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BCL-2 Expression is Prognostic for Improved Survival in Non-small Cell Lung Cancer

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Objective: We used a large patient population to identify immunohistochemical biomarkers to enable improved prognostication in patients with non-small cell lung carcinoma (NSCLC).

Methods: A tissue microarray was constructed using duplicate 0.6 mm cores of formalin-fixed paraffin-embedded tissue blocks from 609 patients with NSCLC. Immunohistochemical was used to detect 11 biomarkers including epidermal growth factor receptor, Her2, Her3, p53, p63, bcl-1, bcl-2, Thyroid transcription factor, carcinoembryonic antigen, chromogranin, and synaptophysin. A clinical database was generated prospectively at the time of tissue collection. Survival outcomes were obtained from a Provincial Cancer Registry database. Univariate and multivariate analyses were performed to look for a relationship between biomarker expression, smoking history, and survival.

Results: Survival data for 535 cases were available. As of June 2005, 429 patients (80%) had died; of these 286 (54%) died of lung cancer, 117 (22%) died of other known causes, and for 26 (5%) the cause of death was not available. Univariate analysis revealed that bcl-2 ($p = 0.007$) was the only biomarker prognostic for improved overall survival (OS). bcl-2 ($p = 0.021$) and p63 ($p = 0.025$) were both found to be prognostic for improved disease-specific survival (DSS). Multivariate analysis (using age and biomarker expression) revealed that bcl-2 expression is prognostic for improved OS ($p = 0.005$) and DSS ($p = 0.021$).

Conclusions: Our results suggest that bcl-2 expression is prognostic for improved OS and DSS in NSCLC. Testing for bcl-2 expression in a prospective study will help to determine its clinical relevance in prognostication.

Key Words: Non-small cell lung cancer, Prognostic markers, Immunohistochemistry, bcl-2, EGFR.

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Lung cancer is the most prevalent of all cancers in North America. Non-small cell lung cancer (NSCLC) comprises the majority of lung cancer and there are a number of factors known to affect prognosis. The most useful prognostic factor is stage of the disease.[1] Other prognostic factors include surgically resectability of the tumor[2] and pathologic features such as tumor size,[3] histologic subtype,[4] lymphatic and blood vessel invasion,[5] and tumor differentiation.[6] Useful clinical prognostic factors include performance statutes and weight loss.[7] Recently, there has been a great deal of interest in using molecular markers to refine prognosis.

Immunohistochemical (IHC) markers in NSCLC have been the subject of a recent review[8] that divided them into several groups based on the proposed function of the molecule. One such group is molecules responsible for the self-sufficiency of growth pathway, and includes epidermal growth factor receptor (EGFR), Her 2, and cyclin D1. Studies of the prognostic value of EGFR expression reported variable results, some suggesting a negative association,[9,10] and other studies failing to find any prognostic significance.[11,12] Studies of Her 2 have also revealed conflicting results.[13–15] Cyclin D1, which causes rearrangement of bcl-1,[16] has been found to be associated with a negative prognosis.[17]

A second group of markers studied are those involved in resistance to apoptosis, particularly p53 and bcl-2. Studies of p53 have found conflicting results regarding prognosis,[18–20] whereas its analogue p63 has been suggested to have a positive prognostic value.[21] The prognostic utility of bcl-2 was recently reviewed in a meta-analysis that concluded that bcl-2 expression is associated with a positive prognosis.[22]

Other markers that have been examined include chromogranin (Ch) and synaptophysin (SNP), both neuroendocrine markers with unclear prognostic significance. Thyroid transcription factor (TTF-1), a lung cancer tumor marker,[23] and carcinoembryonic antigen (CEA)[24] are also potentially useful markers with uncertain prognostic significance.

In a review of IHC markers of prognosis in NSCLC, Zhu et al. described the inconsistent results found with most markers.[8] These findings may be explained by the markers studied thus far having limited prognostic utility, or possibly because of variable methodologies and populations used.[8] Added to these issues are the small sizes of many of the studies, which have been combined in meta-analyses that are difficult to interpret because of methodological variability.

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To address these issues, we took a large patient population and used tissue microarray technology to enable staining of numerous tissue samples at once, thereby minimizing variability associated with multiple staining techniques, to examine 11 biomarkers for their association with prognosis.

