THE *TP53* GENOTYPE BUT NOT IMMUNOHISTOCHEMICAL RESULT IS PREDICTIVE OF RESPONSE TO CISPLATIN-BASED NEOADJUVANT THERAPY IN STAGE III NON-SMALL CELL LUNG CANCER

Daniela Kandioler-Eckersberger, MD^a
S. Kappel, PhD, MD^a
M. Mittlböck, PhD^c
G. Dekan, MD^d
C. Ludwig, PhD^a
E. Janschek, MD^a
R. Pirker, MD^b
E. Wolner, MD^a
F. Eckersberger, MD^a

Background: The cytotoxic effects of cisplatin and anthracyclins have been attributed to apoptosis induction, which has been recognized as a major function of the *TP53* gene. The *TP53* gene appears to be mutated in about 50% of cases of non-small cell lung cancer. A possible dependence of chemotherapy response on *TP53* genotype was evaluated retrospectively in a group of patients with advanced non-small cell lung cancer and induction treatment. **Methods:** Patients with complete or partial remission were compared with those with stable or progressive disease with respect to *TP53* genotype and overall survival. Mutations in the *TP53* gene were detected by complete direct sequencing (exons 2-11). **Results:** A normal *TP53* genotype proved to be significantly associated with major response to chemotherapy ($P < .001$). Overall, no association was found between p53 protein expression and *TP53* genotype. A normal *TP53* genotype was found to be highly sensitive in predicting response to treatment, whereas a mutant genotype was revealed to be specific in predicting lack of response. The difference in overall length of survival was significant between patients exhibiting a normal *TP53* genotype (corresponding to those whose disease responded to chemotherapy) and patients showing mutant *TP53* genotype (corresponding to those who had disease resistant to chemotherapy, $P = .027$). **Conclusions:** In a small cohort of patients with advanced non-small cell lung cancer we found a direct link between normal *TP53* genotype and response to cisplatin-based induction treatment and also between mutant genotype and resistance to treatment, whereas p53 immunohistochemical result was predictive of neither. (J Thorac Cardiovasc Surg 1999;117:744-50)

Approximately 40% of patients with non-small cell lung cancer (NSCLC) are initially seen with a locally advanced stage of disease (stage III). Even if the tumors are surgically accessible (stage IIIA), survival among patients with advanced disease is poor (5-year survival 5%-10%).¹ Preoperative chemotherapy has

been shown to improve disease-free and overall survivals. The benefits have been directly related to the degree of clinical response to chemotherapy and to subsequent complete surgical resection.^{2,3} The most effective combined treatment regimens for NSCLC are based on cisplatin and induce a relevant clinical response in about 50% of patients.^{4,5} Thus about half of these patients do not benefit oncologically; they do not achieve remission, nor are they able to undergo radical resection after induction treatment. Predictive markers will therefore have major implications for clinical treatment in upcoming randomized trials.

TP53 gene alterations are present in about 50% of NSCLCs, and their prognostic implications have been evaluated in diverse clinical studies.^{6,7} Tumor response to anticancer therapies has been recognized to be largely based on apoptosis induction. Active p53 has emerged as an important modulator of DNA damage-induced apoptosis.⁸ A mutated *TP53* gene could be related to resistance to ionizing radiation and DNA-damaging

From the Departments of Thoracic and Cardiovascular Surgery,^a Internal Medicine,^b Medical Computer Sciences,^c and Clinical Pathology,^d University of Vienna, Waehringer-Guertel 18-20, A-1090 Vienna, Austria.

Supported by the Österreichische Nationalbank, project number 7003.

Received for publication June 17, 1998; revisions requested Aug 10, 1998; revisions received Sept 21, 1998; accepted for publication Oct 19, 1998.

Address for reprints: Daniela Kandioler-Eckersberger, MD, Department of Surgery, University of Vienna-Medical School, Waehringer Guertel 18-20, A-1090 Vienna, Austria.

