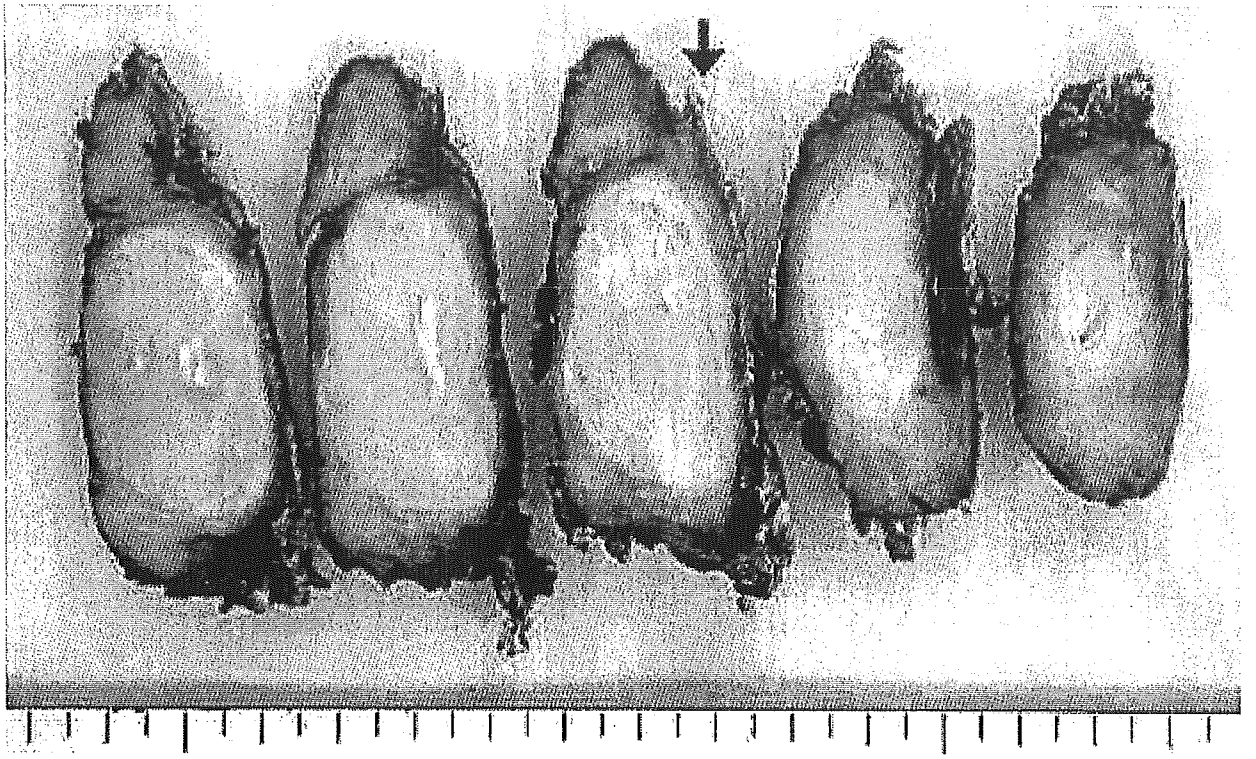


Figure 3
Macroscopic findings of the tumor. The tumor showing the continuity of the vagus nerve (arrow head) was whitish in color and oval shaped while measuring 5 × 3 cm in diameter.