METHODS

Case Selection

Cases of primary NSCLC from patients with early stage (stage I and II) disease, diagnosed between 1978 and 2002, were identified from the archives of St. Paul's hospital (A large tertiary center in Vancouver, British Columbia). Other than resectable lung cancer, no other specific selection criteria were used. Tissue blocks were used to construct a duplicate core tissue microarray. Carcinoids, atypical carcinoids, large cell neuroendocrine carcinomas, and metastatic tumors were all excluded.

Tissue Microarray Construction

Tissue microarrays were constructed as previously described.[25] Briefly, areas containing tumor were marked on the paraffin tissue blocks. Sector maps were designed using Microsoft Excel (Redmond, WA) spreadsheets to identify the location of each specimen on the array blocks. Duplicate 0.6-mm tissue cores were used to construct the tissue microarrays with an arraying machine (Beecher Instruments Sun Prairie, WI). Array blocks were sectioned to produce serial 4-μm sections, and the first section was stained with hematoxylin and eosin to assess adequacy. The remaining sections were stored at room temperature for 3 weeks before immunostaining.

Biomarker Selection

Eleven biomarkers were chosen for assessment. The markers were selected based on previous data suggesting possible prognostic utility outlined in the introduction, and antibody availability. The methods and results of the staining for the neuroendocrine markers (Ch and SNP) have been previously reported.[26]

Immunohistochemistry and Scoring

Staining for each marker was conducted using standard antibodies (Table 1). The staining techniques varied according to the manufactures recommendations. All samples were evaluated and scored by two pathologists blinded to outcome information. The scoring was performed independently and the discrepant scores were resolved by double-scoping and discussion of cases in question. A third pathologist was used as an arbitrator for cases in which a consensus could not be reached. The scoring system for the 11 biomarkers is summarized in Table 2. The final results were reported as negative (score = 0), positive (score = 1, 2, or 3), or uninterpretable, with the exception of Her2 staining in which 0 and 1+ are considered negative and 2+ and 3+ positive. Details of Her2 scoring are reported elsewhere.[27] Staining in nontumor cells was not considered. The scores were entered into blank sector maps of the corresponding array at the time of scoring, and uninterpretable results were eliminated from further consideration.

TABLE 1. Biomarker Immunohistochemistry Assays

Biomarker	Supplier	Clone	Dilution	Antigen Retrival
p53	Dako	D0-7	1:400	30 min heat with CC1
p63	Cell Marque	4A4	1:200	30 min heat with CC1
bcl-1	Neomarkers	sp4	1:100	30 min heat with CC1
bcl-2	Dako	124	1:20	30 min heat with CC1
TTF	Dako	8G7G3/1	1:100	30 min heat with CC1
CEA-mono	Ventana	TF-3H8-1	1:5	No antigen retrieval
EGFR	Zymed	31G7	1:20	Protease 2–24 min
Her2	Neomarkers	sp3	1:500	30 min heat with CC1
Her3	Neomarkers	poly	1:10	30 min heat with CC1

Data Analysis and Statistics

Data on the score sheets were converted into an Excel spreadsheet format using the TMA-Deconvoluter program as previously described.[28] Score results for the duplicate cores were consolidated into 1 score with higher positive staining results always superseding weaker positive, negative, or uninterpretable staining results.

Survival estimates were calculated for each outcome (overall survival (OS) and disease-specific survival (DSS)), and a log-rank statistic was used to test for differences between groups. Log-rank statistics were used to look for any relationships between biomarker expression and survival. A significant difference was declared if the p value from a two-tailed test was less than 0.05. Multivariate analysis using age, and biomarker expression were tested using cox regression analysis. Statistical calculations were performed using SPSS 13.0 software (SPSS, Chicago, IL).

RESULTS

The tissue microarray was constructed from 609 patients with early stage (stage I and II) NSCLC who had their tumors resected between 1978 and 2002 (Table 3). A total of 588 of 609 cases were available for inclusion in the analysis. Hematoxylin–eosin stained sections were reviewed and subclassified as follows: 243 adenocarcinoma (ACA), 272 squamous cell carcinoma (SCC), 35 large cell carcinoma, 32 non-small cell carcinoma NOS, and 6 other (carcinoma, giant cell carcinoma). Twenty-one cases were excluded because on review, they were not considered to represent primary lung tumor (12 cases), exhibited neuroendocrine differentiation (three cases) or no pathologic diagnosis was available (six cases). Table 4 provides a summary of the scoring results for each biomarker. The results for Ch and SNP have been previously reported.[26] No prognostic significance was noted for these two neuroendocrine markers.