Copyright © 1999 by Mosby, Inc.

0022-5223/99 \$8.00 + 0 12/1/95291

agents, including etoposide and cisplatin, in vitro.^{9,10} Overexpression of p53 was shown to be linked to an absence of pathologic response in patients with locally advanced NSCLC.¹¹ However, the absence of immunostaining did not necessarily correlate with a response to cisplatin-based chemotherapy in that study. Thus immunostaining does not appear to be reliable as a predictor of response to treatment.

Our study was designed to determine the value of *TP53* genotype for the prediction of chemotherapy response. Because this phenomenon is evaluated on the molecular genetic level, the potential failures of immunohistochemistry are avoided. This article reports the correlation between *TP53* genotype and response to cisplatin-based induction chemotherapy, as well as the correlation with overall survival, among patients with advanced NSCLC (stage IIIA or IIIB). Additionally, we found no association between p53 immunohistochemical characteristics and *TP53* genotype, which explains the absence of a correlation between p53 immunohistochemical results and response.

Materials and methods

The study included patients with stage IIIA and IIIB NSCLC after cisplatin-based induction treatment. Patients eligible for the study were those who, between 1995 and 1997, had undergone subsequent surgical resection or thoracotomy with biopsy after induction treatment. These criteria were selected to enable histopathologic confirmation of response to chemotherapy. Our study therefore does not reflect the response rates observed in randomized studies; it included more patients with responses to chemotherapy than with resistance.

Patients' cancers were staged by mediastinoscopy before chemotherapy. Chemotherapy was administered intravenously and consisted of cisplatin (30 mg/m²) in combination with ifosfamide (1 g/m²), both administered from days 1 to 3 and repeated every 28 days for a total of 3 cycles. Clinical response was assessed by radiography and confirmed by histopathologic studies. Patients with complete or partial remission according to Union Internationale Contre le Cancer criteria were considered to be responders, whereas those with stable or progressive disease were considered to be nonresponders.

Statistical methods. Patients whose disease responded to chemotherapy were compared with those whose disease was resistant to chemotherapy. Associations between chemotherapy response and *TP53* genotype as well as p53 immunohistochemical results were tested with the Fisher exact test. Sensitivity (the probability that normal *TP53* genotype characterizes responders) and specificity (the probability that *TP53* mutant characterizes nonresponders) and corresponding 95% confidence intervals are given. Sensitivity and specificity were calculated for *TP53* immunohistochemical results in predicting mutant or wild type *TP53* genotype. The

Kaplan-Meier estimation of survival time after neoadjuvant therapy for *TP53* wild type (corresponding to responders) and *TP53* mutant (corresponding to nonresponders) genotypes were calculated, and a log-rank test was used to compare the 2 groups.^{12,13}

Tumor tissue samples for *TP53* analysis and histologic examination were obtained before chemotherapy was started. Tissue for *TP53* sequencing was snap frozen and stored in liquid nitrogen until analysis. Total genomic tumor DNA was extracted with a standard phenol-chloroform extraction method.

Genomic DNA (500 ng) was amplified by polymerase chain reaction (PCR) with *TP53*-specific 20-nucleotide primers placed in the adjacent intron regions of exons 2 to 3, 4, 5, 6, 7, 8 to 9, 10, and 11, as previously described.¹⁴

The quality and quantity of amplified DNA were checked on precast 4% to 20% acrylamide and bis-acrylamide gels (Novex, San Diego, Calif). As a reference standard we used pBR322 DNA-*MspI* digest (Clontech Lab Inc, Palo Alto, Calif).

Enzymatic pretreatment of polymerase chain reaction products. Before sequencing of PCR products, residual single-stranded primers and remaining deoxynucleoside triphosphate were removed from the PCR mixture. We subjected a 2- μ L volume of a PCR amplification product to a combination of exonuclease I and shrimp alkaline phosphatase (United States Biochemical, Cleveland, Ohio) and incubated at 37°C for 60 minutes, followed by 15 minutes of enzyme inactivation at 72°C.

