p53 Gene Deletion Predicts for Poor Survival and Non-Response to Therapy With Purine Analogs in Chronic B-Cell Leukemias

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Conventional cytogenetic analysis in B-cell chronic lymphocytic leukemia (B-CLL) has been very difficult, and the prognostic significance of specific chromosome aberrations is under discussion. Recent improvements in fluorescence in situ hybridization (ISH) techniques have provided an alternative approach for the detection of chromosome aberrations. Here, an interphase cytogenetic study was performed to analyze the incidence and prognostic significance of a p53 gene deletion in B-CLL and related disorders. We studied mononuclear cells from 100 patients with chronic B-cell leukemias (B-CLL, 90 patients; B-prolymphocytic leukemia (B-PLL), 7; Waldenström's macroglobulinemia (WM), 3) by fluorescence ISH with a genomic p53 DNA probe. In a subset of patients, additional G-banding analysis and single strand conformation polymorphism (SSCP) analysis was performed. Seventeen of the 100 patients (17%; B-CLL, 11 of 90 (12%); WM, 1 of 3; B-PLL, 5 of 7) exhibited a monoallelic p53 gene deletion by ISH. G-banding analysis demonstrated abnormalities of chromosome 17 in 13 of these 17 patients, all leading to loss of band 17p13. SSCP analysis showed aberrant bands in 9 of 14 patients with a p53 gene deletion. None of 12 patients with a p53 gene deletion compared with 20 of 36 patients (56%) without a deletion responded to therapy with fludarabine or pentostatin (P < .001). The difference in survival probabilities from the time of diagnosis and from the start of treatment with purine analogs between the two groups was highly significant (P < .001). In multivariate analysis, p53 gene deletion was the strongest prognostic factor for survival. In conclusion, p53 gene deletion predicts for non-response to therapy with purine analogs and for poor survival in chronic B-cell leukemias.

B-CELL CHRONIC lymphocytic leukemia (B-CLL) is the most common leukemia in adults. It is characterized by a highly variable clinical course, with some patients living for 20 years or longer, while others die of their disease within a few months. The decision to treat a patient is mainly based on clinical and laboratory features indicating active disease. Over the past few years, great interest in new techniques has been evoked by the development of purine analogs, and many patients achieving long-lasting remissions. In contrast, patients with a normal karyotype or abnormalities of band 13q14 seem to have a favorable outcome.

In most studies of conventional banding analysis in B-CLL and related disorders, loss of the short arm of chromosome 17, to which the p53 tumor suppressor gene localizes, has not been identified as a frequent chromosome aberration. Nevertheless, evidence for a role of p53 in lymphoid malignancies including B-CLL came from a study demonstrating p53 gene mutations in these diseases by single strand conformational polymorphism (SSCP) analysis and by sequencing of the polymerase chain reaction (PCR)-amplified fragments. Gaidano et al found p53 mutations in 6 of 40 (15%) patients with B-CLL. In two subsequent SSCP studies in B-CLL, p53 gene mutations were found at a frequency of 10% and 15%. Point mutations coupled with deletion of the second allele is one of the characteristics of a recessively acting tumor suppressor gene such as p53. Matations can be detected by PCR amplification of the coding region followed by SSCP analysis or by direct sequencing of the PCR products. Allelic loss of the p53 gene has so far most commonly been demonstrated using restriction fragment length polymorphism (RFLP) analysis. Fluorescence in situ hybridization (ISH) techniques using single-copy DNA sequences have provided an alternative approach for the detection of gene deletions. Compared with RFLP analysis, fluorescence ISH allows analysis on the single-cell level and, thus, a quantification of the cells carrying the deletion. We have recently shown that loss of the retinoblastoma tumor suppressor gene in B-CLL can be detected with high sensitivity by fluorescence ISH.

We report on the incidence of p53 gene deletions as assessed by fluorescence ISH with a genomic p53 DNA probe
and on the clinical implication of this aberration in a large cohort of patients with chronic B-cell leukemias including B-CLL, B-prolymphocytic leukemia (B-PLL), and Waldenström's macroglobulinemia (WM).

