagctaatccagaggtggcttggcagccactccaaaccaggtctacaatttttgctcccgaa</td>
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Table 2. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Very low, low, and intermediate risk, n (%)</th>
<th>High risk and overtly malignant, n (%)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>36 (60)</td>
<td>24 (40)</td>
<td>60</td>
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</tr>
<tr>
<td>Ages &lt;60 years</td>
<td>14 (52)</td>
<td>13 (48)</td>
<td>27</td>
<td>0.30†</td>
</tr>
<tr>
<td>Ages ≥60 years</td>
<td>22 (67)</td>
<td>11 (33)</td>
<td>33</td>
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<td>Male</td>
<td>18 (50)</td>
<td>18 (50)</td>
<td>36</td>
<td>0.06†</td>
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<tr>
<td>Female</td>
<td>18 (75)</td>
<td>6 (25)</td>
<td>24</td>
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<td>Resection quality</td>
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<td></td>
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<td>0.003†</td>
</tr>
<tr>
<td>R0</td>
<td>36 (68)</td>
<td>17 (32)</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>22 (67)</td>
<td>11 (33)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>0</td>
<td>5 (100)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
<td>0.164†</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>27 (66)</td>
<td>14 (34)</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Small bowel</td>
<td>8 (62)</td>
<td>5 (38)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>0</td>
<td>3 (100)</td>
<td>3</td>
<td></td>
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<tr>
<td>Other</td>
<td>0</td>
<td>1 (100)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td></td>
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<td>0.237†</td>
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<tr>
<td>Wild-type</td>
<td>4 (22)</td>
<td>14 (78)</td>
<td>18</td>
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<tr>
<td>Mutation</td>
<td>5 (46)</td>
<td>6 (54)</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Exon 11 mutations were found in 11 of 29 subjects. No exon 9 mutation was observed.
†Risk groups according to the GIST consensus criteria 2001.
*Fisher’s exact test.
†Χ² test.

Table 3. Cross-table of risk groups according to the GIST consensus criteria 2001

<table>
<thead>
<tr>
<th>Microsatellites</th>
<th>Associated oncogene</th>
<th>Very low, low, and intermediate risk, n (%)</th>
<th>High risk and overtly malignant, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>36 (60.0)</td>
<td>24 (40.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Chromosomes 17, 9, 13, 3, 18, and 22</td>
<td>p53, p16, RB, DPC4, NF, other</td>
<td>26 (43.3)</td>
<td>10 (6.7)</td>
<td>0.011</td>
</tr>
<tr>
<td>0-2 LOH</td>
<td></td>
<td>33 (55.0)</td>
<td>18 (30.0)</td>
<td>0.030</td>
</tr>
<tr>
<td>3-11 LOH</td>
<td></td>
<td>36 (60.0)</td>
<td>18 (30.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>Chromosome 17 (n = 3)</td>
<td>p53</td>
<td>36 (60.0)</td>
<td>18 (30.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>0-1 LOH</td>
<td></td>
<td>26 (43.3)</td>
<td>10 (6.7)</td>
<td>0.011</td>
</tr>
<tr>
<td>2-3 LOH</td>
<td></td>
<td>10 (16.7)</td>
<td>18 (30.0)</td>
<td>0.030</td>
</tr>
<tr>
<td>Chromosome 9 (n = 3)</td>
<td>p16</td>
<td>36 (60.0)</td>
<td>21 (35.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>0-1 LOH</td>
<td></td>
<td>36 (60.0)</td>
<td>18 (30.0)</td>
<td>0.030</td>
</tr>
<tr>
<td>2-3 LOH</td>
<td></td>
<td>10 (16.7)</td>
<td>6 (10.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>Chromosome 13 (n = 3)</td>
<td>RB</td>
<td>33 (55.0)</td>
<td>18 (30.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>0-1 LOH</td>
<td></td>
<td>33 (55.0)</td>
<td>18 (30.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>2-3 LOH</td>
<td></td>
<td>3 (5.0)</td>
<td>6 (10.0)</td>
<td>0.077</td>
</tr>
<tr>
<td>Chromosome 3 (n = 2)</td>
<td>DPC4</td>
<td>29 (48.3)</td>
<td>16 (26.7)</td>
<td>0.224</td>
</tr>
<tr>
<td>0 LOH</td>
<td></td>
<td>5 (8.3)</td>
<td>8 (13.3)</td>
<td>0.383</td>
</tr>
<tr>
<td>1-2 LOH</td>
<td></td>
<td>7 (11.7)</td>
<td>8 (13.3)</td>
<td>0.383</td>
</tr>
<tr>
<td>Chromosome 18 (n = 1)</td>
<td>Unknown</td>
<td>29 (48.3)</td>
<td>17 (28.3)</td>
<td>0.383</td>
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<tr>
<td>0 LOH</td>
<td></td>
<td>5 (8.3)</td>
<td>8 (13.3)</td>
<td>0.383</td>
</tr>
<tr>
<td>1 LOH</td>
<td></td>
<td>7 (11.7)</td>
<td>8 (13.3)</td>
<td>0.383</td>
</tr>
<tr>
<td>Chromosome 22 (n = 9)</td>
<td>NF, unknown</td>
<td>30 (50.0)</td>
<td>10 (16.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>0-2</td>
<td></td>
<td>6 (10.0)</td>
<td>14 (23.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Twenty-one microsatellite markers (D17S520, D17S796, D17S804, D9S162, D9S171, D9S1748, RB12, D13S284, D13S262, D3S1038, D3S1286, D18S51, D22S683, D22S685, D22S446, D22S420, D22S425, D22S445, D22S689, D22S303, and D22S311).
†Fisher’s exact test.
products were analyzed using the ABI Prism Genetic Analyzer 310c (Applied Biosystems, Darmstadt, Germany). Data were processed using GeneScan software.