Outcome data were available for 535 patients up to 26.5 years (median 3.52 years; range 34–9696 days) and these were used for survival analysis. As of June 2005, 429 patients (80%) had died; of these 286 (54%) died of lung cancer, 117 (22%) died of other known causes, and for 26 (5%) the cause of death was not available (Table 3). The latter cases were excluded from the DSS analysis.

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TABLE 2. Antibody Scoring System

Stain Name	Staining Pattern	Score 0	Score 1	Score 2	Score 3
p53	Nuclear	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
p63	Nuclear	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
Bcl-1	Cytoplasmic	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
Bcl-2	Cytoplasmic	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
TTF1	Nuclear	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
CEA	Cytoplasmic	<5	5–50 weak/moderate	>5 strong OR >50 weak/moderate	N/A
EGFR	Nuclear/cytoplasmic	<5	5–25	25–75	>75
Her2	Membranous (see methods)	N/A	N/A	N/A	N/A
Her3	Nuclear/cytoplasmic	0	Any cells, weak	Any cells, strong	N/A

TABLE 3. Patient Characteristics (Outcomes as of June 2005)

Number (#) of male patients; Number of female patients	400; 209
Age of diagnosis (mean; range)	63; 35–82
Ever smokers (#; percent)	438; 95%
$n = 459$	
No. of years of smoking (mean; range)	40; 0–69
Total # of patients with outcome data	535
Median survival (yr)	3.5 yr
Range of survival (d)	34–9673 d
Follow up (mean, range of yr)	5.8; 4–26.5
No. of living	106 (20%)
No. of deceased	429 (80%)
No. of died from lung cancer	286 (54%)
No. of died of other known causes	117 (22%)
No. of with cause of death unavailable	26 (5%)

TABLE 4. Biomarker Expression Scoring Results (See Table 2 for a Description of the Scoring System)

Biomarker	Score: 0 (%)	Score: 1 (%)	Score: 2 (%)	Score: 3 (%)
p53 ($n = 486$)	46.4	21.1	32.4	N/A
p63 ($n = 476$)	64.2	14.3	21.6	N/A
Bcl-1 ($n = 471$)	25.6	33.1	41.3	N/A
Bcl-2 ($n = 470$)	72.1	13.0	14.9	N/A
TTF ($n = 482$)	67.3	11.4	21.3	N/A
CEA ($n = 473$)	55.7	23.0	21.3	N/A
EGFR ($n = 477$)	25.9	14.9	33.5	25.7
Her 2 ($n = 473$)	86.3	10.5	2.3	0.9
Her 3 ($n = 475$)	47.5	49.4	3.1	N/A

Univariate analysis was performed to look for an association between biomarker expression and both OS and DSS. Statistically significant associations were found for bcl-2 with OS and for both bcl-2 and p63 with DSS (Table 5). Of 470 samples successfully stained for bcl-2, 131 (28%) had positive expression for bcl-2 (59/194 adenocarcinoma, 59/217 squamous cell carcinoma, 7/25 NSCLC not otherwise specified, 6/30 large cell carcinoma, 0/4 other). Of the 470 samples, 451 had outcome data available. bcl-2 was the only biomarker found to have a statistically significant correlation with OS ($p = 0.007$) (Figure 1) and DSS ($p = 0.021$) (Figure 2).

A total of 476 samples were successfully stained for p63, of which 171 (36%) positively expressed p63 (14/199 adenocarcinoma, 148/218 squamous cell carcinoma, 5/25 NSCLC not otherwise specified, 3/30 large cell carcinoma, 1/4 other). Of the 476 samples, 457 had outcome data available. p63 expression was found to be associated with DSS ($p = 0.025$) (Figure 3). Four hundred seventy-eight samples were successfully stained for EGFR. A total of 353/477 (74%) expressed EGFR (118/200 adenocarcinoma, 191/217 squamous cell carcinoma, 19/25 NSCLC not otherwise specified, 23/31 large cell carcinoma, 2/4 other). Of the 477 samples, 459 had outcome data available. EGFR expression was not associated with OS or DSS in all NSCLC.

