Meta-Analyses of $p53$ Tumor Suppressor Gene Alterations and Clinicopathological Features in Resected Lung Cancers

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Abstract

$p53$ alterations are the most common genetic lesions observed in lung cancers. Because of the limited size of individual studies, the distributions of $p53$ alterations by clinicopathological features have not been well characterized. Here, we present meta-analyses describing the occurrence of $p53$ alterations by patient/tumor characteristics in resected lung cancer. The association between $p53$ alterations (gene and/or protein) and a variety of variables were evaluated by calculating pooled odds ratios (OR) and confidence intervals (CI). $p53$ alterations were detected in 46.8% of 4684 non-small cell lung cancers. $p53$ alterations occurred more frequently in the more strongly smoking-associated histotypes: squamous cell (51.2%) and large cell (53.7%) carcinomas versus adenocarcinomas (38.8%); OR (squamous versus adenocarcinoma) = 1.81, 95% CI = 1.55–2.11. $p53$ alterations were found to be associated with $T_{1–4}$, $N_{0–3}$, stage I–III, differentiation, and sex: OR ($T_{3}$ versus $T_{1}$) = 1.62 (95% CI = 0.99–2.65), OR (NSCLC versus SCLC) = 1.65 (95% CI = 1.27–2.15), OR (stage III versus stage I) = 1.98 (95% CI = 1.35–2.89), OR (poorly and moderately versus well-differentiated) = 3.04 (95% CI = 1.56–5.94), and OR (male versus female) = 1.39 (95% CI = 1.10–1.75). No strong associations between $p53$ and smoking or neoploidy were observed. Lung cancer studies of $p53$ and smoking need to consider the effect of histotype, and prognostic studies of $p53$ should adjust for the effects of $T$ and $N$ or stage and histotype. The apparent association between $p53$ and sex may be confounded by histotype and must be evaluated by multivariate studies.

Introduction

$p53$ tumor suppressor gene alterations are the most common acquired genetic lesion observed in cancers, in general as well as in lung cancer (1–3). Since 1989, a large number of studies have evaluated $p53$ alterations in lung tumors and, in particular, because of tissue availability, in NSCLCs. In contrast, SCLCs, because they are usually systemic at time of diagnosis and respond better to chemotherapy, have been infrequently treated by surgical excision (4). Most studies provided descriptive information regarding the distribution of $p53$ alterations by patient and clinicopathological features. The common objective of the set of meta-analyses presented here was to overcome the limited power of individual studies and produce quantitative pooled statistics evaluating the associations between $p53$ alterations and fundamental clinicopathological features in NSCLC.

Materials and Methods

Studies involving $p53$ and lung cancer published in English-language peer-reviewed journals were identified by MEDLINE searches, repeated approximately every 6 months between the spring of 1995 and the spring of 1997, and by review of citations in the bibliographies of these and related articles. Data describing $p53$ status by histology, $T$ (tumor size and extension), $N$ (nodal status), $M$ (metastasis), stage, sex, differentiation, proliferation, aneuploidy, and $ras$ oncogene status were abstracted and evaluated in pooled analyses. The distributions of $p53$ alterations by other patient and clinicopathological variables were reported too inconsistently for pooled estimates to be precise or were not reported in categorical units that are suitable for pooling. Studies dealing exclusively with SCLC were excluded from analysis, and in mixed studies of NSCLC and SCLC cases, where possible, data on SCLC were excluded from the meta-analyses.

Pooled proportions and 95% CIs for $p53$ alterations by clinicopathological features were calculated by weighting the proportion of individual studies by the inverse variance of the proportion. This method gives greater weighting to larger and more precise studies. Pooled ORs and 95% CIs were calculated to compare the association between $p53$ alterations and patient and tumor characteristics. The pooled ORs were calculated weighting individual study effect estimates by the inverse of their variance using fixed effects or random effects models using the method of DerSimonian and Laird (5–7).

