Clinical Significance of \(p53\) Mutations in Relapsed T-Cell Acute Lymphoblastic Leukemia

By M.B. Dicicinni, J. Yu, M. Hsiao, S. Mukherjee, L. E. Shao, and A. L. Yu

In T-cell acute lymphoblastic leukemia (T-ALL), \(p53\) gene mutations were found in 12 of 51 patients in first relapse (24%). In a retrospective study, bone marrow samples at diagnosis were obtained from 9 of the 12 relapsed patients with \(p53\) mutation; only one patient was found to harbor a \(p53\) mutation at diagnosis. No further \(p53\) mutations were identified in 18 unpaired diagnosis T-ALL samples. This is the first report of a \(p53\) mutation in T-ALL at diagnosis. \(p53\) mutations in relapsed T-ALL were clinically relevant. Patients with \(p53\) mutations experience a shorter duration of survival than those patients without \(p53\) mutations. Additionally, patients with \(p53\) mutations were significantly less likely to have achieved a complete second remission from reinduction therapy than those patients without \(p53\) mutations and experienced a shorter duration of survival from relapse even when a second reinduction is obtained. Though primarily identified only at relapse, \(p53\) mutations were also associated with a decreased duration of first remission and overall decrease in survival from diagnosis. Patients with \(p53\) mutations had a 3.8-fold increase in risk of death than those patients without \(p53\) mutations. These findings suggest that \(p53\) mutation is associated with poor clinical outcome that is characterized by (1) a shortened duration of survival after first relapse; (2) a reduced response to reinduction therapy; (3) a shortened duration of first remission; and, hence, (4) an overall decreased duration of survival and increased risk of death.

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MATERIALS AND METHODS

Source of patient samples. In 1988, the Pediatric Oncology Group (POG) activated protocol POG No. 8862: “Treatment of first marrow and/or extramedullary relapse of childhood T-ALL with combination chemotherapy including 2’-deoxycoformycin.” As part of the studies specified in this protocol, a leukemic sample of each patient at relapse was submitted for our studies after obtaining informed consent from the patients or their parents, in accordance with a protocol approved by the Committee on Investigation Involving Human Subjects at the University of California, San Diego. In the event of the identification of a \(p53\) mutation at relapse, the corresponding diagnosis sample, stored frozen in the central cell bank of the Pediatric Oncology Group at St Jude Children Research Hospital (Memphis, TN), was obtained for analysis. The T-ALL samples were obtained from bone marrow or peripheral blood; the mononuclear cells (MNC) were harvested by centrifugation over Ficoll-Paque density gradient (specific gravity 1.077 g/mL; Pharmacia Fine Chemicals, Piscataway, NJ) at 2,000 rpm for 30 minutes. The patient population consisted of 25% female and 75% male, consistent with the male preponderance of T-ALL. There were 70% white, 13% black, 12% Hispanic, 1.5% Filipino, 1% American Indian, 1% Chinese, and 1.5% others.

Preparation of DNA. Whole-cell lysates were prepared by incubating approximately \(1 \times 10^6\) cells at 55°C in 200 \(\mu\)L of lysis buffer containing 100 mmol/L Tris-HCl, pH 8.3, 500 mmol/L KCl, 20 mmol/L MgCl\(_2\), 0.1% gelatin, 0.5% NP-40, 0.5% Tween (Sigma Chemical Co, St Louis, MO), and 10 mg/mL Proteinase K (Sigma). After a 2-hour incubation, samples were heated at 95°C for 5 min.

utes, cooled, and micro-centrifuged at 12,000 rpm for 5 minutes at 4°C. The supernatants were frozen and saved for subsequent analysis.