MATERIALS AND METHODS

Patients. During October 1990 and February 1994, 90 patients with B-CLL, seven patients with B-PLL, and three patients with WM were studied. Sixty-five patients were male and 35 female; the ages ranged from 33 to 83 years (median, 57 years). The diagnosis of B-CLL required a persistent lymphocytosis of greater than 5,000/μL. Immunophenotypic data were available for 87 of 93 patients with B-CLL or WM. All leukemias were CD19+, and 86 of 87 were CD5+; leukemic cells from 1 patient with WM did not express the CD5 antigen. All 7 patients with B-PLL had greater than 55% prolymphocytes in the blood; leukemic cells from the 7 patients strongly expressed surface immunoglobulin and the CD22 antigen.

The disease was staged according to Rai et al.19 at diagnosis and at the time of initiation of treatment with purine analogs. At diagnosis, complete information on the clinical data was available in 93 of 100 patients: 11 patients had Rai stage 0; 17, stage 1; 53, stage 2; 4, stage 3; and 8, stage 4 disease.

The majority of patients presented at our institution before cytogenetic analysis became a routine investigation in B-CLL. Therefore, in many patients the analysis was performed several months or even several years after diagnosis (mean, 44 months; range, 0 to 235 months). No sequential cytogenetic analyses were done. Thirty-five patients were previously untreated, 30 patients had received one, and 35 patients two or more chemotherapeutic regimens before cytogenetic analysis. Forty-three B-CLL patients were treated with fludarabine (25 mg/m2/d for 5 consecutive days, every 4 weeks, for a maximum of 12 cycles); 36 of these patients were refractory, and 7 patients were responsive to the last prior chemotherapy. Refractory disease was defined as failure to achieve a remission of at least 2 months' duration after therapy with alkylating agents. All seven B-PLL patients were entered on a treatment protocol of the European Organization for Research and Treatment of Cancer (EORTC) using pentostatin.20 One patient received pentostatin as initial therapy, while the remaining 6 patients were refractory to prior chemotherapy. Response to treatment was assessed according to the criteria of the National Cancer Institute (NCI)-sponsored Working Group.21

Fluorescence ISH. A chromosome 17-specific cosmid library constructed by D. Nizetic (Imperial Cancer Research Fund [ICRF], London, UK) from digests of DNA from '4X cell line (LCL 127) was screened with the p53 cDNA probe.22 Four positive clones were detected (ICRFc105BO195-75, ICRFc105CO275-77, ICRFc105EO675-78, and ICRFc105AO144-79). In all patients, the four overlapping cosmid clones were used for ISH. Cosmid clone c518 (D21S55) mapping to 2q22.3 (provided by Dr Katherine Klinger, Integrated Genetics, Framingham, MA) served as a positive control for hybridization efficiency. For further characterization of derivative chromosomes involving number 17, the following DNA probes were used: PBS17, a chromosome 17-specific DNA library (provided by Dr Joe Gray, University of California at San Francisco, San Francisco, CA); D8Z1, D17Z1, and D18Z1, recognizing centromeric and pericentromeric sequences of chromosomes 8, 17, and 18, respectively (Oncor Sciences, Gaithersburg, MA); and cosmid clone HO2115, mapping to band 17q11.2.23 The probes were labeled by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany). ISH was performed as described.17,24 To prevent false-positive results caused by inadequate hybridization or chromatin loss, analysis was only performed on slides with high hybridization efficiency, indicated by two c518 signals in more than 90% of the nuclei. Fluorescence signals were enumerated in 150 to 500 interphase cells. Illustrations were produced using a cooled charged coupled device (CCD) camera (KAPPAG, Gleichen, Germany).