Sequencing. Pretreated PCR products were diluted 1:2 and were directly sequenced with the Thermo Sequinase radiolabeled terminator cycle sequencing kit (United States Biochemical, Cleveland, Ohio), which provides 4 radioactively labeled dideoxynucleotide terminators (guanine, adenine, thymine, and cytosine). Because of linkage of radioactive label and chain termination, only properly terminated DNA chains are visible. A 5.5- μ L volume of enzymatically pretreated PCR product was finally added to the reaction mix. Cycle sequencing for linear increase of the DNA product during sequencing reaction was then performed as described in the kit, with deoxyguanosine triphosphate for termination master mix and phosphorus 33-labeled dideoxynucleotides. The cycle sequencing program was identical with the primer-specific PCR amplification program. The reaction was stopped on ice by adding 4 μ L of STOP solution, and samples were stored at -20°C until sequencing gels could be loaded (eg, the next day). After the 8% acrylamide and bis-acrylamide gels were run, the latter were dried at 70° in a vacuum drying apparatus and directly exposed to a film (Bio Max MR; Kodak, New Haven, Conn).

Phosphorus 33 labels and the high sensitivity of the film allowed overnight exposure. The obtained sequences were almost free of background activity, without stop artifacts, and were easy to read. Mutations were confirmed by complete reanalysis.

The p53 immunohistochemical testing was performed according to the method published by Rush and colleagues¹¹

Table I. Clinical data of 24 neoadjuvantly treated patients with NSCLC with respect to TP53 genotype and p53 immunohistochemical results

Case	Genotype	IHC	Histologic type	Preoperative	
				Stage	TN
663	Wild type	ND	Adenocarcinoma	IIIA	T3 N1
856	Wild type	ND	Squamous cell	IIIA	T1 N2
231	Wild type	80	Adenocarcinoma	IIIA	T3 N1
184	Wild type	<10	Squamous cell	IIIA	T3 N2
769	Wild type	80	Squamous cell	IIIB	T2 N3
151	Wild type	90	Squamous cell	IIIA	T2 N2
568	Wild type	<10	Adenocarcinoma	IIIB	T3 N3
377	Wild type	90	Adenosquamous	IIIA	T1 N2
402	Wild type	80	Squamous cell	IIIA	T3 N2
207	Wild type	<10	Large cell	IIIA	T2 N2
423	Wild type	90	Squamous cell	IIIB	T2 N3
140	Wild type	<10	Adenocarcinoma	IIIB	T2 N3
407	Wild type	70	Squamous cell	IIIA	T2 N2
528	Wild type	70	Adenocarcinoma	IIIB	T2 N3
108	Wild type	70	Adenosquamous	IIIA	T3 N0
267	Wild type	<10	Squamous cell	IIIA	T3 N2
42	Mutant	<10	Adenocarcinoma	IIIA	T2 N2
238	Mutant	70	Adenocarcinoma	IIIA	T3 N1
854	Mutant	30	Squamous cell	IIIB	T3 N3
855	Mutant	70	Squamous cell	IIIB	T4 N0
145	Mutant	<10	Squamous cell	IIIB	T3 N2
641	Mutant	>90	Adenocarcinoma	IIIA	T2 N2
777	Mutant	80	Squamous cell	IIIA	T2 N2
657	Mutant	30	Adenocarcinoma	IIIA	T3 N0

IHC, Immunohistochemical result; ND, not determined; CR, complete remission; R0, complete resection; PR, partial remission; R1, marginal resection; SD, stable disease; PD, progressive disease; R2, incomplete resection.

*Diffuse single tumor cells.

†No lymph nodes found.

with the monoclonal antibody pAb1801 (Oncogene, Uniondale, NY). Microscopic examination for the p53 staining product was scored as follows: *negative*, fewer than 10% of cells staining; *positive*, more than 10%.