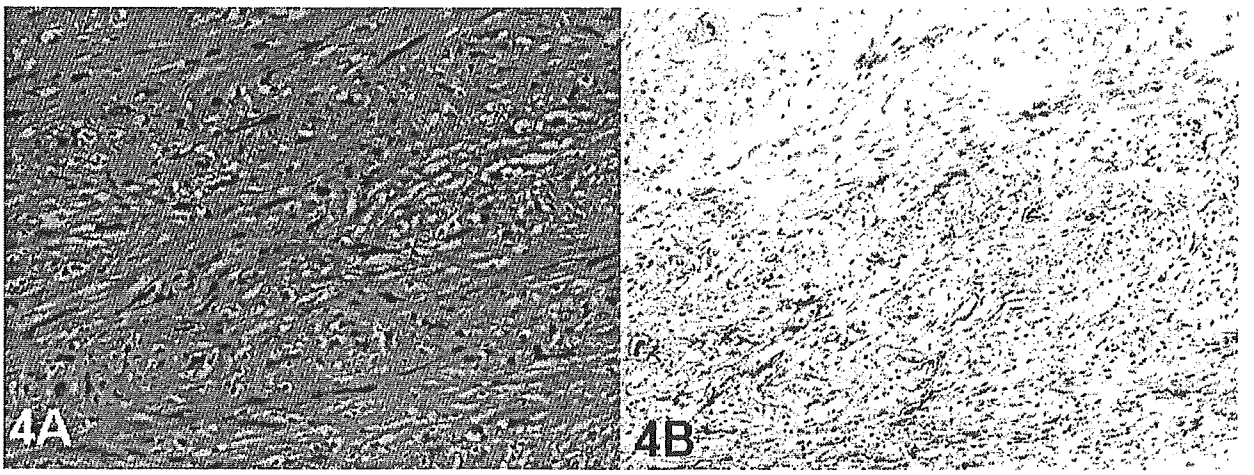


Figure 4
Histological findings of the tumor. 4A) The tumor was found to consist of a few of viable atypical spindle cells with hyperchromatic nuclei (Hematoxilin-eosin staining x200) 4B). Tumor cells were positive for S-100 protein (immunohistochemical staining, original magnification: X200).

multimodality treatment proved to be effective and the patient is now doing well without any recurrence.

We initially misdiagnosed this patient's disease to be non-small cell lung cancer. The reason for this was partly due to the cytological findings which indicated undifferentiated carcinoma. In general, an exact diagnosis cannot always be made based on the findings of aspiration cytology alone. The second reason for a misdiagnosis in this case was due to the patient's symptoms which included hoarseness and Horner's syndrome. Apical lung cancer involving both the vagus and the sympathetic nerve is occasionally observed. However, to the best of our knowledge, the present case is considered to be the first case demonstrating malignant schwannoma of the vagus nerve involving the sympathetic nerve.

Conclusion

Malignant schwannoma of the upper mediastinum arising from the vagus nerve is rare. The multimodality treatment administered in this case, including induction chemo-radiotherapy and surgery, proved to be effective.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FS: conceived of the study, participated in its design and coordination and drafted the manuscript.

RM and TO: carried out the literature search and helped in drafting the manuscript.

HW: participated in the design of the study and helped in drafting the manuscript.

KN: performed histological examination and provided photographs.

YI: shaped the idea for the study, coordinated the study and edited the manuscript.

All authors read and approved the final manuscript.

Acknowledgements

We thank Mr. Brian Quinn for critical comments on the manuscript.

Written consent was obtained from the patient for the publication of this case.

References

1. Davis RD, Oldham HN, Sabiston DC: **Primary cysts and neoplasms of the mediastinum: recent changes in clinical presentation, methods of diagnosis, management, and results.** *Ann Thorac Surg* 1987, 44:229-237.
2. Travis WD, Colby TV, Corrin B: **World Health Organization International Histological Classification of Tumors. Histological typing of Lung and Pleural Tumors, 3 rd ed.** Berlin: Springer Verlag; 1999.
3. Ichinose Y, Fukuyama Y, Asoh H, Ushijima C, Okamoto T, Ikeda J, Okamoto J, Sakai M: **Induction chemoradiotherapy and surgical resection for selected stage IIIB non-small cell lung cancer.** *Ann Thorac Surg* 2003, **76:**1810-1814.
4. Sugio K, Inoue T, Inoue K, Tateishi M, Ishida T, Sugimachi K: **Neurogenic tumors of the mediastinum originated from the vagus nerve.** *Eur J Surg Oncol* 1995, **21:**214-216.
5. Maebeya S, Miyoshi S, Fujiwara K, Sekii H, Suzuka T, Yoshimasu T, Naito Y, Nishino E: **Malignant schwannoma of the intrathoracic vagus nerve:report of a case.** *Surg Today* 1993, **23:**1078-1080.
6. Yano T, Hara N, Ichinose Y, Maeda K, Yokoyama H, Ohta M: **An intrathoracic vagus nerve schwannoma invading the trachea.** *Surg Today* 1993, **23:**1113-1115.
7. Suzuki H, Yamaguchi Y, Kimura H, Shiba M, Baba M, Hiroshima K: **Malignant mediastinal schwannoma associated with von Recklinghausen's disease.** *J Jpn Assn Thorac Surg* 1996, 44:864-868.
8. Marchevsky AM: **Mediastinal tumors of peripheral nerve system origin.** *Semin Diagn Pathol* 1999, **16:**65-78.
9. Lodding P, Kindblom LG, Angervall L: **Epithelioid malignant schwannoma. A study of 14 cases.** *Virchows Arch* 1986, 409 A:433-451.
10. Fukai I, Masaoka A, Yamakawa Y, Niwa H, Eimoto T: **Mediastinal malignant epithelioid schwannoma.** *Chest* 1995, **108:**574-575.