The $\chi^2$ statistic and ANOVA were used to evaluate inter-study heterogeneity, i.e., whether all of the study effect estimates came from a single population or different populations and whether interstudy variance was a significant contributor to the overall variance. When pooled studies were found to be heterogeneous, then a pooled OR based on the random effects model (ORR) was obtained. Otherwise, pooled ORs based on the fixed effects model (ORF) were presented. The fixed effects

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3 The abbreviations used are: NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; CI, confidence interval; OR, odds ratio; IHC, immunohistochemistry; BAC, bronchiolo-alveolar carcinoma.
model assumes that all of the studies have the same underlying effect estimates and only the individual study sampling variance \( (\sigma^2_{\text{intrastudy}}) \) accounts for differences in the study effect estimates. In the random effects model, some of the differences between study effect estimates are attributed to sampling from a population of different possible effect estimates, so the total variance is determined by intra- and interstudy variance \( (\sigma^2_{\text{intrastudy}} + \sigma^2_{\text{interstudy}}) \).

Data were stored and analyzed using Excel 97 spreadsheets (Microsoft Corporation, Redmond, WA), SAS Institute Inc., Cary, NC, and the EPIMETA program Version 1.1 (Centers for Disease Control and Prevention, Atlanta, GA). When a contingency table of results for a study contained empty cells, 0.5 was added to each cell. Calculations were performed using the natural log of the ORs [ln(OR)] and asymptotic SEs (8).

These meta-analyses were not weighted by study quality because efforts to control bias in individual studies are subjective and it is difficult to quantify biases that have occurred in studies and the impact that they have on effect estimates (9). Consideration of the effect of bias is particularly important when the pooled effect estimate is influenced by one or a few studies, which was not generally the case in these meta-analyses. Given that most studies reported univariate relations between p53 and variables, it was not possible with these data to evaluate the role that confounding biases played in apparent associations.

To ensure study quality and prevent duplication of results, we excluded meeting abstracts and non-peer-reviewed material, such as letters, from the pooled analyses. Several studies were excluded because they included patients described in other publications (study that was excluded/study that was included): Refs. 10/11, 12/13, 14/15, 16/17, 18/19, 20/21, 22/23, and 24/25. Larger sample size and more detailed variable description, rather than sequential order, determined the selection of which of the two duplicating studies was included in the meta-analyses. As far as the authors were aware, for any pooled summary statistic, all subjects were included only once.

In many studies, p53 alterations were detected as p53 gene mutations in tumors by amplifying conserved regions of the p53 gene using PCR technology and then screening for mutations using single-strand conformation polymorphism analysis. Exons that were found to exhibit altered electrophoretic mobility by single-strand conformation polymorphism were then sequenced to identify the nature of the mutations. Alternative methods of mutation detection, such as denaturing gradient gel electrophoresis, were also used in a few studies. Other studies, instead of evaluating the p53 gene for mutations, evaluated p53 protein accumulation in tumor tissue using IHC methods. In most cases, IHC-stained tissue sections were evaluated microscopically, but in a few studies, p53 protein accumulation was measured by flow cytometry or ELISA of tissue extracts. Wild-type p53 protein has a short half-life (15–20 min) and does not normally accumulate intracellularly. In contrast, many mutant forms of p53 protein and, in particular, those resulting from missense mutations, have extended half-lives (4–12 h), which leads to immunohistochemically detectable amounts of intracellular p53 protein (26).

Pooled summary statistics were calculated for variables with p53 alterations evaluated by p53 gene mutation, p53 protein accumulation, and both combined, when numbers allowed and results were not heterogeneous. To reduce potential heterogeneity, calculations were performed excluding special samples that are considered to be unique with regard to sampling or p53 assay method. The descriptive study by Takahashi et al. (27) was excluded because it was restricted to intronic point mutations, and the study by McDonald et al. (28) was excluded because it evaluated p53 mutations only at codon 249. Studies of lung cancer patients with unusual exposures, such as uranium miners (29, 30), mustard gas workers (31), and atomic bomb exposure (32), were excluded from pooled analyses. Studies, or parts of studies, using tumor cell cultures and not tumor tissue directly were excluded from pooled analyses (23, 33–37), as were studies that used flow cytometry (38, 39) or ELISA of tumor extracts (40) and not tissue IHC to evaluate p53 protein accumulation. Flow cytometry and ELISA may not be comparable to the IHC methods used in other studies, and data were not presented in the categorical format necessary for pooling. Pooled statistics were not reported for pooled groups totaling fewer than 50 individuals. The \( \kappa \) statistic was used to evaluate agreement between p53 alteration detected by gene mutation and protein accumulation when both assays were performed on the same sample.