G-banding analysis. G-banding analysis was performed as described25 in 51 consecutive patients and another 6 selected patients
who had been shown to have a p53 gene deletion by fluorescence ISH. Between 6 and 30 metaphase spreads (mean, 18) were analyzed per case. Karyotypes were designated according to the International System for Cytogenetic Nomenclature (ISCN 1991).26

SSCP analysis. SSCP analysis was performed in 14 of 17 patients exhibiting a monoallelic p53 deletion by fluorescence ISH. No material was available in the remaining 3 patients. Double-stranded PCR fragments were amplified from approximately 100 ng of genomic DNA using Taq Polymerase (Promega, Heidelberg, Germany), under conditions recommended by the manufacturer, and primers used to amplify specific oligonucleotide primers. Reactions were performed in a thermocycler (Perkin Elmer, Foster City, CA) for 40 cycles, each cycle comprising incubation for 1 minute at 94°C, 1 minute at 58°C, and 90 seconds at 72°C. The primers used to amplify exons 5 through 8 were previously described.27 The sequences of specific oligonucleotides for exon 9 were as follows: F81 (CCT lTC CC), derived from intron 8 and intron 9 sequences, respectively; F91 (TCC ACT TGA TAA GAG GTC GAA ATA TTC TCC), both derived from exon 9 sequences. Exons 5, 6, and 8 were amplified as a single fragment (primer pairs F6, R7). The reaction was performed as previously described.25 The sequences of specific oligonucleotides for exon 9 were as follows: F81 (CCT lTC CC), derived from intron 8 and intron 9 sequences, respectively; F91 (TCC ACT TGA TAA GAG GTC GAA ATA TTC TCC), both derived from exon 9 sequences. Exons 5, 6, and 8 were amplified as a single fragment (primer pairs F6, R7). The reaction was performed as previously described.25

**Table 1. Cytogenetic and SSCP Analysis Data of the 17 Patients With a p53 Deletion**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>p53 Signals by Fluorescence ISH (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>54.1</td>
<td>89.1</td>
<td>91.3</td>
<td>89.8</td>
<td>21.6</td>
<td>79.0</td>
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<td>79.7</td>
<td>54.3</td>
<td>91.1</td>
<td>18.0</td>
<td>59.1</td>
<td>26.4</td>
<td>90.0</td>
<td>64.7</td>
<td></td>
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<tr>
<td>Two</td>
<td>45.9</td>
<td>9.9</td>
<td>6.3</td>
<td>8.2</td>
<td>78.1</td>
<td>11.2</td>
<td>45.8</td>
<td>59.9</td>
<td>13.0</td>
<td>45.1</td>
<td>8.0</td>
<td>80.6</td>
<td>33.6</td>
<td>71.2</td>
<td>36.0</td>
<td>32.8</td>
<td></td>
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<tr>
<td>Aberrant Band by SSCP Analysis (exon)</td>
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<td>ND</td>
<td>8</td>
<td>5/6</td>
<td>5/6</td>
<td>ND</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>6</td>
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<td>8</td>
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</tbody>
</table>

Abbreviations: ND, not done; 5/6, exons 5 and 6 amplified as a single fragment.