**Mutation analysis.** For KIT exon 9 and 11 mutation analysis, PCR was done and DNA sequences and respective primers were used as reported previously (15, 16). Sequencing reactions of exons 9 and 11 were done by MWG. The respective forward and reverse primers and the sequences of the amplified fragments are depicted on Table 1.

**LOH analysis.** PCR was repeated at least thrice in case of positive results and twice in case of negative testing. Normal DNA from the patient served as a negative control. When homozygous in a locus, a patient was defined to be noninformative in that site. Different chromosomes bearing the microsatellite markers D17S520, D17S796, D17S804, D9S162, D9S171, D9S1748, RB12, D13S284, D13S262, D3S1038, D3S1286, D18S51, D22S683, D22S685, D22S446, D22S420, D22S425, D22S445, D22S689, D22S303, and D22S311 were analyzed. Nine primers on chromosome 22 were derived from a study on 42 GISTs, which linked the pathogenesis of GIST to the neurofibromatosis gene NF2 (17). The other primers were proven to efficiently indicate LOH in relevant regions in different carcinoma and sarcoma studies (9, 12, 18). These included microsatellite regions on chromosomes 3, 9, 13, 17, and 18. The respective oncogenes and tumor suppressor genes were p53, p16, DPC4, RB, and VHL:

Tumor samples and healthy tissue from every patient were analyzed and compared by gel electrophoresis. A total of 21 microsatellite primers was tested. Among these, sensitivity and specificity were calculated as described (17). LOH was defined as loss of allelic balance as described before (9). Briefly, a reduction in the intensity of one allele in the target sample of >50% was considered to represent LOH and the presence of new shifted alleles (appearance of new bands) indicated microsatellite instability. Alterations were confirmed by reamplification.

All data, including sex, age, histopathologic data, such as size, lymph node metastases, tumor type, and stage, were obtained from the clinical and pathologic records. Clinical follow-up was effected by studying the clinical charts of the patients and by contacting them on an outpatient basis or by phone call. Patients were informed about the ongoing study, and all patients recruited into the study gave informed consent.

**Statistical analysis.** Disease-specific, overall, and disease-free survivals were assessed separately. All types of survival were calculated with the Kaplan-Meier method with 95% confidence intervals. The log-rank test allowed for comparison of the curves. Cox regression analysis allowed for multivariate analysis of survival risk factors. Candidate variables, such as gender, age, tumor location, tumor size, depth, infiltration, resection technique, number of operations, and the Fletcher score, were explored using χ² tests and the Fisher’s exact test. Two-tailed tests were considered significant at a P < 0.05. Analysis was done using

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**Fig. 1.** Overall (A) and disease-free (B) survivals in very low, low-risk, and intermediate-risk GISTs (n = 36; top curve) versus high-risk and overtly malignant GISTs (n = 24).

**Fig. 2.** Overall (A) and disease-free (B) survivals in patients with zero to two LOH (n = 32; top curve) and three or more LOH (n = 28) in their primary tumors.
the Statistical Package for the Social Sciences software version 11.0 (SPSS, Inc., Chicago, IL).

Results

Patient characteristics are depicted in Table 2. Eleven (18.3%) of 60 patients showed metastases at presentation and classified as overtly malignant. The consensus classification 2001 (19) revealed that 13 (21.7%) of 60 were high-risk GISTs. No correlation was observed between the consensus criteria 2001 and sex, age, or the primary tumor site. All patients with GISTs of low, intermediate, and high risks were R0 resected. Imatinib therapy was given to five patients who relapsed after October 2001. Out of these patients, two of five were of intermediate-risk GISTs, whereas three of five were high-risk GISTs according to the consensus criteria 2001.