Results

Table I shows TP53 genotype and p53 immunohistochemical results, listed with preoperative stage and TN classification, clinical response, type of resection, and histopathologic stage, for 24 patients who had received induction chemotherapy for stage III NSCLC. Preoperative stages IIIA and IIIB were equally distributed in the 2 groups. There were no differences in histologic subtype between the 2 groups. Altogether, 11 squamous cell carcinomas, 6 adenocarcinomas, 6 adenosquamous carcinomas, and 1 large cell carcinoma were included. Sixteen patients had major responses (complete or partial remission), whereas eight patients had stable or progressive disease. Of 4 patients who had complete clinical responses, 2 showed complete pathologic remission (8%). The correlation between

TP53 genotype and response is shown in Table II. A normal TP53 genotype was present in all 16 patients who had major responses. A mutant TP53 gene was found in 8 of 8 patients who had clinically stable disease (n = 5) or progressive disease (n = 3). No association between p53 protein expression and TP53 genotype was found (Table II). Sensitivity and specificity of p53 immunohistochemical testing in indicating TP53 genotype were as high as 75% and 35%, respectively. Corresponding 95% confidence intervals were 34.91% to 96.82% and 12.76% to 64.86%, showing a weak sensitivity of p53 immunohistochemical testing in indicating mutant genotype and no specificity of immunohistochemical testing in indicating wild type genotype.

In comparing response to chemotherapy and TP53 genotype, clear associations between normal TP53 genotype and chemotherapy response and between mutant genotype and chemotherapy resistance were observed ($P < .001$, Table II). Sensitivity and specificity of TP53 gene analysis to predict response and non-response were high (both 100% for the small number

<i>Clinical response</i>	<i>pTN</i>	<i>Grading</i>	<i>Operation</i>	<i>Resection</i>
CR	T0 N0	x	Extended pneumonectomy	R0
CR	T0 N0	2-3	Lobectomy	R0
CR	Tx* N0	x	Pneumonectomy	R0
CR	T1 N0	x	Pneumonectomy	R0
PR	T2 N2	2	Pneumonectomy	R0
PR	T0 N1	2	Lobectomy	R0
PR	T3 N0	2	Extended pneumonectomy	R0
PR	T1 N1	3	Bilobectomy	R0
PR	T1 N2	2-3	Lobectomy	R0
PR	T2 N0	3	Lobectomy	R0
PR	T2 N0	1-2	Lobectomy plus vascular sleeve	R0
PR	T2 N1	2	Pneumonectomy	R1
PR	T2 N1	2	Extended pneumonectomy	R1
PR	T2 Nx†	2-3	Lobectomy	R0
PR	T2 N0	3	Lobectomy	R0
PR	T2 N0	2	Pneumonectomy	R0
SD	T2 N2	3	Pneumonectomy	R0
SD	T4 N1	1-2	Extended pneumonectomy	R1
SD	T4 N3	2-3	Biopsy	Biopsy only
SD	T4 N0	2-3	Biopsy	Biopsy only
SD	T4 N2	3	Biopsy	Biopsy only
PD	T4 N2	2	Biopsy	Biopsy only
PD	T3 N2	3	Sleeve resection	R0
PD	T4 N0	2	Lobectomy	R2

of patients included). The corresponding 95% confidence intervals were 82.93% to 100% for sensitivity and 68.77% to 100% for specificity (the wide ranges of the confidence intervals resulted from the small numbers of patients).

Patients were followed up for a median of 27 months. The 75% overall survival time was 28 months among patients whose disease responded to chemotherapy and who had no *TP53* gene mutations, whereas it was 8 months in the group with unresponsive disease and *TP53* mutations. With respect to overall survival the difference between patients with mutated and unmutated tumors was statistically significant ($P = .027$, Fig 1).