Polymerase chain reaction (PCR) single-stranded conformational polymorphism (SSCP). PCR amplification of exon 5 of the p53 gene for SSCP was performed on a Perkin-Elmer-Cetus model 9600 GeneAmp PCR System (Perkin-Elmer-Cetus, Norwalk, CT) with 10 pmol of \( \gamma ^{32} \)P-ATP labeled primers and 1 U Taq DNA polymerase (GIBCO BRL, Gaithersburg, MD) in PCR buffer (12 mmol/L Tris-HCl, pH 8.3, 60 mmol/L KCl, 2.3 mmol/L MgCl2; 0.12% gelatin, 0.01% each NP-40 and Tween) containing 250 \( \mu \)mol/L each dNTP. After an initial denaturation of 2 minutes at 94°C, amplification proceeded for 35 cycles with cycling parameters optimized at 94°C for 1 minute, 58°C for 30 seconds and 72°C for 45 seconds, which was followed by a final extension at 72°C for 6 minutes. Exon 4 amplification was performed using the same procedure as exon 5 except (1) 25 \( \mu \)mol/L for each dNTP was used, (2) \( \alpha ^{32} \)P-dATP (10 \( \mu \)Ci) was used to label PCR products instead of kinased primers, and (3) an annealing temperature of 60°C was applied. Amplification primers for exon 5 (MH20 and MH22) and for sequencing were described earlier. Amplification primers for exon 4 are MH60 (\( 5' - GAGGACCTGGTCCTCTGACT-3' \)) and MBD4 (\( 5' - CGGCCAGGATTTAGGTC-3' \)).

For SSCP analysis, \( 1 \mu L \) of PCR amplification mixture was diluted 100-fold in 0.1% sodium dodecyl sulfate (SDS)/10 mmol/L EDTA. An aliquot was diluted a further twofold in \( 2 \times \) loading buffer (95% formamide, 20 mmol/L EDTA, 20 mmol/L NaOH, 0.025% each bromphenol blue/xylene cyanole). The samples were boiled for 5 minutes, immediately cooled on ice, and 5 \( \mu L \) was loaded onto a 6% non-denaturing polyacrylamide gel. Samples were electrophoresed at 35 W in TBE buffer (90 mmol/L Tris-borate, 2 mmol/L EDTA) and run at room temperature. SSCP analysis of exon 4 was performed after digestion of PCR products with restriction enzyme Xcm I (New England Biolabs, Beverly, MA). This enzyme yields two asymmetric fragments, a 210-bp 5' fragment and a 158-bp 3' fragment, allowing for an increase in the sensitivity of SSCP and the localization of any mutations to the 5' or 3' portion of the exon.

DNA sequencing and dideoxy fingerprinting (ddF). PCR product spanning either exons 4-5 or exons 4-6 were subjected to a dideoxynucleotide sequencing reaction using the dsDNA Cycle Sequencing System (GIBCO BRL) using a nested end-labeled primer according to the manufacturer’s instructions. Sequencing of exon 4 entailed PCR amplification and agarose gel purification followed by TA Cloning (Invitrogen, San Diego, CA). Positive colonies were selected, grown into mini-cultures, and mini-preparations of the DNA used for sequencing. For ddF analysis, the sequencing reaction was performed with dideoxy ATP only. This reaction is stopped with \( 2 \times \) SSCP loading buffer plus 0.1% SDS. Samples are then denatured at 95°C for 5 minutes, cooled on ice, and resolved by 8% non-denaturing polyacrylamide gel electrophoresis.

Statistical analysis. Data on response to therapy was analyzed by \( \chi^2 \) analysis. Data on duration of remission were performed by \( t \)-test, adjusting the degrees of freedom for unequal variances. Survival was assessed using the Kaplan-Meier method with statistical significance of the survival curves determined by the Wilcoxon test of equality.