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Fig 2. Analysis of the p53 gene by fluorescence ISH. (A) Metaphase spread and one interphase cell of patient no. 8 with B-CLL and a monoallelic p53 gene deletion. Note the two control fluorescent signals (Cy-3) detected via fluorescein isothiocyanate (FITC) and only one p53 signal (detected via 4,6-diamidino-2-phenyl-indole dihydrochloride (DAPI) exhibiting two control signals (Cy-3) and only one p53 signal (Cy-3). (B) Interphase cells of patient no. 7 with B-CLL counterstained with 4,6-diamidino-2 phenyl-indole dihydrochloride (DAPI) exhibiting two control signals (Cy-3) and only one p53 signal (Cy-3). (C) Partial metaphase spread of patient no. 7 with B-CLL and a der(17;18)(q10;10) after colhybridization with chromosome 17-specific library DNA (pBS17; FITC) and p53 (Cy-3). Note one normal chromosome 17 with a p53 signal (arrow) and the derivative chromosome 17 lacking the p53 signal (arrowhead). (D) Metaphase spread of patient no. 7. Note the localization of one chromosome 17 (Cy-3) and one chromosome 18 (FITC) centromere, indicating the dicentric nature of the derivative chromosome (arrow). (E) Metaphase spread of patient no. 8 with B-CLL. Loss of the p53 gene is caused by formation of an i(17q). Note the two HO2155 signals (mapping to band 17q11.2; FITC) on the i(17q) (arrow) and the third HO2155 signal on the normal chromosome 17, and only one p53 signal (Cy-3).
and the Fisher's exact test (binary variables). Univariate logistic regression was used to identify possible relevant prognostic factors for a response to therapy. Survival time, measured from the date of diagnosis and from the date of initiation of therapy with fludarabine or pentostatin, was plotted from life tables using Kaplan-Meier estimates. Differences between two survival curves were analyzed by the log-rank test. The proportional hazards regression model of Cox was used for the multivariate analysis of survival time data, where model selection was performed by a bootstrap resampling procedure. All statistical calculations were performed using the software packages S-Plus (Nath-Soft, Inc, Seattle, WA) and StatXact (CYTEL Software Corp, Cambridge, MA).
RESULTS

Fluorescence ISH. To define the cut-off level for the diagnosis of a p53 gene deletion, hybridization experiments of blood specimens from 5 probands were performed. By analogy to our previous study on the incidence of RB-1 deletion in B-CLL, the cut-off level was defined by the mean + 3 standard deviations (SD) of the frequency of control cells exhibiting only one p53 signal (mean, 2.0%; SD, 0.339; cut-off level).

Figure 1 shows the percentage of nuclei with one p53 hybridization signal of the 5 controls and the patients with B-CLL (n = 90), WM (n = 3), and B-PLL (n = 7). Seventeen of the 100 (17%) patients [B-CLL, 11 of 90 (12%); WM, 1 of 3; B-PLL, 5 of 7] exhibited a p53 gene deletion. The percentage of nuclei with one p53 signal ranged from 19.3% to 93.6% (Table 1). As Fig 1 shows, there was a clear distinction between the groups with and without a p53 gene deletion. Photomicrographs of dual-color hybridizations are shown in Fig 2.

G-banding analysis. Fifty-seven patients, including all 17 patients with a p53 gene deletion identified by fluorescence ISH, were studied by G-banding analysis. Assessable metaphase spreads were found in 50 of 57 (88%) cases. Of 50 patients, 27 (54%) had clonal chromosome abnormalities. Of 17 patients with a p53 gene deletion, 13 (76%) had chromosome 17 aberrations resulting in loss of 17p material (Table 1). Nine patients exhibited unbalanced translocations: der(12)t(12;17)(p13;q23), patient no. 3; der(8;17)(q10;q10), patient no. 9; der(17)t(Y;17)(q11;p11), patient no. 12; der(9)t(9;17)(q34;q11), patient no. 15; der(17)t(13;17)(q14;p11), patient no. 17; and der(17;18)(q10;q10), patients no. 1, 4, 7, and 10. The der(8;17)(q10;q10) in patient no. 8 and the der(17;18)(q10;q10) in the latter 4 patients were proven to contain alphoid sequences of both translocation partners, as shown by fluorescence ISH using D8Z1, D17Z1, and D18Z1 (Fig 2D). Patient no. 8 had an isochromosome i(17q) (Fig 2E); patient no. 13, a deletion del(17)(p11); and patients no. 14 and 16, a monosomy 17. Two patients (patients no. 2 and 6) with a p53 gene deletion had a normal karyotype, and in 2 patients (patients no. 5 and 11) no assessable metaphases were found. Partial karyotypes of patients with chromosome 17 aberrations are shown in Fig 3.