Results

Information for 5207 individuals from 77 studies was reviewed for inclusion in the meta-analyses. A table that summarizes the studies (11, 13, 15, 16, 17, 19, 21, 25, 34, 41–96) and shows which studies were used in the separate components of the meta-analyses is available from M. C. T.

| p53 Gene Mutation and p53 Protein IHC Correlation. Data were available for 291 individuals from eight studies whose tumors had been evaluated for both p53 gene mutations and p53 protein accumulation (58, 62, 63, 67, 83, 84, 90, 92). The most common p53 antibodies used in IHC were PAb1801, DO-7, and CM-1. The pooled results are described in Table 1. The agreement proportion was 66%, and the \( \kappa \) statistic was 0.33.

<table>
<thead>
<tr>
<th>p53 Gene Mutation and p53 Protein IHC Correlation.</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant</td>
<td>81 (28%)</td>
<td>29 (10%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>70 (24%)</td>
<td>111 (38%)</td>
</tr>
<tr>
<td>Total</td>
<td>151 (52%)</td>
<td>140 (48%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 1 The combined results from eight studies evaluating p53 gene mutation and protein accumulation detected by IHC.</th>
<th>p53 gene analysis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Mutant</td>
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<tr>
<td>Wild-type</td>
<td>29 (10%)</td>
<td>111 (38%)</td>
</tr>
<tr>
<td>Total</td>
<td>110 (38%)</td>
<td>181 (62%)</td>
</tr>
</tbody>
</table>

Lung Cancer Type and Histology. p53 mutations were observed in 42.1% of NSCLCs (95% CI, 39.7–44.5%), and p53 protein accumulation detected by IHC was observed in 49.1% of NSCLCs (95% CI, 47.5–50.8%). The proportion of p53 alterations detected by protein IHC was significantly greater than that detected by p53 gene mutation. The proportion of p53 alterations detected by p53 gene mutation and/or p53 protein accumulation combined was 46.8% (95% CI, 45.4–48.2%). The combined sample 95% CI does not include the point estimate for p53 proportions by either assay group alone. Normally, significantly different statistics should not be pooled because the combined figure does not accurately reflect either of the component groups, but because it is such a widely reported statistic, an overall estimate for p53 alterations detected by both assays is presented here. The statistical difference in proportions of p53 alterations in NSCLC detected by the two assay methods does not reflect a striking variation in estimate magnitudes, as much as it does the high precision that accompanies samples of such sizes.

The proportion of p53 alterations in NSCLC also differed...
by histological type. Squamous cell and large cell carcinomas had significantly higher proportions of p53 alterations than did adenocarcinomas, regardless of how p53 was assayed (Table 2; Fig. 1). p53 alterations (gene and/or protein) were observed in 51.2% of squamous cell carcinomas (95% CI, 49.0–53.3%), 53.7% of large cell carcinomas (95% CI, 48.5–59.0%), and 38.8% of adenocarcinomas (95% CI, 36.6–41.1%). The pooled ORs comparing p53 alterations in squamous cell carcinomas to adenocarcinomas was 1.81 (95% CI, 1.55–2.11). Only four studies presented data for p53 protein accumulation detected by IHC for BACs, a subtype of adenocarcinoma. The pooled proportion of p53 protein accumulation in BAC was 12.8% (95% CI, 4.8–20.7%; Table 2). The total numbers of adenosquamous carcinomas and of carcinoid lung cancers were fewer than 50 per group when stratified by mutation versus protein analyses. Their analyses are not presented because the frequency estimates were imprecise and heterogeneous by p53 analytic method.

This overview focused on NSCLC, but 20 studies also

![Fig. 1](image-url) Proportions of p53 alterations, with 95% CIs, in pooled lung cancers by histotype and p53 assay. MT, mutation; AdC, adenocarcinoma; SqCC, squamous cell carcinoma. Numbers of studies for each histotype are in brackets.