LOH was found in all tumors (Table 3). LOH was detected mostly simultaneously at different microsatellite marker sites. Twenty-eight (46.7%) of 60 showed more than two LOH in 21 microsatellite marker sites. The frequency of LOH in one single microsatellite marker varied from 1.7% to 28.3% among all tumors. Frequent LOH was found on chromosomes 22 and 17. The correlation of LOH positivity and the histopathologic scoring was significant ($P < 0.001$, $\chi^2$ test). As shown in Table 3, groups of at least two microsatellite markers were intentionally targeted at a respective locus (e.g., D9S162, D9S1748, and D9S171 on 9q), except for chromosome 18 where a single marker was used, and allowed for detection of the targeted oncogene. For specificity and sensitivity reasons, we arbitrarily defined that two or more LOH should be considered positive whereas zero to one LOH was regarded as negative. Noninformative status was encountered inconsistently, the percentages of which are found in the Genome Database of the National Center for Biotechnology Information for each locus, and meant that the normal tissue of the patient was displaying homozygosity, thus not allowing for LOH detection at that site, or that PCR was not successful at more than three repetitions. Allelic shifting as in microsatellite instability was detected only sporadically and did not allow for statistical analysis (data not shown). Exon 11 KIT mutations occurred more frequently in high-risk and overtly malignant GISTs, although not statistically significant. Sequence alterations were encountered in KIT exon 11 in 11 of 29 samples as point mutations in 4 patients (W557G, V560D, and L576P) and deletions in 7 patients (Del555-560, 570-578, 554-558, and 557-558). No mutations were found in KIT exon 9 among the 29 patients studied (Table 2).

After a median observation time of 33.3 months (95% confidence interval, 23.9-42.6), overall and disease-free survivals were best for patients with tumors of very low, low, and intermediate risks with only 6 of 36 death events, whereas 14 of 24 high-risk and metastasized patients had died ($P = 0.005$ and $P < 0.001$, log-rank test; Fig. 1A and B). Likewise, LOH significantly predicted overall and disease-free survivals ($P = 0.013$ and 0.003, respectively; Fig. 2A and B) and the effect was detrimental particularly at presence of LOH on chromosome 17 ($P < 0.001$; Fig. 3A and B). The effect of LOH in chromosome 22 for prognosis was not significant on the 5% level ($P = 0.11$, log-rank test; curve not shown).

Because of the unequal distribution of patients among risk groups and the consecutive statistical problems, we allocated patients with tumors of very low, low, and intermediate risks (according to the consensus criteria 2001) into one group and patients with tumors of high risk and with overtly malignant tumors into a second group (Fig. 1). This seemed legitimate, as at explorative data analysis, we observed a detrimental effect on prognosis particularly for tumors of the second group. As a general approach, we analyzed overall, disease-specific, and disease-free survivals, but for definitive evaluation, we used overall and disease-free survivals.

Secondly, we assessed the frequency of LOH in individual marker sites (Table 4). We calculated LOH risk by a score summarizing all LOH in an individual patient.

Because of the variable LOH pattern among different tumors, we arbitrarily grouped patients into those that displayed two or less LOH versus patients with more than two LOH and thus obtained approximately equal groups of patients (32 versus 28, respectively). Survival analysis revealed that a LOH score $\geq 2$ could significantly predict overall and disease-free survivals (Fig. 2). Further analyses revealed that survival was not correlated with isolated onset of LOH in the chromosome 22.
region but very significantly with isolated LOH in the chromosome 17p12-13 region (Fig. 3). No such independent prognostic value was found for the other loci on the chromosomes 3, 9, 13, and 18. This applied to all types of survival, including overall, disease-specific, and disease-free survivals (data not shown).

To estimate the independent prognostic value of sex, age, LOH, and the consensus risk criteria, we did Cox regression analysis. We found that LOH on chromosome 17 (three markers, D17S520, D17S796, and D17S804) could independently predict overall survival ($P = 0.029$; Table 5), whereas other marker sites did not ($P = 0.266$).

**Discussion**

LOH has been implicated in prognosis of several malignancies and has gained a strong effect in clinical settings, such as adjuvant therapy in colon cancer (20). In this study, 21 LOH markers were analyzed in 60 GISTs. A strong association between these LOH and the degree of malignancy (2001 consensus criteria) was observed. This was expected, as several previous studies have shown associations between DNA alterations and GIST malignancy (2, 6, 17, 21, 22). As long-term follow-up data were available from our patients, the prognostic role of LOH could also be evaluated in this study.

First, we found an effect for LOH on prognosis in GIST and have made an attempt to define risk groups among GIST patients according to the number of LOH observed in an individual patient. These analyses showed that the presence of more than two LOH sites was linked to poor prognosis. This was not unexpected, as a high number of LOH is a hallmark of high regenerative instability.