There were no significant associations between biomarker expression and survival (OS and DSS) noted within histologic subtypes (other than what would be expected by chance alone), (Tables 6 and 7). For bcl-2, the relationship between expression and OS was not significant within histologic subtypes, other than in the small group of NSCLC not otherwise specified adenocarcinoma ($p = 0.087$), squamous cell carcinoma ($p = 0.14$), NSCLC not otherwise specified ($p = 0.035$), large cell carcinoma ($p = 0.162$). These findings may be related to the smaller sample size within the individual histologic subgroups.

A multivariate analysis model using age and biomarker staining pattern was used to look for an association between biomarker expression and OS. The only biomarker found to have a significant association with survival was bcl-2. After allowing for age, bcl-2 expression remained significantly associated with increased survival, both OS ($p = 0.005$) and DSS ($p = 0.021$) for all NSCLC. A further multivariate analysis was conducted including age, gender, biomarker expression, and histologic subtype. Stage was not included in this analysis, because although all patients were early stage, detailed staging information (stage I versus II) was not available for most patients. The results again confirmed that bcl-2 expression was associated with OS ($p = 0.004$) and DSS ($p = 0.018$).

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TABLE 5. Univariate Analysis of the Prognostic Significance of Biomarkers on Overall Survival (OS) and Disease-Specific Survival (DSS)

Biomarker	Association with OS (All Non-small Cell Lung Cancer) (*P*)	Association with DSS (All Non-small Cell Lung Cancer) (*P*)
p53 (n = 467)	0.65	0.137
p63 (n = 457)	0.29	0.025 (longer DSS)
Bcl-1 (n = 452)	0.054	0.434
Bcl-2 (n = 451)	0.007 (longer OS)	0.021 (longer DSS)
TTF (n = 463)	0.188	0.799
CEA (n = 454)	0.2	0.443
EGFR (n = 459)	0.233	0.809
Her 2 (n = 455)	0.611	0.635
Her 3 (n = 457)	0.83	0.214

OS, overall survival; DSS, disease-specific survival.

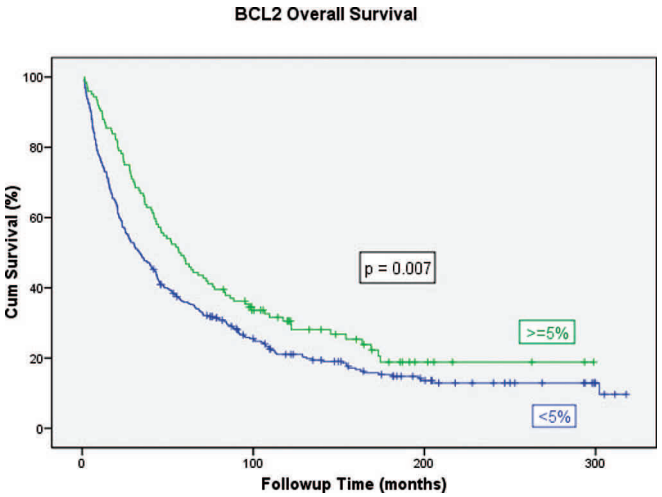

FIGURE 1. Kaplan Meier curve of univariate analysis of bcl-2 expression and overall survival (Total n = 451; ≥5% was interpreted as positive staining [n = 124]; <5% was interpreted as negative staining [n = 327]).

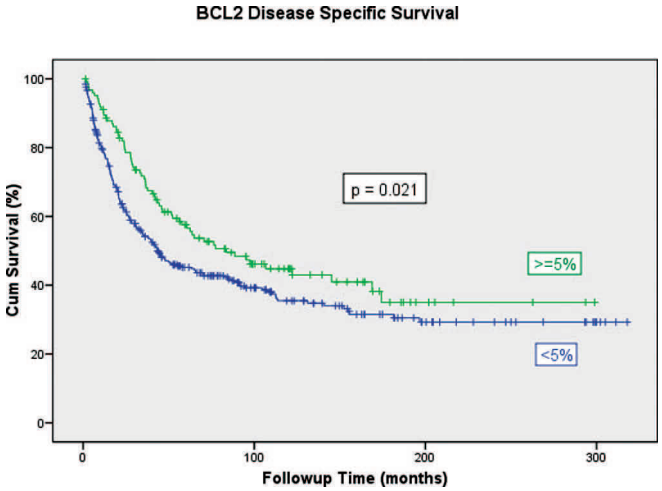

FIGURE 2. Kaplan Meier curve of univariate analysis of bcl-2 expression and disease-specific survival (Total n = 451; ≥5% was interpreted as positive staining [n = 124]; <5% was interpreted as negative staining [n = 327]).