The frequency of other recurring chromosome abnormalities in B-CELL, such as trisomy 12 (8%) and abnormalities of band 13q14 (12%) and 14q32 (8%), was similar to that of previous cytogenetic studies in B-CELL.4

SSCP analysis. Shifts in the electrophoretic mobility were detected in 9 of 14 (64%) specimens analyzed (Table 1). Two aberrant bands occurred in exon 5 (patients no. 6 and 10), and one in exon 6 (patient no. 13); two additional aberrant bands were detected in the PCR fragment comprising exons 5 and 6 (patients no. 4 and 11; Fig 4). Three specimens had aberrant bands in exon 8 (patients no. 5, 14, and 16) and one in exon 9 (patient no. 1). In 5 patients (patients no. 3, 7, 9, 15, and 17), no abnormalities were detected. All 5 patients had the p53 gene deletion in the majority (greater than 50%) of nuclei by ISH and also had loss of 17p13 by G-banding analysis.

Correlations with clinical and laboratory data. The clinical characteristics and laboratory data of the patients with and without a p53 gene deletion are shown in Table 2. There was no significant difference in age, sex, Rai stage, hemoglobin level, platelet count, lactate dehydrogenase level, spleenomegaly, and degree of lymphadenopathy between the two groups. Patients with a p53 gene deletion had significantly lower albumin levels (40 g/L v 45 g/L; P < .01) and higher alkaline phosphatase levels (154 IU/L v 128 IU/L; P = .02), and they tended to have higher white blood cell (WBC) counts.
Table 2. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th></th>
<th>No p53 Deletion (N = 83)</th>
<th>p53 Deletion (N = 17)</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>57</td>
<td>64</td>
<td>.07</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>63</td>
<td>76</td>
<td>.40</td>
</tr>
<tr>
<td>Stage at diagnosis (%)*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rai 0-2</td>
<td>82</td>
<td>76</td>
<td>.22</td>
</tr>
<tr>
<td>Rai 3-4</td>
<td>10</td>
<td>24</td>
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<tr>
<td>WBC count ( \times 10^3/\mu L )</td>
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<td>145</td>
<td>.05</td>
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<td>Hemoglobin level (g/dL)</td>
<td>12.0</td>
<td>11.4</td>
<td>.47</td>
</tr>
<tr>
<td>Platelet count ( \times 10^3/\mu L )</td>
<td>154</td>
<td>123</td>
<td>.18</td>
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<tr>
<td>Lactate dehydrogenase level (IU/L)</td>
<td>194</td>
<td>221</td>
<td>.15</td>
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<tr>
<td>Alkaline phosphatase level (IU/L)</td>
<td>128</td>
<td>154</td>
<td>.02</td>
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<tr>
<td>Albumin level (g/L)</td>
<td>45</td>
<td>40</td>
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<tr>
<td>Splenomegaly (%)</td>
<td>83</td>
<td>82</td>
<td>.68</td>
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<tr>
<td>Lymph nodes ( \geq 5 \text{ cm} ) (%)</td>
<td>30</td>
<td>29</td>
<td>1.00</td>
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<tr>
<td>Time from diagnosis to first treatment (d)</td>
<td>396</td>
<td>59</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Median values are given for quantitative variables.
* No data on stage at diagnosis available in 7 patients.

(145,000/\mu L v 45,000/\mu L; \( P = .05 \)). Patients with a p53 gene deletion had a significantly shorter treatment-free interval (59 days v 396 days; \( P < .01 \)). Figure 5 shows the survival probabilities of the two patient groups. The difference between the two curves was highly significant (\( P < .001 \)). The median survival time from the date of diagnosis of the patients with a p53 gene deletion was 2.1 years compared with 10.3 years in the patients without a deletion. The predictive value of p53 gene deletion was as striking when only the 90 patients with B-CLL, and not the 3 patients with WM and the 7 patients with B-PLL, were included in the analysis. The difference between the survival curves of the B-CLL patients with and without a p53 gene deletion was highly significant (\( P < .001 \); Fig 6). In contrast, no significant difference in the survival curves (\( P = .24 \); Fig 7) was found when comparing the disease categories B-CLL and B-PLL.