The analysis of the role of individual markers confirmed high frequency and association with malignancy of LOH on chromosome 22 as suggested by Pylkkanen et al. (2, 17). As we know from previous studies, NF2, which lies on chromosome 22, plays a major role in the pathogenesis of GIST. However, as to be expected for an early alteration in disease development, LOH at chromosome 22 failed to be decisively linked to survival.

| Table 4. Incidence of positive microsatellite markers (Cont’d) |
|-----------------|-----------------|-----------------|
|  | n (%) | Valid % |
| D17S520 | Negative | 36 (60.0) | 81.8 |
|  | Positive | 8 (13.3) | 18.2 |
|  | NA* | 16 (26.7) |
| D17S796 | Negative | 33 (55.0) | 84.6 |
|  | Positive | 6 (10.0) | 15.4 |
|  | NA | 21 (35.0) |
| D17S804 | Negative | 29 (48.3) | 82.9 |
|  | Positive | 6 (10.0) | 17.1 |
|  | NA | 25 (41.7) |
| D9S162 | Negative | 31 (51.7) | 81.6 |
|  | Positive | 7 (11.7) | 18.4 |
|  | NA | 22 (36.7) |
| D9S171 | Negative | 35 (58.3) | 85.4 |
|  | Positive | 6 (10.0) | 14.6 |
|  | NA | 19 (31.7) |
| D9S1748 | Negative | 34 (56.7) | 85.0 |
|  | Positive | 6 (10.0) | 15.0 |
|  | NA | 20 (33.3) |
| RB12  | Negative | 26 (43.3) | 96.3 |
|  | Positive | 1 (1.7) | 3.7 |
|  | NA | 33 (55.0) |
| D13S284 | Negative | 21 (35.0) | 100.0 |
|  | NA | 39 (65.0) |
| D13S262 | Negative | 33 (55.0) | 80.5 |
|  | Positive | 8 (13.3) | 19.5 |
|  | NA | 19 (31.7) |
| D3S1038 | Negative | 38 (63.3) | 82.6 |
|  | Positive | 8 (13.3) | 17.4 |
|  | NA | 14 (23.3) |
| D3S1286 | Negative | 30 (50.0) | 78.9 |
|  | Positive | 8 (13.3) | 21.1 |
|  | NA | 22 (36.7) |

*Not available (e.g., homozygote locus).
Unexpectedly, we found the strongest effect on overall and disease-free survivals for LOH on chromosome 17. The p53 locus has been implicated in tumor growth in many malignant tumors. Our candidate tumor suppressor gene on 17p is p53. A role of p53 was indeed recently suggested in a study analyzing 105 GISTs by immunohistochemistry (13). The authors found p53 to be differentially expressed in GIST of different sites and suggested an independently prognostic role for p53 immunohistochemistry in gastric GIST. For this study, we carefully classified our patients according to the criteria of the GIST consensus group 2001. This classification has been widely accepted for the risk assessment of resected localized tumors. In our patient group, the consensus criteria well reflect patient outcome. However, some uncertainty about outcome in the consensus criteria 2001 remains. Our data show a significant effect of LOH in multiple microsatellite loci on disease-free, disease-specific, and overall survivals, which displays a high practicability and reproducibility in laboratory practice. LOH analysis might be a valuable additional diagnostic tool for estimating prognosis.

Future studies should include comparative genomic hybridization and single-nucleotide polymorphism to identify novel allelic imbalances in GIST. Moreover, looking out, in contrast to histopathology and immunohistochemistry, LOH analysis can be easily done from the blood (12). Under the assumption that tumors shed DNA into the bloodstream, a therapeutic monitoring of resected and nonresected GIST may seem conceivable.

In summary, this LOH study in a set of 60 GISTs with long-term follow-up data reveals a strong prognostic relevance of chromosomal imbalances in GIST. This applies for the number of LOH but also specifically for LOH at 17p.

### Table 5. Multivariate Cox analysis of risk variables for overall survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative risk (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIST consensus score 2001 (very low risk, low-risk, and intermediate-risk tumors vs high-risk and malignant tumors)</td>
<td>4.237 (1.397-12.851)</td>
<td>0.011</td>
</tr>
<tr>
<td>LOH on chromosome 17p (&gt;2 vs 1 or 0 LOH)</td>
<td>2.140 (1.110-4.124)</td>
<td>0.023</td>
</tr>
<tr>
<td>Sex</td>
<td>1.527 (0.561-4.156)</td>
<td>0.407</td>
</tr>
<tr>
<td>Age (&gt;60 vs ≤60 years)</td>
<td>1.033 (0.991-1.077)</td>
<td>0.126</td>
</tr>
</tbody>
</table>

References