### Table 2. Relative frequencies of pooled lung cancers with p53 alterations (95% CIs) by histotype

<table>
<thead>
<tr>
<th>Histological type</th>
<th>p53 gene mutations or protein accumulation</th>
<th>p53 gene mutations</th>
<th>p53 protein accumulation by IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f (n)</td>
<td>f (n)</td>
<td>f (n)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>0.468 (0.454–0.482) (4684 [66])</td>
<td>0.421 (0.397–0.445) (1500 [26])</td>
<td>0.491 (0.475–0.508) (3184 [40])</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0.512 (0.490–0.533) (1883 [54])</td>
<td>0.494 (0.456–0.532) (540 [22])</td>
<td>0.520 (0.494–0.545) (1343 [32])</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0.388 (0.366–0.411) (1743 [50])</td>
<td>0.354 (0.319–0.390) (693 [23])</td>
<td>0.411 (0.382–0.440) (1050 [27])</td>
</tr>
<tr>
<td>BAC</td>
<td>0.537 (0.485–0.590) (280 [31])</td>
<td>0.542 (0.457–0.627) (105 [15])</td>
<td>0.532 (0.468–0.600) (175 [16])</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f, frequency (95% CI); n, number of individuals [number of studies] pooled.
included data for a total of 163 SCLC cases (Table 2). This number constitutes 3.4% of the total number of patient tumors evaluated in the pooled analyses. p53 gene mutations were detected in 58.9% (95% CI, 47.7–70.1) of 59 SCLCs reported in eight studies, and p53 IHC-detected protein accumulation occurred in 34.7% (95% CI, 27.1–42.3%) of 104 SCLCs reported in 12 studies.

Seventeen of 1358 normal lung tissues adjacent to neoplastic tissues were found to be p53 protein positive by IHC, and all of these cases came from one study (74).

### Stage.
Pooled proportions and ORs describing p53 alterations (gene and/or protein) by T and N and stage are reported in Figs. 2 and 3, Pooled proportions and ORs describing p53 alterations respectively, and Table 3. The general tendency for the proportion of p53 alterations to increase with T and N is apparent in Fig. 2. Comparison of p53 alterations (gene and/or protein) in T3 to T1 and in N1–3 to N0 yielded pooled ORs of 1.62 (95% CI, 1.67–2.84) and 1.95 (95% CI, 1.12–3.41), respectively. The pooled OR for stage IV was elevated: 1.22 (95% CI, 0.71–7.93). All but one of these studies (19) evaluated p53 protein accumulation by IHC, and the findings of the single study using mutational analysis were compatible with those of the other studies (Table 3).

### Proliferation.
Ten studies evaluated the association between proliferation and p53 alterations: five found significant positive associations, one observed a trend toward a significant positive association, and the remaining four found no significant associations (Table 4). Data suitable for pooling (dichotomous for both variables) were available from four of these studies. Significant interstudy heterogeneity was present and the pooled OR based on the random effects model was 2.30 (95% CI, 0.30–17.43). Although all of these studies used IHC to evaluate p53 protein accumulation, proliferation assays varied between studies (Table 4) and may have accounted for some of the heterogeneity of the pooled results.

### Aneuploidy.
The pooled OR and 95% CI indicate that no significant interstudy heterogeneity was present and the pooled OR based on the random effects model was 2.30 (95% CI, 0.30–17.43). Although all of these studies used IHC to evaluate p53 protein accumulation, proliferation assays varied between studies (Table 4) and may have accounted for some of the heterogeneity of the pooled results.

### ras Oncogene.
Although not described in all studies, K-ras mutations were predominantly studied. Concerning p53 alterations and ras oncogene activation, the results of the reviewed studies were heterogeneous, and the pooled OR estimates were imprecise (Table 3). Mutational analysis suggested an inverse association, whereas p53 protein accumulation suggested a positive association.