Of our 17 patients with a p53 gene deletion, 3 were studied at diagnosis, 2 within 8 months, and another 2 within 11 months from diagnosis. In the remaining 10 patients, the median time interval from diagnosis to study was 32 months. Although our study was not performed prospectively, these data demonstrate that p53 gene deletion was not a late event in a significant number of patients. One patient (patient no. 12) with WM and a p53 gene deletion survives longer than 20 years. The diagnosis of B-CLL was made in 1972, and there was an indolent course of the disease over almost 20 years. In 1991, the patient presented at our institution with signs of disease progression such as marked B symptoms, splenomegaly, polyserositis, and a monoclonal IgM of 45 g/L. The patient's specimen had a low percentage of nuclei (18%) with only one p53 hybridization signal, suggesting a clonal evolution of the disease in this patient.

One hundred patients were entered on the Cox proportional hazards model for the analysis of the survival time.
from diagnosis (Table 3). Adjusting for age, sex, stage, WBC count, hemoglobin level, platelet count, lactate dehydrogenase level, alkaline phosphatase level, albumin level, splenomegaly, degree of lymphadenopathy, and p53 gene deletion, four variables gave independent prognostic information: p53 gene deletion (P < .001), age (P < .001), Rai stage (P = .003), and hemoglobin level (P = .047). Patients with a p53 gene deletion had an estimated sevenfold greater risk of death than patients without a deletion. All patients were also studied for the presence of trisomy 12 and retinoblastoma gene deletion using fluorescence ISH (data not shown). In survival analysis, both abnormalities had no significant impact on survival.

Fifty patients received therapy with purine analogs. The response to therapy depended strongly on the presence of a p53 gene deletion. None of the 12 patients with a deletion responded to therapy with fludarabine (n = 7) or pentostatin (n = 5), while 20 of 36 (56%) patients without a deletion who were assessable for response achieved a remission (P < .001). Because of the small number of patients, only univariate statistical analysis seemed reasonable and was applied to search for possible relevant prognostic factors. Three parameters gave a P value of less than 5%: p53 gene deletion (P < .001), the number of prior chemotherapies (P = .002), and the serum albumin level (P = .018). Whether or not the patient was refractory to prior chemotherapy with alkylating agents had no significant impact on the response (P = .22); this may be due to the small group of patients (n = 7) who still were responsive to prior therapy with alkylating agents.

The median survival time after start of therapy in the patients with a p53 gene deletion was 7 months, whereas the median survival time of the patients without a deletion has not yet been reached. Figure 8 shows the survival probabilities of the two patient groups after start of therapy with fludarabine or pentostatin. The difference between the two curves was highly significant (P < .001). To search for independent prognostic factors for the survival time beginning with the date of therapy, we again started with univariate models. Two parameters seemed to be important: p53 gene deletion (P < .001) and serum albumin level (P = .011). Using the bootstrap procedure to build a multivariate Cox regression model resulted in a model that finally included only p53 gene deletion. No other parameter was identified as an additional independent prognostic factor for the survival time.

**DISCUSSION**

Using interphase cytogenetic analysis, deletion of the p53 tumor suppressor gene was identified in 17 of 100 patients with chronic B-cell leukemia, including B-CLL, B-PLL, and WM. By analogy with a previous study on the incidence of the retinoblastoma gene deletion in B-CLL,\(^7,18\) our data demonstrate that deletions of a specific gene can be routinely diagnosed with high sensitivity by fluorescence ISH.

In most cytogenetic studies in B-CLL or related disorders, chromosome 17 had not been reported to be frequently involved in chromosome aberrations.\(^4,11\) In the Second International Working Party on Chromosomes in CLL (IWCCLL), which compiled data from 662 patients,\(^11\) 4% of the assessable patients had structural aberrations of chromosome 17. In only one single-center study using conventional banding analysis,\(^31\) the incidence of chromosome 17 aberrations was comparable with that found in our interphase cytogenetic study. Five of 31 (16%) assessable cases had abnormalities of chromosome 17, all leading to a deletion of the short arm.