The sequence analysis of the *TP53* mutation of patient 855 is shown in Fig 2. All detected mutations are characterized in Table III. The mutations affected the highly conserved regions of the *TP53* gene (exons 5-8) in addition to exons 4 and 10 of the *TP53* gene.

Fourteen of 16 patients in the responder group and 2 of 8 patients in the nonresponder group (both with stage IIIA disease) could undergo complete resection. Four of 8 patients in the nonresponder group had pleural carcinoma at the time of thoracotomy. Three patients in the

nonresponder group with stage IIIB disease underwent thoracotomy to confirm the unresectability suspected on the basis of computed tomographic imaging.

Discussion

Induction chemotherapy has been reported to improve the prognosis of stage III disease in patients with NSCLC.¹⁵⁻¹⁷ From 40% to 50% of patients do not have a response to chemotherapy, however, and to date we have not been able to predict before chemotherapy which patients these will be. The *TP53* mutation frequency has been reported to be as high as 50% in NSCLC.¹⁸ In vitro a correlation between p53 status of tumor cell lines and apoptosis induction in response to cytotoxic treatment has been suggested.¹⁹ Until now, however, no investigation successfully elucidated a possible clinical association between response to chemotherapy and the presence or absence of a normal *TP53* gene. In a clinical evaluation of patients with NSCLC, Rush and associates¹¹ found p53 protein overexpression to be linked to the absence of pathologic response in patients with locally advanced NSCLC, whereas the absence of immunostaining did not neces-

Fig 1. Kaplan-Meier estimation of survival in patients with and without *TP53* mutations. A significant survival advantage was found for patients with normal *TP53* genotype. Patients with normal *TP53* genotype were found to correspond to those with treatment response.

Fig 2. Sequencing analysis showing part of exon 7 of the *TP53* gene from normal and tumor DNA of patient 855 (with NSCLC unresponsive to cisplatin-based therapy). Tumor sequence shows mutation in codon 248, a cytosine to thymine transition (arrow).

sarily correlate with a response to cisplatin-based chemotherapy in that study. This is exactly what we found when we looked at p53 immunohistochemical results and response in our patients—p53 overexpression was present in 6 of 8 tumors in the nonresponder group but also in 9 of 16 tumors that did respond to chemotherapy. When we analyzed the *TP53* genotype, however, we found a direct link both between mutant genotype and resistance and between normal genotype and response.

Table II. Correlations between *TP53* genotype and chemotherapy response and between *TP53* genotype and p53 immunohistochemical result

	Genotype	
	Mutant	Wild type
Response		
Complete or partial remission	0	16
Stable or progressive disease	8	0
Immunohistochemical result		
Positive	5	9
Negative	3	5

Ever since p53 immunohistochemical staining was introduced, it has commonly been used as a fast and easy method. However, the interpretation of staining results remains a controversial subject.^{20,21} For example, deletion and insertion mutations result in truncation of the p53 messenger RNA. Truncated messenger RNA most probably will not be processed into a p53 protein, resulting in a negative immunostaining result despite the presence of a functionally important mutation. Currently available p53 antibodies mainly detect overexpression of p53 protein on the basis of protein stabilization, which is mainly due to point mutation. In addition, p53 overexpression also has been shown to result from *TP53* gene activation and a high translation rate in response to DNA damage, without the presence of a mutation in the gene. High levels of p53 protein therefore may also indicate the gene's functional effort to arrest cell cycling or to initiate apoptosis in cells that are potentially malignant.