RESULTS

p53 mutations in T-ALL at relapse. To confirm the frequent incidence of p53 mutation in relapsed T-ALL, and to expand our database of 36 relapse T-ALL patients for investigating the clinical relevance of p53 mutations, another 15 T-ALL samples were obtained from patients at first relapse and investigated for p53 status. Using DNA from these cells, exons 4 to 8 of the p53 gene were subjected to mutational analysis by SSCP. Of the 15 new relapse patients studied, 2 patients were found to have p53 mutations in this region. Figure 1 shows the results of p53 analysis of exon 4 of patient 1. As shown in Fig 1A, a mutation in the 5' portion of the exon was detected with both the mutant and wild-type bands being evident. To confirm this mutation, PCR amplified DNA was subcloned before subjected to ddF analysis and sequencing. The ddF analysis showed subclones with electrophoretic mobilities indicative of wild-type and mutant genotypes (Fig 1B). The sequence of the subclone with the mutant conformation identifies a point mutation in the localization of any mutations to the S' or 3' portion of the exon.

Fig 1. Detection of point mutations of exon 4 of the p53 gene in relapse T-ALL. (A) PCR-SSCP analysis of a healthy person’s MNC DNA (control DNA) (lane 1) versus 3 relapse T-ALL patients (lanes 2 through 4) was performed after cleavage with Xcm 1, yielding two asymmetric fragments. The 5' and 3' portions of the PCR-amplified products are indicated. Arrows indicate the different electrophoretic mobilities of the individual single strands of the 5' portion of exon 4 corresponding to the wild-type alleles of all patients and the mutant alleles of patient 1 (lane 4). (B) Exon 4 of DNA from patient 1 was compared, purified, subcloned and subjected to ddF analysis as described in Materials and Methods. The ddF profile of two subclones, one exhibiting a mutant conformation (lane 2), and the other exhibiting a wild-type conformation (lane 3) are shown relative to control DNA (lane 1). Arrows indicate the different electrophoretic mobilities of the individual single strands of DNA corresponding to the mutant alleles. (C) The sequence of the subclones shown in (B) with mutant (lane 2) and wild-type conformation (lane 3) are shown relative to control DNA (lane 1) in the region of codon 52. Sequencing was performed with MH 41 primer. Reading proceeds from the bottom of the gel upwards and is shown 5' (bottom) to 3' (top). The base substitution is indicated by an asterisk.

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ROLE OF p53 MUTATIONS IN T-ALL

Table 1. Summary of the p53 Mutations Found in Relapse T-ALL Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Amino Acid Change</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exon 4</td>
<td>CAA → TAA</td>
<td>Gln → Stop</td>
</tr>
<tr>
<td>2</td>
<td>Exon 4</td>
<td>CGT → CTT</td>
<td>Arg → Leu</td>
</tr>
<tr>
<td>3</td>
<td>Intron 4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>Exon 5</td>
<td>TGC → AGC</td>
<td>Cys → Ser</td>
</tr>
<tr>
<td>5</td>
<td>Exon 5</td>
<td>TGC → AGC</td>
<td>Cys → Ser</td>
</tr>
<tr>
<td>6</td>
<td>Exon 5</td>
<td>TGC → TAC</td>
<td>Cys → Tyr</td>
</tr>
<tr>
<td>7</td>
<td>Exon 5</td>
<td>TGC → TCC</td>
<td>Cys → Ser</td>
</tr>
<tr>
<td>8</td>
<td>Exon 5</td>
<td>TGG → TGA</td>
<td>Trp → Stop</td>
</tr>
<tr>
<td>9</td>
<td>Exon 5</td>
<td>GCC → ACC</td>
<td>Ala → Thr</td>
</tr>
<tr>
<td>10</td>
<td>Exon 5</td>
<td>AGC → GCG</td>
<td>Gln → Arg</td>
</tr>
<tr>
<td>11</td>
<td>Exon 6</td>
<td>CGC → GCC</td>
<td>Arg → Gly</td>
</tr>
<tr>
<td>12</td>
<td>Exon 8</td>
<td>CGG → GGG</td>
<td>Arg → Gly</td>
</tr>
</tbody>
</table>

p53 mutations were found in 12 of 51 T-ALL patients at the time of relapse using PCR-SSCP analysis as described in Materials and Methods and reference 3. 