### Sex.
Twenty-six studies evaluated the association between tumor p53 alterations and sex. Of these, three reported a significantly larger proportion of tumor p53 alterations in males than in females (11, 17, 91). For meta-analysis, tumor p53 and sex data were available for 1674 patients from 22 studies. p53 alterations were observed more often in males than in females. p53 alterations (mutation and/or protein) occurred in 18.4% (95% CI, 45.5–51.1%) of tumors from males and in 32.1% (95% CI, 28.4–35.8%) of tumors from females (OR = 1.39; 95% CI, 1.10–1.75; Table 3).

### Table 3 p53 association with patient and tumor variables pooled over NSCLC studies

<table>
<thead>
<tr>
<th>Variable</th>
<th>p53 gene and/or protein alterations</th>
<th>p53 gene mutations</th>
<th>p53 protein IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR&lt;sub&gt;F&lt;/sub&gt;</td>
<td>n</td>
<td>OR&lt;sub&gt;F&lt;/sub&gt;</td>
<td>OR&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td>Histology (squamous cell vs. adenocarcinoma)</td>
<td>1.81&lt;sup&gt;a&lt;/sup&gt; (1.55–2.11) 3629 [59]</td>
<td>2.18&lt;sup&gt;b&lt;/sup&gt; (1.67–2.84) 1235 [24]</td>
<td>1.65&lt;sup&gt;b&lt;/sup&gt; (1.36–1.99) 2394 [35]</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; vs. T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1.62&lt;sup&gt;a&lt;/sup&gt; (0.99–2.65) 343 [11]</td>
<td>1.19&lt;sup&gt;b&lt;/sup&gt; (0.35–4.04) 61 [4]</td>
<td>2.37&lt;sup&gt;b&lt;/sup&gt; (0.71–7.93) 282 [7]</td>
</tr>
<tr>
<td>N&lt;sub&gt;1–3&lt;/sub&gt; vs. N&lt;sub&gt;0&lt;/sub&gt;</td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt; (1.27–2.13) 1067 [14]</td>
<td>1.63&lt;sup&gt;b&lt;/sup&gt; (0.85–3.11) 216 [5]</td>
<td>1.66&lt;sup&gt;b&lt;/sup&gt; (1.24–2.21) 851 [9]</td>
</tr>
<tr>
<td>Stage II vs. stage I</td>
<td>1.22&lt;sup&gt;a&lt;/sup&gt; (0.91–1.66) 1198 [25]</td>
<td>1.29&lt;sup&gt;b&lt;/sup&gt; (0.77–2.18) 422 [12]</td>
<td>1.19&lt;sup&gt;b&lt;/sup&gt; (0.82–1.72) 776 [13]</td>
</tr>
<tr>
<td>Stage III vs. stage I</td>
<td>1.98&lt;sup&gt;a&lt;/sup&gt; (1.35–2.89) 1617 [27]</td>
<td>1.86&lt;sup&gt;b&lt;/sup&gt; (1.27–2.72) 565 [12]</td>
<td>1.95&lt;sup&gt;b&lt;/sup&gt; (1.12–3.41) 1052 [15]</td>
</tr>
<tr>
<td>Differentiation (poor and moderately vs. well)</td>
<td>3.04&lt;sup&gt;b&lt;/sup&gt; (1.56–5.94) 492 [9]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferation (high vs. low)</td>
<td>See results in last column.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aneuploidy (aneuploid vs. diploid)</td>
<td>0.92&lt;sup&gt;b&lt;/sup&gt; (0.45–1.88) 202 [4]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras oncogene activation (activated vs. wild-type)</td>
<td>Heterogeneous results not pooled. 0.38&lt;sup&gt;b&lt;/sup&gt; (0.05–3.29) 209 [4]</td>
<td>1.39&lt;sup&gt;b&lt;/sup&gt; (0.74–2.59) 210 [4]</td>
<td></td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>1.39&lt;sup&gt;a&lt;/sup&gt; (1.10–1.75) 1674 [22]</td>
<td>1.59&lt;sup&gt;b&lt;/sup&gt; (1.16–2.19) 847 [13]</td>
<td>1.20&lt;sup&gt;b&lt;/sup&gt; (0.86–1.68) 827 [9]</td>
</tr>
</tbody>
</table>