Our study shows for the first time that exact predictive information concerning response to cisplatin-based neoadjuvant chemotherapy in patients with NSCLC can be obtained from *TP53* genotype but not from p53 immunohistochemical staining. The abilities of DNA sequencing and immunohistochemical testing to detect mutations in the *TP53* gene were compared in a study on 316 patients with breast cancer. Immunohistochemical testing was found to produce false-negative results in 33% of cases and false-positive results in 30%.²² In addition, *TP53* sequencing data yielded better prognostic information in the reported cohort of patients with breast cancer. In our study the major end point was treatment response, not survival. Because we found a 100% correlation of response with *TP53* genotype, however, we correlated survival and *TP53* genotype to determine whether the established link between survival and response would hold true for our patients. We think that the significant survival advantage we

Table III. Characterization of *TP53* gene mutations

Case	Mutation	Exon	Codon	Normal sequence	Mutant sequence	Amino acid change
42	8-base deletion	4	82-84	5'-ACA CCG GCG GCC CCT-3'	5'-ACA CCC T-3'	Frameshift
238	A-G transition	6	195	5'-CTT ATC CGA GTG-3'	5'-CTT ACC CGA GTG-3'	Ile-Thr
854	C-T transversion	5	163	5'-ATC TAG AAG-3'	5'-ATC TAG AAG-3'	Tyr-STOP
855	C-T transition	7	248	5'-AAC CGG AGG-3'	5'-AAC TGG AGG-3'	Arg-Trp
145	Complex	10			Complex	Frameshift
641	G-T transversion	8	273	5'-GTG CGT GTT TGT-3'	5'-GTG CTT GTT TGT-3'	Arg-Leu
777	1-base deletion	5	151	5'-CCC CCG CCC GG-3'	5'-CC CCG GCC CGG-3'	Frameshift
657	Complex	10			Complex	Frameshift

Ile, Isoleucine; Thr, threonine; Tyr, tyrosine; Arg, arginine; Trp, tryptophan; Leu, leucine.

found in the group with a normal *TP53* genotype additionally confirms our conclusions.

The direct correlation we found between *TP53* genotype and chemotherapy responsiveness may appear surprising, because our study did not deal with other mechanisms of apoptosis induction. However, the most frequent genetic abnormalities in NSCLC are *TP53* and *ras* mutations, with mutation rates of 50% and 30%, respectively.²³ Rosell and colleagues² found no relationship between good response and the absence or presence of *K-ras* mutations, whereas Kishimoto and associates⁶ found that *TP53* mutations and *K-ras* mutations occurred independently in primary NSCLC. Although *c-myc* mutations are rarely seen in NSCLC, other cell cycle-affecting proteins and tumor suppressor genes appear to be, at least in part, under the control of *TP53*.²³ *TP53* may be essential for the pathway of DNA damage-induced apoptosis that is important for the cytotoxic effects of cisplatin and anthracyclins.

In our study the observed *TP53* mutation frequency was 33%, compared with 55% in unselected cases of NSCLC. Because we wanted histopathologic confirmation of clinical response, we included twice as many patients undergoing surgery for response to chemotherapy than patients undergoing surgery despite resistance to chemotherapy (in a randomized trial, one would expect at least 50% not to have a response). The lower mutation frequency was therefore exactly as expected and additionally underlines our findings.

In locally advanced NSCLC a favorable prognosis is linked to response to induction treatment. Because of response rates, however, half of these patients undergo a toxic and expensive treatment without any benefit for survival or quality of life. Some patients (with stage IIIA disease) may even miss the chance of resection because of progression during the course of treatment, whereas optimal response to chemotherapy could result in radical resection of primarily inoperable tumors

(stage IIIB). Limitation of induction treatment to patients who are likely to respond should therefore be the goal. We found that response to neoadjuvant therapy based on cisplatin and ifosfamide was directly related to a normal *TP53* genotype, for the first time providing clinical evidence of the *TP53* dependent cytotoxicity of these substances. Before *TP53* genotype is introduced as a marker for possibly predicting clinical response of NSCLC to neoadjuvant treatment, however, this marker needs to be confirmed in a larger prospective, randomized trial and needs to be tested for other standard therapies.

We thank Jenny Hirsch for her assistance in preparing the manuscript.