Abbreviation: N/A, not applicable.

* bp no. 13049 [Genbank (Los Alamos, NM) accession no. x54156].

Data compiled from this study and previous study.3

codon 52 from CAA to TAA leading to a stop codon; sequence analysis of the subclone with the ddF profile matching that of MNC DNA from a healthy person confirmed the wild-type sequence at codon 52 as Gln (CAA) (Fig 1C). Because this relapse sample contained only 33% leukemic blasts, our findings of both mutant and wild-type alleles may be accounted for by the mixture of normal marrow cells with wild-type p53 and leukemic cells harboring a homozygous mutation at codon 52. Alternatively, it is also possible that the relapse leukemic cells of this patient had a heterozygous mutation. This pattern was also observed in a patient (no. 8) with a mutation in exon 5, that is, both wild-type and mutant genotypes were observed (data not shown). Of particular note is the fact that both of these new mutations form stop codons that were not observed in our previous study.3

A detailed SSCP analysis of exons 6, 7, and 8 showed no additional mutations in any of the T-ALL patient samples investigated here. Results in exons 4, 5, and 6 were also confirmed by ddF analysis in most patients (data not shown). A summary of the mutations found in this study as well as those previously identified3 are shown in Table 1. The mutations are distributed in exons 4, 5, and 8 with the majority of mutations found in exon 5 (8 of 12; 67%). Table 1 also shows the diversity of the mutation genotype. In these studies we detected missense or nonsense mutations; no insertion or deletion mutations were detected.

p53 mutations in T-ALL at diagnosis. Previous studies have suggested that p53 exists only as wild-type at diagnosis.4 Indeed, our own preliminary investigation into p53 status in T-ALL at diagnosis has also detected only wild-type p53.5 However, if patients exhibit p53 mutations at relapse, they may be more likely to harbor p53 mutations at diagnosis than relapse patients with normal p53. Therefore, focusing on the 12 relapse patients identified as having p53 mutations (Table 1), we obtained cryopreserved T-ALL samples from 9 of these patients harvested at the time of diagnosis and instituted at retrospective analysis of p53 status. Mutation analysis of these diagnosis samples showed one patient (no. 8) with an SSCP and ddF migration profile indicative of a mutation (Fig 2, A and B). Furthermore, migration profiles were analogous to those for the relapse sample from this patient (data not shown), suggesting that the mutation was identical to that observed at relapse. Sequence analysis confirmed that this was indeed the case with a mutation in codon 146 from TGG to TGA (Trp to stop codon) (Fig 2C). Mutation and sequence analysis also show that, unlike at relapse, only the mutated allele is detected at diagnosis. Because this diagnosis sample contained 92% leukemic blasts, patient 8 likely harbors either a homozygous mutation or has undergone loss of the normal allele. However, the possibility that this mutation may exist as a germ-line mutation in one of the p53 alleles cannot be excluded. An additional six unpaired diagnostic samples showed no further mutations. To date, this is the first identification of a T-ALL patient with a p53 mutation at diagnosis.

p53 mutation, T-ALL patient survival, and response to reinduction chemotherapy. Using the pool of 51 relapse T-ALL patients in which p53 status was investigated (and this study), of which 12 were determined to harbor a p53 gene mutation (Table 1), we sought to delineate the clinical relevance of p53 mutations with respect to clinical responses to treatment as well as the clinical outcome of the patients. We first examined whether p53 mutations were associated with alterations in patient survival after first relapse. As seen in Fig 3, survival curve analysis demonstrates that those 12 relapse T-ALL patients who harbor p53 mutations have a significantly shorter duration of survival after relapse than those 39 patients without p53 mutations (P = .013).