<sup>a</sup>OR<sub>F</sub>: OR based on fixed/random effects models (95% CI; n, number of individuals [number of studies] pooled.
<sup>b</sup>Value represents OR.<br><sup>c</sup>Value represents OR.<br>
Discussion

Correlation between p53 Gene Mutation and p53 Protein IHC

Several studies concluded that there was good agreement between p53 gene mutation assays and p53 protein IHC by using Fisher’s exact test or the \( \chi^2 \) test (20, 83, 92). However, these tests are not appropriate for an analysis of concordance because small \( P \)s indicate simply that the null hypothesis of no difference should be rejected. Other studies used percentage agreement to describe the concordance between p53 gene and protein analyses. This method tends to exaggerate the true agreement between two methods because many results agree due to chance alone.

In the pooled analysis of 291 lung tumors evaluated for both p53 mutations and protein accumulation, the proportion agreeing was 0.66, and the \( \kappa \) statistic was 0.33 (Table 1). In this case, the low \( \kappa \) should not be interpreted as an indicator of poor reproducibility, but rather as an indication that the two assay methods are measuring different aspects of complex molecular events. When disagreement occurred between the two approaches measuring p53 dysfunction, IHC-positive/mutation-negative tumors occurred 2.4 times more often as the reverse.

In the meta-analyses of p53, pooled analyses were presented for p53 gene mutation and protein accumulation separately, where numbers allowed. Despite the lack of direct concordance, p53 mutational analyses agreed with p53 protein analyses in estimating the frequency of p53 in a wide variety of variables, estimating associations (ORs), and demonstrating p53 dose response with stage (Table 3). These similar findings are based on aggregate data and may suffer from ecological bias. Nevertheless, they do suggest that, at least in some cases, p53 gene and protein analysis may be measuring similar phenomena and that, in some circumstances, p53 protein IHC may be a useful surrogate for mutational analysis.

Substantive Findings

These meta-analyses of p53 and clinicopathological characteristics incorporated data from a large number of individuals from...
numerous studies of NSCLCs. Many significant associations were detected that, until now, had not been clearly established. **Lung Cancer Type and Histology.** p53 alterations occurred in a significantly greater proportion of squamous cell and large cell carcinomas than in adenocarcinomas. The former two are more strongly associated with smoking than are adenocarcinomas (97). This relationship was observed regardless of whether p53 mutations or p53 protein accumulation were evaluated separately or in combination.

p53 protein accumulation occurred in a significantly lower proportion of BACs than in adenocarcinomas as a group (Table 2; Fig. 1). BACs are considered a subtype of adenocarcinoma, and most studies in the meta-analysis included BACs under the broader classification of adenocarcinoma. This implies that the overall estimate of the proportion of p53 alterations in adenocarcinoma may be shifted downward because of the inclusion of BAC. Further study of p53 occurrence in adenocarcinoma by subtype will help clarify whether differences exist between BAC and other subtypes of adenocarcinoma and between non-BAC adenocarcinoma and squamous cell carcinoma.

All studies reviewed, excluding one outlier study (74), failed to detect p53 alterations in normal tissue. In contrast, several studies found p53 alterations in metaplastic and dysplastic lung tissues adjacent to neoplastic NSCLC tissue. The prevalence of these p53 alterations in many studies became elevated at or near the severe dysplasia phase of carcinogenesis (49, 50, 53, 59, 65, 66).

**Stage.** p53 alterations occurred in a higher proportion of tumors with higher T, N, and stage, regardless of whether p53 alterations were assayed by gene mutation, protein accumulation, or both. A dose-response relationship between p53 alterations and stage was observed for stages I–III (Fig. 3). Although the estimated proportion of p53 alterations in stage IV was lower than in stage III, this estimate was based on small numbers and had a wide CI. Stage IV NSCLCs are seldom treated by surgical excision, so estimates based on these excised tumors may not be fully representative of stage IV NSCLCs. Thus, the present meta-analysis cannot characterize completely the role p53 plays in the advancement of lung cancer stage.