REFERENCES

1. Shepherd FA. Future directions in the treatment of lung cancer. *Semin Oncol* 1994;21:48S-62S.
2. Rosell R, Gomez-Codina J, Camps C, Maestre J, Padille J, Canto A, et al. A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small-cell lung cancer. *N Engl J Med* 1994;330:153-8.
3. Eberhardt W, Wilke H, Stamatis G, Stuschke M, Harstrick A, Menker H, et al. Preoperative chemotherapy followed by concurrent chemoradiation therapy based on hyperfractionated accelerated radiotherapy and definitive surgery in locally advanced non-small-cell lung cancer: mature results of a phase II trial. *J Clin Oncol* 1998;16:622-34.
4. Dillman RO, Herndon J, Seagren SL, Eaton WL Jr, Green MR. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of cancer and leukemia Group B trial (CALGB) 8433 Trial. *J Natl Cancer Inst* 1996;88:1210-5.
5. Rosell R, Font A, Pifarre A, Canela M, Aurel J, Arellano A. The role of induction (neoadjuvant) chemotherapy in stage IIIA NSCLC. *Chest* 1996;109:102S-6S.
6. Kishimoto Y, Murakami Y, Shiraishi M, Hayashi K, Sekiya T. Aberrations of the p53 tumor suppressor gene in human non-small cell carcinomas of the lung. *Cancer Res* 1992;52:4799-804.
7. Gazdar AF. Molecular markers for the diagnosis and prognosis of lung cancer. *Cancer* 1992;69:1592-9.

8. Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. *J Natl Cancer Inst* 1996;88:1442-55.
9. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993;74:957-67.
10. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 1993;362:847-52.
11. Rusch V, Klimstra D, Venkatraman E, Oliver J, Martini N, Gralla R, et al. Aberrant p53 expression predicts clinical resistance to cisplatin-based chemotherapy in locally advanced non-small cell lung cancer. *Cancer Res* 1995;55:5038-42.
12. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
13. Peto R, Peto J. Asymptotically efficient rank invariant procedures. *J R Stat Soc* 1972;135:185-207.
14. Lehmann TA, Bennett WP, Metcalf RA, Welsh JA, Ecker J, Modali RV, et al. p53 mutations, *ras* mutation, and p53-heat shock 70 protein complexes in human lung carcinoma cell lines. *Cancer Res* 1991;51:4090-6.
15. Roth JA, Fossella F, Komaki R, Ryan MB, Putnam JB Jr, Lee LS, et al. A randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. *J Natl Cancer Inst* 1994;86:673-80.
16. Rusch VW, Albain KS, Crowley JJ, Rice TW, Lonchyna V, McKenna R, et al. Surgical resection of stage IIIA and stage IIIB non-small-cell lung cancer after concurrent induction chemotherapy: a Southwest Oncology Group trial. *J Thorac Cardiovasc Surg* 1993;105:97-106.
17. Dillman RO, Seagren SL, Propert KJ, Guerra J, Eaton WL, Perry MC, et al. A randomized trial of induction chemotherapy plus high-dose radiation versus radiation alone in stage III non-small-cell lung cancer. *N Engl J Med* 1990;323:940-5.
18. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994;54:4855-78.
19. Kastan MB, Canman CE, Leonard CJ. p53, cell cycle control and apoptosis: implications for cancer. *Cancer Metastasis Rev* 1995;14:3-15.
20. Fisher CJ, Gillett CE, Vojtesek B, Barnes DM, Millis RR. Problems with p53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. *Br J Cancer* 1994;69:26-31.
21. Wynford-Thomas D. p53 in tumor pathology: can we trust immunohistochemistry? *J Pathol* 1992;166:329-30.
22. Sjögren S, Inganäs M, Norberg T, Lindgren A, Nordgren H, Holmberg L, Bergh J. The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. *J Natl Cancer Inst* 1996;88:173-82.
23. Salgia R, Skarin AT. Molecular abnormalities in lung cancer. *J Clin Oncol* 1998;16:1207-17.