To determine if the presence of p53 mutations was associated with an altered response to reinduction therapy, patients were divided into four subgroups based on their ability to achieve a second complete remission versus no complete
compared to a diagnosis of p53. 2) and is shown single strands of data, summarized in Table 2, show that among the 12 remission and the presence or absence of p53 mutations. The data, summarized in Table 2, show that among the 12 relapsed patients with p53 mutations, only 4 (33%) achieved second complete remission, whereas 27 of 39 (69%) without p53 mutations were reinduced to complete remission (P = .026). Thus, on average, twice as many patients without p53 mutations than with p53 mutations can achieve a second complete remission from second-line therapy. Therefore, we conclude that p53 mutations are associated with poorer response to reinduction therapy.

Next, the postrelapse duration of survival was examined in relation to p53 mutations and reinduction status. A survival curve comparison (Fig 4) among the four patient subgroups (as listed in Table 2) shows that significant differences in survival exist among the four subgroups (P < .001). A comparison of the survival curves of only those patients who obtained a second complete remission suggests that those 4 patients with p53 mutations have a survival duration shorter than those 27 patients without p53 mutations. However, the large differences in sample size of these two groups precluded an accurate statistical analysis. Patients with no second remission, regardless of p53 status, experience significantly shorter survivals than those patients who obtained a second complete remission but do not have p53 mutations (P < .001). This is particularly meaningful because the survival curves of patients with p53 mutations who obtained a second complete remission are similar to those of patients who did not achieve second remission. Together, these three subgroups of patients are not statistically different from each other (P > .3). These data confirm that p53 mutations in T-ALL are associated with a shortened duration of survival after relapse, and this shortened duration of survival exists even after successful reinduction therapy.

p53 mutation, duration of first remission, and overall duration of survival. Although generally only detected at relapse, we hypothesized that if p53 mutations exist at diagnosis but are undetected (because of their presence in only a small percentage of tumor cells, for example), or if they develop over the course of the disease, they may influence the duration of remission. We thus sought to determine whether p53 mutations at relapse have any bearing on the duration of first remission. It was found that the duration of

<table>
<thead>
<tr>
<th>p53 Mutation Status</th>
<th>Complete Second Remission</th>
<th>No Second Remission</th>
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<tbody>
<tr>
<td>No p53 mutation</td>
<td>27/30 (69%)</td>
<td>12/39 (31%)</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>4/12 (33%)</td>
<td>8/12 (67%)</td>
</tr>
</tbody>
</table>

P = .026, p53 mutations were determined by PCR-SSCP analysis as described in Materials and Methods. There were 39 T-ALL patients without p53 mutation and 12 patients with p53 mutation at the time of relapse. The numbers represent the patients with or without p53 mutations who achieved second complete and no second remission. The criteria for complete and no second remission are according to protocol no. 8862.
remission of those 39 patients without p53 mutations (20.5 ± 13.7 months) is significantly longer than that observed for those 12 patients with p53 mutations (13.1 ± 6.8 months; P = .016). These data imply that although mutations of the p53 gene are infrequently detected at diagnosis, they play some roles before their detection at relapse. Because p53 mutations are associated with a shortened duration of remission as well as a shortened duration of survival after first relapse, an association with a shortened overall duration of survival from diagnosis would be expected. Indeed, as shown in Fig 5, those 39 patients without p53 mutations have a significantly longer survival than those 12 patients with p53 mutations (P = .002). Proportional hazard analysis shows that patients with p53 mutations have a 3.7-fold increased risk of dying than those patients without p53 mutations (P = .0002).

The data present a strong association of p53 mutations at relapse with poor prognosis in T-ALL. However, there remained the possibility that other poor prognostic factors such as high white blood cell (WBC) count may have contributed to the poor outcome of those patients with p53 mutations at diagnosis. This does not appear to be the case. Patients without p53 mutations had WBC counts ranging from 0.9 × 10⁹/μL to 780 × 10⁹/μL (mean = 182 ± 195; n = 38), whereas patients with p53 mutations had WBC counts ranging from 5.2 × 10⁹/μL to 390 × 10⁹/μL (mean = 145 ± 149; n = 12) (P > .1); data on one patient was missing.