**Differentiation.** Poorly and moderately differentiated NSCLC had similar proportions of p53 alterations (52.7 and 56.7%, Fig. 3). Proportions of p53 alterations, with 95% CIs, in pooled NSCLCs by ascending groupings of stage.
respectively), and for this reason, they were combined into one category for analysis. Poorly and moderately differentiated tumors compared to well-differentiated tumors had a significantly greater proportion of p53 alterations (mutation and/or protein). Differentiation criteria vary by histological types, whereas in the meta-analysis, it was necessary to pool differentiation overall NSCLC. Whether this association between p53 and differentiation applies equally in all histological types awaits evaluation in stratified or multivariate analysis.

**Proliferation.** Highly proliferating NSCLC were found to have a higher proportion of p53 IHC positivity. The study results were heterogeneous, and the CI for the random effects model OR did include the null value. It should be noted that four of six studies that found a significant positive association were excluded from analysis (Table 4). These preliminary findings suggest that an association between p53 and proliferation may exist. An updated and more inclusive meta-analysis may be able to clarify the association between p53 and proliferation and characterize the sources and nature of the heterogeneity.

**Sex.** Males had a higher frequency of p53 alterations than females, and this observation was consistently observed when alterations were analyzed by gene mutation or protein accumulation separately or in combination. The higher proportion of p53 alterations observed in males compared to females may have resulted from males having relatively more squamous cell carcinomas, which have a higher proportion of p53 alterations than do adenocarcinomas, which occur more frequently in women. Whether p53 alterations are associated with sex independently of histotype remains to be determined in multivariate analysis.

**Aneuploidy.** Wild-type p53 is involved in the regulation of the number of centrosomes in a cell and the number of spindles in mitosis, and cells in culture lacking both p53 alleles rapidly become aneuploid, whereas cells with wild-type p53 remain diploid during their passage in culture (98, 99). Although this information suggests an association between p53 alterations and aneuploidy, no strong association was observed in meta-analysis. The pooled effect estimates were near the null but may be the result of random sampling variation. Further study is required to determine whether p53 alterations are associated with ras mutations and whether the association is heterogeneous by p53 assay method.

### Problems and Issues in Meta-Analysis

It is unlikely that publication bias had an important influence in this set of meta-analyses because, in most of the pooled studies, the frequencies of p53 alterations by clinicopathological variables were not considered to be part of the primary hypotheses under investigation and were reported as incidental findings. Furthermore, the large majority of associations between p53 and clinicopathological variables in the pooled studies were nonsignificant.

The classification of variables such as sex, T, N, stage, differentiation, and histology are generally standardized internationally throughout medical research institutes. Many studies failed to report what criteria they used to classify TNM, stage, or histology. Most studies that did specify these criteria reported using the international staging system of the American Joint Committee on Cancer/International Union Against Cancer for staging (100–102) and the WHO criteria for histological classification (103). The latter also describes criteria for differentiation. This suggests that staging and histological classification were more or less uniform across studies. There are no apparent reasons to suspect a strong differential mismeasurement of p53 status by clinicopathological variables. Despite possible nondifferential measurement errors, which generally tend to bias effect estimates toward the null (104), several of the pooled estimates in these meta-analyses demonstrated significant associations.

The pooled analyses were univariate and cannot evaluate independent association or effect modification. Understanding the association between p53 and clinicopathological variables, as well as p53’s association with other important variables, such as smoking and survival in NSCLCs, can only be made through multivariate analysis including relevant covariate/confounder data. Few published articles provided data on all relevant variables for each study participant, and some studies may not have collected data on all relevant variables. For this reason, the independent associations and interactions of p53 with clinicopathological variables in NSCLC will most likely be resolved in the future through large independent studies, as