The increase in incidence of 17pl deletions found by fluorescence ISH underlines the importance of obtaining cytogenetic data beyond the level of metaphase cells in this disease, which is very difficult to study by conventional banding analysis. In B-CLL cases without clonal aberrations, mitotic cells often stem from nonleukemic T lymphocytes, as demonstrated by the study of Autio et al.\(^32\) Interphase cytogenetics allows the detection of numerical and structural chromosome abnormalities, not only on metaphase spreads, but also in interphase nuclei.\(^33\) Thus, interphase cytogenetics provides an important approach to assess the true incidence of chromosome aberrations in B-CLL. Recently, the discrepancy between metaphase and interphase cytogenetic analysis has been demonstrated for other recurring numerical and structural chromosome abnormalities in B-CLL, such as trisomy 12 and 13q deletions.\(^9,17,18,25,34,37\) In our study, 4 of 17 (24%) patients with a p53 gene deletion either had no assessable mitotic cells (2 patients) or had a normal karyotype (2 patients) by G-banding analysis. Nevertheless, 13 of 17 patients exhibited abnormalities of chromosome 17, all leading to loss of band 17p13, to which the p53 gene localizes.

**Table 3. Cox Regression Model for Survival Time From Diagnosis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>Hazard Ratio</th>
<th>95% Confidence Limits</th>
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<td>Age</td>
<td>&lt;.001</td>
<td>1.083</td>
<td>1.036-1.131</td>
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<td>Rai stage</td>
<td>.003</td>
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<td>1.685-12.654</td>
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<td>Hemoglobin level</td>
<td>.047</td>
<td>0.830</td>
<td>0.691-0.997</td>
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</tbody>
</table>

Selected variables are listed in order of entry in stepwise analysis (N = 93).

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Breakpoints frequently occurred within the centromeric or pericentromeric region; 4 B-CLL patients had an unbalanced whole-arm translocation der(17;18)(q10;q10) (Figs 2C through D and 3A) that by fluorescence ISH was shown to contain centromeric sequences of both translocation partners. To our knowledge, this aberration has not been previously reported in this disease.

The fact that the majority of cases with loss of the p53 gene had microscopically rather large deletions of 17p by G-banding analysis raises the question whether p53 is the target gene of these deletions. Evidence for a pathogenetic role of p53 in our patients comes from the results of the SSCP analysis. Nine of 14 patients studied exhibited aberrant bands; ie, in 9 cases, both p53 alleles were mutated, one by deletion and the other by point mutation. There are several possible explanations why, in the remaining 5 patients in our study, no point mutations were found. First, point mutations are detected by SSCP analysis in only 80% to 90% of cases.38 Second, the SSCP analysis was restricted to exons 5 to 9, where most of the mutations have so far been identified. Mutations may reside outside this highly conserved region of the gene, although at a much lower frequency.16,19 Finally, allelic deletion may represent only the first of two mutation events, with an unaffected second allele. Data from other SSCP studies support the concept that the p53 gene plays a role in the pathogenesis of lymphoid malignancies. In B-CLL, point mutations of the p53 gene have been found in 10% to 15% of cases.11,14 Furthermore, p53 gene mutations have been identified in acute lymphoblastic leukemia cell lines and in tumor biopsies from malignant lymphomas of follicular or diffuse histology.04-44