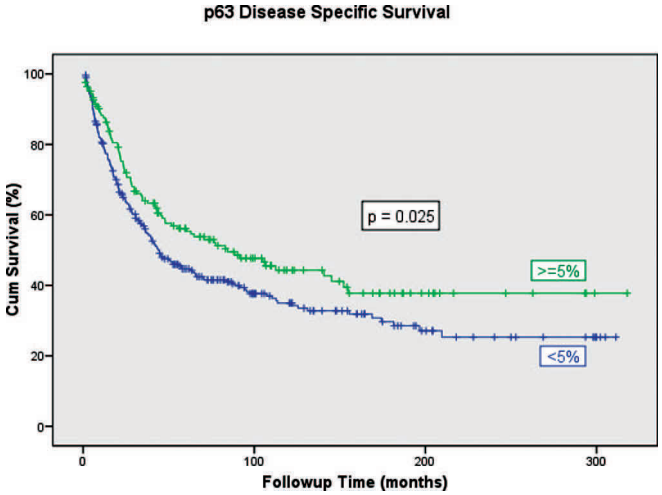

FIGURE 3. Kaplan Meier curve of univariate analysis of p63 expression and disease-specific survival (Total n = 457; ≥5% was interpreted as positive staining [n = 165]; <5% was interpreted as negative staining [n = 292]).

DISCUSSION

A NSCLC tissue microarray was used to investigate the associations between IHC markers and survival. Univariate analyses demonstrated significant associations for bcl-2 with OS, and both bcl-2 and p63 with DSS. After allowing for the effect of age, only the association with bcl-2 expression remained.

There has been extensive research on IHC biomarkers in NSCLC with limited success in identifying markers that have clinical utility. To date, none of the markers tested have significant prognostic utility, which may reflect heterogeneity in the methods used in these studies. Specifically, this relates to variability in antibody use, scoring methods, and the different disease stages of patients included. This heterogeneity is especially important when interpreting the numerous meta-analyses that have been performed in this area. Through the use of tissue microarray technology, we were able to overcome many of these issues and study a large population to identify several biomarkers that seem to have prognostic utility, the most compelling being bcl-2.

bcl-2 is a proto-oncogene involved in cellular apoptosis. A meta-analysis performed in 2003 suggested that overexpression of bcl-2 in NSCLC was associated with improved survival.[22] Using univariate analysis, we confirmed this, finding bcl-2 expression to be associated with both OS (p = 0.007) and DSS (p = 0.021). Multivariate analysis using age and marker expression also revealed an association between bcl-2 expression and both OS (p = 0.012) and DSS (p = 0.029). These results suggest that bcl-2 expression is prognostic for improved survival. In contrast, none of the other biomarkers we examined demonstrated a strong association with survival. Overexpression of p63, an analogue of p53, has

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TABLE 6. Univariate Analysis of the Prognostic Significance of Biomarkers on Overall Survival (OS) Based on Histology

Biomarker	Adenocarcinoma (*P*)	Squamous Cell Carcinoma (*P*)
p53	0.28	0.41
p63	0.92	0.35
Bcl-1	0.003	0.24
Bcl-2	0.087	0.14
TTF	0.052	0.52
CEA	0.02	0.82
EGFR	0.27	0.113
Her 2	0.766	0.663
Her 3	0.773	0.364

TABLE 7. Univariate Analysis of the Prognostic Significance of Biomarkers on Disease-Specific Survival (DSS) Based on Histology

Biomarker	Adenocarcinoma (*P*)	Squamous Cell Carcinoma (*P*)
p53	0.21	0.72
p63	0.81	0.039
Bcl-1	0.2	0.545
Bcl-2	0.28	0.112
TTF	0.44	0.761
CEA	0.06	0.633
EGFR	0.44	0.012
Her 2	0.53	0.951
Her 3	0.76	0.085

EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; TTF, Thyroid transcription factor.

previously been demonstrated to be associated with improved survival.[21] We also found p63 expression to be associated with DSS in all NSCLC ($p = 0.025$) using univariate analysis, but this association was no longer present after allowing for the effect of age. We did not find any association of EGFR expression and survival, in keeping with the findings of a previous meta-analysis.[12]

It is unclear why expression of bcl-2 is associated with improved prognosis. On the basis of the current understanding of bcl-2 function, one would expect that bcl-2 expression would enable cells to escape apoptosis and therefore would be associated with a worse prognosis. One possible explanation for why bcl-2 expression may be associated with a good prognosis comes from the breast cancer literature. In breast cancer, bcl-2 expression has also been associated with improved prognosis. Martinez-Arribas et al.[29] recently published a study demonstrating that bcl-2 expression is associated with biologic features of tumors, which define a better prognosis (in breast cancer these include hormone receptor expression, absence of c-erb-B2, and mutant p53 expression). Therefore, although bcl-2 may function to promote tumor growth, its expression may be associated with other features of tumors that define a more favorable prognosis.

There is interest in the targeted therapies against bcl-2, and there is ongoing research regarding this therapeutic modality in various tumor sites.[30] In the future, bcl-2 may be found to be a useful predictive marker to predict response to these therapies. There is also a chance that bcl-2 expression may have a predictive role regarding response to nontargeted therapies. There is preliminary evidence[31] that p53 expression predicts response to vinorelbine and cisplatin. Further studies are required to demonstrate whether bcl-2 expression has any predictive role.

The major strength of this study is the use of a large patient population to investigate biomarkers that may have prognostic utility. Because of the large size, we are able to avoid the heterogeneity that has made it difficult to interpret meta-analyses of smaller studies. Our study provides compelling evidence that bcl-2 is an important prognostic marker in NSCLC, as it is associated with improved OS and DSS. p63 may also have a prognostic role although our results are less definitive. This study was limited in that no postsurgical treatment information was available for the patients included in the database, which may have contributed to differences in survival. In addition, detailed staging information (stage I versus II) was not available for most patients. A prospective study is required to determine the clinical relevance of these biomarkers, and our results suggest that bcl-2 is worthy of such a trial. The hope for the future is that biomarkers such as bcl-2 can be used to individualize prognosis and potentially guide therapeutic decisions.

REFERENCES

1. Mountain CF. Revisions in the international system for staging lung cancer. *Chest* 1997;111:1710–1717.
2. Flehinger BJ, Kimmel M, Melamed MR. The effect of surgical treatment on survival from early lung cancer. Implications for screening. *Chest* 1992;101:1013–1018.
3. Lipford EH III, Eggleston JC, Lillemoe KD, et al. Prognostic factors in surgically resected limited-stage, nonsmall cell carcinoma of the lung. *Am J Surg Pathol* 1984;8:357–365.
4. Gail MH, Eagan RT, Feld R, et al. Prognostic factors in patients with resected stage I non-small cell lung cancer. A report from the Lung Cancer Study Group. *Cancer* 1984;54:1802–1813.
5. Macchiarini P, Fontanini G, Hardin MJ, et al. Blood vessel invasion by tumor cells predicts recurrence in completely resected T1 N0 M0 non-small-cell lung cancer. *J Thorac Cardiovasc Surg* 1993;106:80–89.
6. Takise A, Kodama T, Shimosato Y, et al. Histopathologic prognostic factors in adenocarcinomas of the peripheral lung less than 2 cm in diameter. *Cancer* 1988;61:2083–2088.
7. Stanley KE. Prognostic factors for survival in patients with inoperable lung cancer. *J Natl Cancer Inst* 1980;65:25–32.
8. Zhu C-Q, Shih W, Ling C-H, Tsao M-S. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. *J Clin Pathol* 2006;59:790–800.
9. Meert AP, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J* 2002;20:975–981.
10. Niemiec J, Kolodziejski L, Dyczek S. EGFR LI and Ki-67 LI are independent prognostic parameters influencing survivals of surgically treated squamous cell lung cancer patients. *Neoplasma* 2005;52:231–237.
11. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003;21:3798–3807.
12. Nakamura H, Kawasaki N, Taguchi M, et al. Survival impact of epidermal growth factor receptor overexpression in patients with non-small cell lung cancer: a meta-analysis. *Thorax* 2006;61:140–145.
13. Nakamura H, Kawasaki N, Taguchi M, et al. Association of HER-2