Activating Mutations in the Tyrosine Kinase Domain of the Epidermal Growth Factor Receptor Are Associated with Improved Survival in Gefitinib-Treated Chemorefractory Lung Adenocarcinomas

Miguel Taron,[1] Yukito Ichinose,[3] Rafael Rosell,[1] Tony Mok,[5] Bartomeu Massuti,[7] Lurdes Zamora,[1] Jose Luis Mate,[2] Christian Manegold,[8] Mayumi Ono,[4] Cristina Queralt,[1] Thierry Jahan,[9] Jose Javier Sanchez,[10] Maria Sanchez-Ronco,[10] Victor Hsue,[6] David Jablons,[9] Jose Miguel Sanchez,[1] and Teresa Moran[1]

Abstract

Purpose: Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) confer a strong sensitivity to gefitinib, a selective tyrosine kinase inhibitor of EGFR.

Experimental Design: We examined *EGFR* mutations at exons 18, 19, and 21 in tumor tissue from 68 gefitinib-treated, chemorefractory, advanced non–small cell lung cancer patients from the United States, Europe, and Asia and in a highly gefitinib-sensitive non–small cell lung cancer cell line and correlated their presence with response and survival. In addition, in a subgroup of 28 patients for whom the remaining tumor tissue was available, we examined the relationship among *EGFR* mutations, CA repeats in intron 1 of *EGFR*, *EGFR* and *caveolin-1* mRNA levels, and increased *EGFR* gene copy numbers.

Results: Seventeen patients had *EGFR* mutations, all of which were in lung adenocarcinomas. Radiographic response was observed in 16 of 17 (94.1%) patients harboring *EGFR* mutations, in contrast with 6 of 51 (12.6%) with wild-type *EGFR* ($P < 0.0001$). Probability of response increased significantly in never smokers, patients receiving a greater number of prior chemotherapy regimens, Asians, and younger patients. Median survival was not reached for patients with *EGFR* mutations and was 9.9 months for those with wild-type *EGFR* ($P = 0.001$). *EGFR* mutations tended to be associated with increased numbers of CA repeats and increased *EGFR* gene copy numbers but not with *EGFR* and *caveolin-1* mRNA overexpression (P = not significant).

Conclusions: The presence of *EGFR* mutations is a major determinant of gefitinib response, and targeting EGFR should be considered in preference to chemotherapy as first-line treatment in lung adenocarcinomas that have demonstrable *EGFR* mutations.

Platinum-based chemotherapy as first-line treatment in advanced non–small cell lung cancer (NSCLC) yields limited survival benefit. A retrospective analysis of advanced NSCLC patients showed that response rates decreased with each successive chemotherapy regimen: first line, 21%; second line, 16%; third line, 2%; fourth line, 0% (1). Aberrant epidermal growth factor receptor (EGFR) signaling limits sensitivity to anticancer agents, and ligand-independent tyrosine kinase activation of EGFR, often caused by EGFR mutations in the extracellular domain, has been observed in various tumor types, including glioblastoma multiforme (2). Pharmacologic inhibitors of EGFR, such as gefitinib (Iressa), disrupt EGFR activity by binding the ATP pocket within the catalytic domain containing a critical ATP-binding site, Lys^{745} (K745). Gefitinib and related tyrosine kinase inhibitors occasionally yield dramatic and durable "Lazarus responses" (3), yet response rates are variable, with higher rates in patients with adenocarcinoma, female gender, Asian origin, and never-smoker status (4, 5).