Adjusting for WBC counts, the relative risk of dying for patients with p53 mutations is 3.8-fold greater than those without p53 mutations (P = .0008). These results clearly show that p53 mutations at relapse are a poor prognostic factor for T-ALL, independent of initial WBC count.

**DISCUSSION**

In this study, we have investigated the incidence of p53 gene mutations in T-ALL at relapse and have correlated the p53 status with the clinical response to secondline chemotherapy and the clinical course of the patients. Based on this and previous studies, there were 12 cases of p53 mutations in a total of 51 relapse patients. Therefore, our results confirm the relatively high incidence of p53 gene mutations at relapse with a combined incidence rate of 23%. We further establish that p53 mutations contribute to a poor clinical outcome in the following manner: (1) p53 mutations correlated with a shortened duration of survival both from time of relapse as well as from time of diagnosis; (2) p53 mutations, while generally only detected at relapse, nevertheless are associated with a shortened duration of first remission; (3) the presence of p53 mutations is associated with a reduced response rate to reinduction therapy; and (4) p53 mutations correlated with a shortened duration of survival even after successful reinduction therapy.

We have also investigated p53 status in diagnosis T-ALL, and identified one diagnosis T-ALL patient with a p53 mutation out of nine cases in which a mutation was identified at relapse. No mutations were found in an investigation of 18 diagnosis T-ALL patients (and this study), or in 76 cases previously reported. Together, this is the first report of a p53 mutation in 103 diagnosis T-ALL patient samples investigated. This patient relapsed at 9 months and did not respond to secondline therapy. Taken together, our data are suggestive of p53 involvement in the progression of T-ALL, the ability to respond to secondline chemotherapy and ultimately patient survival.

Our finding of an association of p53 mutations with a reduced response rate to reinduction therapy suggests that T-ALL cells with p53 mutations may exhibit an increased resistance to the chemotherapeutic agents. Alterations of the p53 gene have been associated with drug resistance in at least two instances: (1) an increased frequency of N-(phosphonacetyl)-L-aspartate (PALA)-induced gene amplification when cells are challenged with PALA, an inhibitor of uridine biosynthesis; and (2) stimulation of MDR1 promoter activity by mutant p53 and transrepression of MDR1 gene activation by wild-type p53. Missense mutations resulting in amino acid substitutions were detected in 10 of the 12 T-ALL patients identified as harboring a p53 mutation, although their influences on MDR1 gene activity are unknown. Two T-ALL patients exhibit mutations leading to premature stop codons that likely result in the loss of p53 expression. If a normal role of wild-type p53 is to suppress MDR1 expression, then mutations leading to a loss of p53 may be sufficient to allow MDR1 gene expression. Further studies are in progress to examine the MDR1 levels of T-ALL cells and their correlation with p53 status.
The strong inverse correlation of p53 mutations with the duration of first remission suggest that, although undetected at diagnosis, p53 may still be involved in the steps after diagnosis and may be a late event in the mutational cascade leading to relapse. Alternatively, at diagnosis, cells containing p53 mutations may represent a small subset of the total blast population such that they are not detected in our analysis. Indeed, mutations that are present in less than 20% of the cell population are not readily detectable by direct sequencing, and are unlikely to be detected by SSCP or ddPCR. During chemotherapy, those cells containing p53 mutations may be resistant to treatment and may thus be selected to proliferate and become the primary population of cells observed during relapse, a population that is resistant to further chemotherapy. Alternatively, p53 gene mutations may develop spontaneously during the remission phase of the disease, or as a result of mutagenicity of chemotherapeutic agents. At least one other study has also noted the apparent acquisition of a p53 mutation between diagnosis and relapse. As in this study, the source of the relapse mutation, be it a de novo mutation, chemotherapy induced or a selected mutation cannot be determined. We are currently attempting to differentiate these possibilities.