### Table 4

<table>
<thead>
<tr>
<th>Study</th>
<th>p53 measurement (antibody)</th>
<th>Proliferation measurement</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S + A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morkve and Laerum (38)</td>
<td>Flow cytometry (PaB 1801)</td>
<td>Flow cytometry, propidium bromide</td>
<td>24</td>
</tr>
<tr>
<td>Hyoshi et al. (43)</td>
<td>IHC (PaB 1801)</td>
<td>Mitotic index</td>
<td>74</td>
</tr>
<tr>
<td>Volm et al. (52)</td>
<td>IHC (PaB 1801)</td>
<td>Flow cytometry, propidium iodide</td>
<td>56</td>
</tr>
<tr>
<td>Fontanini et al. (54)</td>
<td>IHC (PaB 1801 &amp; PaB 240)</td>
<td>PCNA, Ki-67, and flow cytometry</td>
<td>99</td>
</tr>
<tr>
<td>Marchetti et al. (58)</td>
<td>IHC (PaB 1801 &amp; PaB 240)</td>
<td>Flow cytometry, propidium iodide</td>
<td>53</td>
</tr>
<tr>
<td>Fontanini et al. (65)</td>
<td>IHC (CM-1)</td>
<td>PCNA IHC</td>
<td>30</td>
</tr>
<tr>
<td>Volm et al. (73)</td>
<td>IHC [PaB-6 (DO-1)]</td>
<td>Flow cytometry, propidium iodide</td>
<td>65 (T + A)</td>
</tr>
<tr>
<td>Harpole et al. (79)</td>
<td>IHC (PaB 1801)</td>
<td>Proliferating index Ki-67 IHC</td>
<td>271</td>
</tr>
<tr>
<td>Wiethege et al. (86)</td>
<td>IHC (PaB 1801 &amp; DO-1)</td>
<td>PCNA IHC</td>
<td>279</td>
</tr>
<tr>
<td>Esposito et al. (95)</td>
<td>IHC (DO-7)</td>
<td>PCNA IHC</td>
<td>61</td>
</tr>
<tr>
<td>Subtotals</td>
<td>279</td>
<td>220</td>
<td>386</td>
</tr>
<tr>
<td>Totals</td>
<td>499</td>
<td>513</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> NS, not significant; PCNA, proliferating cell nuclear antigen; S + A, significant positive association; T + A, statistical trend toward a positive association.

<sup>b</sup> Exclusions occurred because data was not categorical (38, 95), or data were not provided (43, 52, 54, 58, 65, 73, 79, 86).
opposed to a multivariate meta-analysis incorporating aggregate data from past studies. In some cases, the pooled effects observed in these meta-analyses, despite being univariate, are suggestive of real associations. For instance, the large and significant difference in proportion of p53 alterations between squamous cell carcinoma and adenocarcinoma is not likely to be the result of confounding bias alone.

### Conclusion

These meta-analyses are the largest compilation of individuals and studies available to date estimating the frequency of p53 alterations by lung cancer type and by selected clinicopathological features and evaluating the association between p53 and lung cancer characteristics. Such estimates of frequency and associations are not possible using the IARC p53 mutation database because they do not include information on p53-negative cases from the case series they document (105). p53 alterations were found to occur in a significantly larger proportion of NSCLCs with higher T, N, and stage; moderately and poorly differentiated (versus well-differentiated) tumors; and males (versus females). p53 alterations (gene and/or protein) occurred in a significantly smaller proportion of adenocarcinomas compared to other NSCLCs and occurred in a significantly smaller proportion of BACs than other NSCLCs, including adenocarcinomas as a group. In NSCLC research, investigators of exposures and p53 and p53 and prognosis must be aware of and consider these associations. p53 was found to be associated with variables that differ by smoking status (e.g., histotype and sex) and with potential prognostic factors (e.g., T, N, stage, differentiation, and proliferation). Thus, studies of exposures, p53, and prognosis in NSCLC should use multivariate analysis to evaluate whether these clinicopathological variables are independently associated with p53, are acting as confounders, or lie on the same causal pathway. Future studies need to clarify the association between p53 and proliferation, ras, and aneuploidy. The findings of this study have presented strong evidence supporting some associations, and the heterogeneity detected in other associations points to future avenues of investigation. It is likely that the descriptive meta-analytic approach used to characterize p53 in NSCLCs can be applied to develop a better understanding of the nature of oncogenes and tumor suppressor genes in other cancers.

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p53 Alterations and Lung Cancer

Cancer Epidemiology, Biomarkers & Prevention

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