The value of EGFR inhibitors as an NSCLC treatment approach has been limited by the lack of reliable methods for predicting which patients are likely to respond. The logical supposition that tumors overexpressing EGFR would respond best to EGFR inhibitors has not been borne out either in preclinical models(6, 7) or in clinical trials (8, 9). However, recent discoveries of *EGFR* mutations in the tyrosine kinase

Authors' Affiliations: [1]Catalan Institute of Oncology, [2]Pathology Department, Hospital Germans Trias i Pujol, Badalona, Spain; [3]National Kyushu Cancer Center, [4]Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [5]Prince of Wales Hospital, [6]St. Teresa Hospital Cancer Centre, Hong Kong, China; [7]Hospital General de Alicante, Alicante, Spain; [8]Heidelberg University Medical Center, Mannheim, Mannheim, Germany; [9]Comprehensive Cancer Center, University of California, San Francisco, California; and [10]Autonomous University of Madrid, Madrid, Spain

Received 12/20/04; revised 3/15/05; accepted 3/21/05.

Grant support: Spanish Ministry of Health grants provided through Red Temática de Investigación Cooperativa de Centros de Cáncer (CO-010) and Red de Centros de Epidemiología y Salud Pública and La Fundació Badalona Contra el Càncer and La Fundación Carvajal.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: The sponsors of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Requests for reprints: Rafael Rosell, Medical Oncology Service, Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Ctra Canyet, s/n 08916 Barcelona, Spain. Phone: 34-93-497-8925; Fax: 34-93-497-8950; E-mail: rrosell@ns.hugtip.scs.es.

doi:10.1158/1078-0432.CCR-04-2618

domain have shed light on the relationship between EGFR and sensitivity to both gefitinib and the related kinase inhibitor erlotinib. Accumulated data from three studies (10–12) show that 25 of 31 (81%) tumors from NSCLC patients with partial response or marked clinical improvement contained mutations in the *EGFR* tyrosine kinase domain. In contrast, none of 29 specimens from patients refractory to EGFR inhibitors had such mutations ($P < 0.0001$). The mutations included small in-frame deletions (746-750) adjacent to K745 (ELREA amino acids) and missense mutations, mainly L858R adjacent to the DFG motif in the COOH-terminal lobe in the activation loop of the kinase (10–12). These *EGFR* mutations are bona fide somatic mutations in NSCLC and have not been identified in other primary tumor types (10, 13, 14), with the exception of colorectal tumors. One of 293 tumors contained a G719S point mutation (15) that had previously been reported in NSCLC (11), and recently, 4 of 33 tumors harbored point mutations in exons 19 and 20 (16). *In vitro* studies of lung cancer cell lines with endogenous *EGFR* mutations displayed elevated activation of downstream antiapoptotic targets like AKT and signal transducer and activation of transcription (STAT5 and STAT3), conferring enhanced gefitinib sensitivity and increased cisplatin resistance (17).

The transcription activity of the *EGFR* gene is closely related to the enhancer region in intron 1 that is located near a polymorphic CA single sequence repeat containing 14 to 21 CA dinucleotides. Decreased numbers (<19) of CA dinucleotides in this CA sequence correlate with increased *EGFR* transcription (18, 19), and in breast cancer, this CA sequence is a frequent target for *EGFR* gene alterations (20). Moreover, interethnic studies have found that Japanese breast cancer patients carry increased numbers (>19) of CA dinucleotides than Caucasian patients (20). It has been shown that the number of repeats itself affects the mutation rate of nucleotide repeats (21).

A variety of cell surface receptors, including EGFR, as well as intracellular signaling molecules, are concentrated in specialized plasma membrane domains known as caveolae (22). *Caveolin-1* mRNA expression is elevated in multidrug-resistant cultured cancer cells (23), and up-regulation of caveolin-1 and caveolae organelles has been observed in drug-resistant human and ovarian cancer cell lines (24). In addition, high *caveolin-1* mRNA expression has been observed in potentially chemoresistant NSCLC cell lines established from metastatic NSCLCs (25). We therefore hypothesized that tumors harboring EGFR mutations might be associated with higher levels of *caveolin-1* mRNA.

In the present study, we examined *EGFR* mutations in tumor tissue from gefitinib-treated, chemorefractory, advanced NSCLC patients from the United States, Europe, and Asia and in a highly gefitinib-sensitive NSCLC cell line (26) and correlated their presence with response and survival. In addition, in a subgroup of patients for whom remaining tumor tissue was available, we examined the relationship among *EGFR* mutations, number of CA repeats, *EGFR* and *caveolin-1* mRNA levels, and increased *EGFR* gene copy numbers.