Many T-ALL patients have no detectable alterations in their p53 gene. Does p53 play a role in T-ALL in these patients? In the absence of p53 mutations, the activity of p53 protein could be modulated through protein-protein interactions, and thereby play a role in tumor pathogenesis. One such candidate protein is the MDM2 oncoprotein, which is often amplified in sarcomas. MDM2 can bind p53 and inhibit p53-mediated transactivation and growth suppression. More recently it was shown that the MDM2 gene is also amplified in 8% to 10% of glioblastomas and anaplastic astrocytomas; none of these tumors with MDM2 amplification contained p53 gene mutations. The possibility of the functional inactivation of p53 among patients without p53 mutation is under current investigation. Other possibilities include defects in the pathway of p53 action, such as deletion or other functional inactivation of the putative p53 target gene, the cell-cycle regulator p21.

Two T-ALL patients were found to have nonsense mutations leading to premature stop codons. A truncated protein will have lost the C-terminal domains responsible for the formation of a functional p53 complex and DNA binding, both essential for p53 function. Furthermore, the loss of the nuclear localization domain makes it unlikely p53 would reach its nuclear target. It is also doubtful that such truncated proteins would be stably expressed and likely these cells are therefore deficient in p53 protein. Unfortunately, no sample was available for protein analysis.

The ability of p53 to suppress tumor cell growth in vitro has been clearly established, and cumulative data have shown that p53 is the most frequently mutated gene in human cancer. However, it has been difficult to establish the clinical consequences of these mutations. In this study we have correlated p53 status in T-ALL with the clinical parameters of survival, remission, and response to therapy. This is the first time p53 has been demonstrated as a prognostic indicator in T-ALL. p53 as a prognostic factor in other cancers has only briefly been investigated, with conflicting results. Studies of p53 immunopositive tumors in breast cancer by different laboratories have shown both an association with a shortened duration of survival and no relationship. Association of an increase in p53 expression with decreased survival was also shown in colorectal tumors, but not in lung carcinoma. However, the increase in p53 expression may not necessarily be associated with p53 mutations, an example of which has been demonstrated in cell lines derived from human lung carcinoma. Therefore, it is important to determine whether p53 mutations exist before a clinical correlation can be made. A report of 109 breast carcinomas has demonstrated that those patients with p53 mutations have a mortality rate significantly higher than the population without p53 mutations. An association of p53 mutation with survival has also been suggested in acute myeloid leukemia. p53 mutations have also recently been shown to be associated with a
poor clinical outcome in B-cell chronic lymphocytic leukemia. As in the present study, mutations were associated with a poorer response to therapy and a shorter duration of survival; no relationship with MDR1 could be established. Our results showing frequent p53 mutation at relapse, but not at diagnosis, suggest p53 involvement in disease progression. A similar association of p53 with disease progression has been reported in follicular lymphoma. These investigators found that p53 immunopositive cells/SSCP-identifiable mutations are rare before histologic transformation, but comprise approximately 25% of the transformed tumors. These studies show the critical prognostic role p53 can play in human cancers.

We have shown in this study that there is a high correlation between postrelapse survival, response to secondline therapy and postdiagnostic duration of remission with the presence of p53 gene mutations. We further hypothesize that p53 gene mutations may influence the drug sensitivity of T-ALL cells. We are currently investigating the in vitro drug sensitivity and the functional state of p53 at diagnosis and relapse of T-ALL to test this hypothesis.

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REFERENCES

30. Bobrow J, Bennett WP, Metcalf RA, Welsh JA, Ecker J,


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MB Diccianni, J Yu, M Hsiao, S Mukherjee, LE Shao and AL Yu