The presence of a p53 gene deletion had strong implications for the clinical course of the disease. Patients with a deletion had a significantly shorter treatment-free interval than patients without a deletion. Although our study was not performed prospectively, the short interval between diagnosis and first treatment suggests that in most patients the deletion occurred early in the course of the disease. Furthermore, p53 gene deletion was strongly associated with short survival time (Fig 5). Poor survival could be assigned to p53 gene deletion rather than disease category, as p53 gene deletion had the same strong predictive value when considering patients with B-CLL only (Fig 6). In multivariate analysis, p53 gene deletion was the strongest prognostic factor for survival, followed by known prognostic factors in B-CLL, such as age, Rai stage, and hemoglobin level (Table 3). Our data are in agreement with a recent SSCP study of the p53 gene in 53 B-CLL patients.14 Adjusting for prognostic factors, patients with p53 gene mutations in this study had a 13-fold greater risk of death than patients without mutations. In the initial SSCP study by Gaidano et al,12 p53 gene mutations appeared to be more frequent in Richter's syndrome, which represents the evolution of B-CLL into a high-grade malignant lymphoma. Furthermore, in the SSCP analyses that were performed in follicular or diffuse lymphomas,41-43 p53 gene mutations were associated with histologic transformation and/or disease progression. Thus, as in many solid tumors,35 p53 gene mutations also seem to confer a growth advantage and a poorer survival rate to patients with a spectrum of lymphoproliferative diseases.

The most important recent improvement in therapy for B-CLL was the introduction of purine analogs, such as fludarabine, pentostatin, and 2-chlorodeoxyadenosine.2 Grever et al16 first demonstrated the antileukemic activity of fludarabine in a group of patients with advanced B-CLL. In the series of 75 patients reported by Keating et al,47 57% of previously treated patients achieved a partial or complete remission. In a study of the long-term follow-up of B-CLL patients treated with fludarabine,48 response to treatment and survival were strongly correlated with the degree of previous therapy, the stage of disease, and whether or not the patients were refractory to alkylating agents. Other parameters associated with survival were the age of the patient and the serum albumin at the start of treatment. The results of our univariate analyses confirm degree of prior chemotherapy and serum albumin level to be correlated with lower response rates to therapy with purine analogs. However, p53 gene deletion again was the strongest predictive variable (Table 4). None of the 12 patients with a p53 deletion responded to therapy with either fludarabine or pentostatin, whereas 20 of 36 (56%) patients without a deletion achieved a remission (P < .001). Furthermore, p53 gene deletion was the strongest prognostic factor for survival after start of therapy (Table 5). So far, too few patients have been treated to perform a meaningful multivariate analysis. Purine analogs may exert part of their effect in B-CLL by the induction of apoptotic cell death.49 On the other hand, p53 has been shown to play an essential role in apoptosis induced by radiation and DNA-damaging chemotherapeutic agents.50 Homozygous or even heterozygous mutations of the p53 gene may be one mechanism by which leukemic cells become resistant to therapy with purine analogs.34

Our data indicate that p53 is one tumor suppressor gene important in the pathogenesis of chronic B-cell leukemias, including B-CLL, B-PLL, and WM. The deletion of the p53 gene identifies a subset of patients with resistance to therapy and poor survival.

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Table 4. Logistic Regression Model for Best Response to Therapy With Fludarabine or Pentostatin: Univariate Analysis (N = 50)

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Odds Ratio</th>
<th>95% Confidence Limits (lower-upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 gene deletion</td>
<td>&lt;.001</td>
<td>0.000</td>
<td>0.000-0.27</td>
</tr>
</tbody>
</table>
| No. of prior
| chemotherapies      | .002 | 0.423 | 0.216-0.830 |
| Albumin level        | .18  | 1.198 | 1.021-1.405 |

Table 5. Cox Regression Model for Survival Time From Therapy With Fludarabine or Pentostatin: Univariate Analyses (N = 50)

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Hazard Ratio</th>
<th>95% Confidence Limits (lower-upper)</th>
</tr>
</thead>
<tbody>
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<td>p53 gene deletion</td>
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<td>6.570</td>
<td>2.490-17.300</td>
</tr>
<tr>
<td>Albumin level</td>
<td>.11</td>
<td>0.881</td>
<td>0.798-0.973</td>
</tr>
</tbody>
</table>
tance; Martin Günsche for help in data collection; Dr Lutz Edler for statistical advice; and Dr Hanswalter Zentgraf for advice in the SSCP analyses.

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p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias

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