Materials and Methods

Patients. Patients with pretreated NSCLC received gefitinib, based on the attending oncologist's decision at the time of chemotherapy failure, at a daily dose of 250 mg given until disease progression. Patients were selected for the present study based on the availability of tumor tissue, without scoring tumor response at the time of selection. Acquisition of tissue specimens and examination of clinical records was approved by the ethics committees of participating institutions. A total of 68 patients were included: 32 Asians (19 Japanese and 13 Chinese) and 36 Caucasians (23 Spanish, nine German, three North American, and one English patient resident in Hong Kong). Assessment of *EGFR* mutations was done for all 68 patients. After this initial analysis, sufficient genomic DNA remained to perform additional related analyses in a subgroup of 28 patients.

Patients were divided into smokers and nonsmokers (having smoked <100 cigarettes in their lifetimes; ref. 27). Tumor response was defined according to the Response Evaluation Criteria in Solid Tumors (28). Survival was calculated from the start of gefitinib treatment. Follow-up was calculated from the start of gefitinib treatment; median follow-up was 11.4 months (range, 1.7-40.3 months).

Epidermal growth factor receptor sequencing. Pure tumor genomic DNA was derived from paraffin-embedded tissue obtained by laser capture microdissection (Palm, Oberlensheim, Germany). For isolation of DNA from deparaffinated, microdissected tissue, the material was incubated with proteinase K and DNA was extracted with phenol-chloroform and ethanol precipitation. Primers for PCR amplification in nested reactions for exons 18, 19, and 21 of EGFR (Genbank accession no. X00558) were as follows: exon 18 (first PCR, forward 5′-CAAATGAGCTGGCAAGTGCCGTGTC-3′ and reverse 5′-GAGTTTCCCA-AACACTCAGTGAAAC-3′; nested PCR, forward 5′-CAAGTGCCGTGTCC-TGGCACCCAAGC-3′ and reverse 5′-CCAAACACTCAGTGAAACAAAG-AG-3′); exon 19 (first PCR, forward 5′-GCAATATCAGCCTTAGGT GCGGCTC-3′ and reverse 5′-CATAGAAAGTGAACATTTAGGATGTG-3′; nested PCR, forward 5′-GTGCATCGCTGGTAACATCC-3′ and reverse 5′-TGTGGAGATGAGCAGGGTCT-3′); exon 21 (first PCR, forward 5′-CTAA-CGTTCGCCAGCCATAAGTCC-3′ and reverse 5′-GCTGCGAGCT-CACCCAGAATGTCTGG-3′; nested PCR, forward 5′-GCTCAGAGCCTGG-CATGAA-3′ and reverse 5′-CATCCTCCCCTGCATGTGT-3′). Sequencing was done using forward and reverse nested primers with the ABI Prism 3100 DNA Analyzer (Applied Biosystems, Foster City, CA). Electropherograms were analyzed for the presence of mutations using Seqscape v2.1.1 software in combination with Factura to mark heterozygous positions. The human NSCLC cell line (PC9) derived from an adenocarcinoma (Kyushu Cancer Center, Fukuoka, Japan) was also examined using the same methods.

CA repeats in intron 1. In the subgroup of 28 patients, genomic DNA from peripheral blood or adjacent normal lung tissue was used to determine the number of CA repeats in intron 1. PCR amplification was done with 50 ng of genomic DNA; the primer sequences specific for this microsatellite marker were as follows: forward 5′-FAMGGGCTCACAG-CAAACTTCTC-3′ and reverse 5′-AAGCCAGACTCGCTCATGTT-3′. One microliter of each PCR product was mixed with 0.5 μL of size standard (GenScan-350 Rox Standard, Applied Biosystems) and denatured in 18 μL of formamide at 95°C for 5 minutes. Separation was done with a four-color laser-induced fluorescence capillary electrophoresis system (ABI Prism 3100 DNA Analyzer, Applied Biosystems). The collected data was evaluated with the GeneScan Analysis Software (Applera, Norwalk, CT). DNA from the tumor cell line Hep-2 was used as a control for PCR amplified microsatellite fragment length.