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overexpression with prognosis in nonsmall cell lung carcinoma: a metaanalysis. *Cancer* 2005;103:1865–1873.
14. Au N H, Cheang M, Huntsman DG, et al. Evaluation of immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers. *J Pathol* 2004;204:101–109.
15. Pelosi G, Del Curto B, Dell'Orto P, et al. Lack of prognostic implications of Her-2/neu abnormalities in 345 stage 1 non-small cell carcinomas (NSCLC) and 2007 stage I-III neuroendocrine tumors (NET) of the lung. *Int J Cancer* 2005;113:101–108.
16. Yang WI, Zukerberg LR, Motokura T. Cyclin D1 (Bcl-1, PRAD1) protein expression in low-grade B-cell lymphomas and reactive hyperplasia. *Am J Pathol* 1994;145:86–96.
17. Caputi M, Groeger AM, EspositoV. Prognostic role of cyclin D1 in lung cancer. *Am J Respir Cell Mol Biol* 1999;20:746–750.
18. Steels E, Paesmans M, Berghmans T, et al. Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. *Eur Respir J* 2001;18:705–719.
19. Mitsudomi T, Hamajima N, Ogawa M, et al. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. *Clin Cancer Res* 2000;6:4055–4063.
20. Tammemagi MC, McLaughlin JR, Bull SB. Meta-analyses of p63 tumor suppressor gene alterations and clinicopathological features in resected lung cancers. *Cancer Epidemiol Biomarkers Prev* 1999;8:625–634.
21. Massion PP, Taflan PM, Rahman SM, et al. Significance of p63 and overexpression in lung cancer development and prognosis. *Cancer Res* 2003;63:7113–7121.
22. Martin B, Peasmans M, Berhmans T, et al. Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* 2003;89:55–64.
23. Martins SJ, Ho VL, Capelozzi TY, et al. Thyroid transcription factor-1 and matrix metalloproteinase-9 with lung adenocarcinoma. *J Clin Oncol* 2005 ASCO Annual Meeting Proceedings supplement 2005;7207.
24. Takamochi K, Nagai K, Suzuki K. Clinical predictors of N2 disease in non-small cell lung cancer. *Chest* 2000;117:1577–1582.
25. Pelosi G, Pasini F, Sonzogni A, et al. Prognostic implications of neuroendocrine differentiation and hormone production in patients with stage I nonsmall cell lung carcinoma. *Cancer* 2003;97:2487–2497.
26. Ionescu D, Treaba D, Gilks CB, et al. Nonsmall cell lung carcinoma with neuroendocrine differentiation—an entity of no clinical or prognostic significance. *Am J Surg Pathol* 2007;31:26–32.
27. Wang S, Saboorian H, Frenkel E, et al. Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: comparison of immunohistochemistry assay with fluorescence in situ hybridization assays. *J Clin Pathol* 2000;53:374–381.
28. Ruckdeschel J, Linnoila RI, Mullshine JL, et al. The impact of neuroendocrine and epithelial differentiation on recurrence and survival in patients with lung cancer [Abstract]. *Proc Am Soc Clin Oncol* 1991;10:248.
29. Martinez-Arribas F, Alvarez T, Del Val G, et al. Bcl-2 expression in breast cancer: a comparative study at the mRNA and protein level. *Anticancer Res* 2007;27:219–222.
30. Manion MK, Fry J, Schwartz PS, Hockenbery DM. Small-molecule inhibitors of Bcl-2. *Curr Opin Invest Drugs* 2006;7:1077–1084.
31. Tsao MS, Aviel-Ronen S, Ding K. P53 protein over-expression but not *p53* gene mutation is a poor prognostic marker and a predictive marker for survival benefit from adjuvant chemotherapy in non-small cell lung cancer (NSCLC) in the JBR. 10 Trial. *J Clin Oncol* 2007; 18S (20 Suppl), 2007:7577.

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