Quantitative PCR. In the subgroup of 28 patients, total RNA was derived from paraffin-embedded tissue obtained by laser capture microdissection. After standard tissue sample deparaffinization using xylene and alcohols, samples were lysed in a Tris-chloride, EDTA, SDS, and proteinase K containing buffer. RNA was then extracted with phenol-chloroform-isoamyl alcohol followed by precipitation with isopropanol in the presence of glycogen and sodium acetate. RNA was resuspended in RNA storage solution (Ambion, Inc., Austin, TX) and treated with DNase I to avoid DNA contamination. cDNA was synthesized using Moloney murine leukemia virus retrotranscriptase enzyme. Template cDNA was added to Taqman Universal Master Mix (Applied Biosystems) in a 12.5-μL reaction with specific primers and

probe for each gene. The primer and probe sets were designed using Primer Express 2.0 Software (Applied Biosystems). Quantification of gene expression was done using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Primers and probe for *EGFR* and *caveolin-1* mRNA expression analysis were designed according to the Ref Seq NM_005228 and NM_001753, respectively (http://www.ncbi.nlm.nih.gov/LocusLink). The primers and labeled fluorescent reporter dye probe were as follows: β-actin, forward 5′-TGAGCGCGGCTACAGCTT-3′, reverse 5′-TCCTTAATGTCACGCACGATTT-3′, probe 5′-FAMACCACCACGGCCGAGCGG-3′TAMRA; EGFR, forward 5′-GGAAATTACCTATGTGCAGAGGAATT-3′, reverse 5′-TAACCAGCCACCCCCTGGAT-3′, MGB probe 5′-FAMTGATCTTTCCTTCTTAAAGAC-3′; Caveolin-1, forward 5′-CGACCCTAAACACCTCAACGA-3′, reverse 5′-GGTTCTGCAATCACATCTTCAAAG-3′, MGB probe 5′-FAMCGTGGTCAAGATTG-3′. Relative gene expression quantification was calculated according to the comparative C_t method using β-actin as an endogenous control and commercial RNA controls (Stratagene, La Jolla, CA) as calibrators. Final results were determined as follows: $2^{-(\Delta Ct\ sample\ -\ \Delta Ct\ calibrator)}$, where ΔC_t values of the calibrator and sample are determined by subtracting the C_t value of the target gene from the value of the *β-actin* gene. In all experiments, only triplicates with a SD of the $C_t < 0.20$ were accepted. In addition, for each sample analyzed, a retrotranscriptase minus control was run in the same plate to assure lack of genomic DNA contamination.

To distinguish between high and low gene expression levels, median levels obtained were used as cutoffs: 3.28 for *EGFR* and 0.52 for *caveolin-1* mRNA expression.

Fluorescence in situ hybridization assay. For each patient in the subgroup of 28 patients, two sections of 3- to 5-μm paraffin-embedded tumor tissue were placed over silenized treated slides. Another section was stained with H&E and confirmed to contain tumor tissue components. The silenized slides were left overnight at 60°C; deparaffinized in two changes of xylene for 10 minutes; rehydrated in 100% ethanol, 90% ethanol, and 70% ethanol for 1 minute each; and left in deionized water for 5 minutes. After tissue hydration, sections were placed in citrate buffer and heated in a microwave twice for 5 minutes at 800 W each. Slices were then digested by proteinase K treatment for 15 minutes at 37°C, fixed with formalin solution (pH 7.5), and washed in 2× SSC buffer. The hybridization was done using Vysis probes (LSI EGFR/CEP 7 Dual Color, Downers Grove, IL) following the manufacturer's instructions. Briefly, 5 μL of the probe solution were added to each slide and covered by a coverslip. Slides and probes were denatured for 3 minutes at 85°C in a slide warmer plaque (Hybrite, Vysis) and left at 37°C overnight. The coverslips were removed and the slides washed in 2× SSC/0.3%NP40 solution for 2 minutes at 72°C followed by an additional wash in 2× SSC/0.3%NP40 solution for 10 seconds at room temperature. Finally, slides were counterstained using a 4′-6′-diamidine-2-phenylindole-containing medium that specifically binds to DNA. For each patient, 100 nuclei from the selected tumor region were analyzed in a fluorescence microscope. The ratio of the average number of EGFR spots/nucleus by the average number of CEP 7 (centromeric chromosome 7) spots/nucleus was used for the scoring criteria. EGFR status in tumors was scored as follows: (a) single copy, up to four specific signals of both EGFR and CEP 7 probes with a ratio equal to 1; (b) polysomy, more than four specific signals of both probes per nucleus and a ratio <2; (c) amplification, more than four specific signals of EGFR probe per nucleus compared with CEP 7 with a ratio >2. Tumors scored as polysomy and/or amplification were labeled as having increased EGFR copy numbers.

Statistical methods. The primary objective of this study was to compare clinical characteristics, response rates, and survival in gefitinib-treated patients with and without mutations in the EGFR tyrosine kinase domain. In the subgroup of 28 patients, further analyses were done to examine the correlation among EGFR mutations, the number of CA repeats in intron 1 of EGFR in normal tissue, *EGFR* and *caveolin-1* mRNA expression levels in tumor tissue, and *EGFR* gene copy numbers.

The nonparametric Mann-Whitney *U* test and one-way ANOVA test were used to analyze differences in EGFR mutation status, number of CA repeats in intron 1 of EGFR, EGFR and caveolin-1 mRNA expression, and *EGFR* gene copy numbers. Normality of the distribution of continuous variables was assessed with the Kolmogorov-Smirnov test. The χ^2 and Fisher's exact tests were used to compare differences in response according to EGFR mutation status, number of CA repeats in intron 1, EGFR and caveolin-1 mRNA expression, and gene copy numbers. Univariate Cox regression models were used to measure hazard ratios. To identify relevant variables of influence, a multivariable logistic regression model was used, and the fit of the models was evaluated with the Hosmer-Lemeshow likelihood ratio test. The Wald test was used to test the statistical significance of each variable in the model. Survival curves were drawn with the Kaplan-Meier product limit method and *P* values assessed with the Tarone-Ware test. All reported *P* values are two sided; $P < 0.05$ was considered statistically significant. SPSS software version 11.5 (SPSS, Inc., Chicago, IL) was used for all analyses.

Results

Table 1 shows characteristics for all patients according to *EGFR* mutation status. Seventeen of the 68 patients harbored *EGFR* mutations in the tyrosine kinase domain. Mutations were not observed in DNA from peripheral blood or adjacent normal lung tissue, indicating that all mutations were somatic. All mutations were identified in adenocarcinomas (Table 1); 10 were heterozygous and six were homozygous (Table 2). Eleven tumors had in-frame nucleotide deletions in exon 19, adjacent to K745; five were delE746-A750, which was also observed in

Table 1. Characteristics of all patients according to *EGFR* mutation status

	EGFR mutation status		*P*
	Mutation	Wild-type	
No. patients	17	51	0.8
Age (range)	60 (34-84)	59 (39-86)	
Sex (%)			
Male	6 (35.3)	39 (76.5)	0.003
Female	11 (64.7)	12 (23.5)	
Histology (%)			
Adenocarcinoma	17 (100)	30 (58.8)	0.007
Large cell carcinoma	—	5 (9.8)	
Squamous cell carcinoma	—	11 (21.6)	
Other	—	5 (9.8)	
Smoking history (%)			
Smokers	3 (17.6)	43 (84.3)	0.0001
Nonsmokers	14 (82.4)	8 (15.7)	
No. prior regimens (range)	1 (0-3)	2 (0-6)	0.04
Response to gefitinib (%)			
Complete and partial response	16 (94.1)	6 (11.8)	<0.0001
Stable disease	1 (5.9)	8 (15.7)	
Progressive disease	—	34 (66.7)	
Not evaluable	—	3 (5.8)	
Duration of gefitinib treatment			
Months (range)	9.4 (1.1-23.1)	4.2 (0.2-41.9)	0.07

Table 2. Clinical characteristics and *EGFR* mutation status in 22 responders to gefitinib

Country of origin	Age	sex	Smoking status	Pathol	Prior regimens	Response	Overall survival (mo)	Survival status	*EGFR* mutation 1 AA sequence	*EGFR* mutation 2 AA sequence	Mutational status
Spain	60	F	Yes	ADC	1	PR	6.7	D	wt		wt
Spain	52	F	Yes	ADC	1	PR	8.3	D	wt		wt
Germany	65	M	Yes	ADC	1	PR	22.1	D	wt		wt
Japan	53	F	No	LCC	3	PR	18.4	D	wt		wt
Japan	76	M	Yes	ADC	3	PR	10.8	A	wt		wt
Japan	68	F	No	ADC	2	PR	24.3	D	wt		wt
Spain	71	F	No	ADC	1	PR	8.9	A	delE746.A750		Hetero
Spain	63	M	Yes	ADC	3	PR	17.8	A	delE746.T751InsA		Hetero
Germany	66	F	No	ADC	2	PR	13.7	D	delE746.S752InsV		Hetero
China	67	M	No	ADC	0	PR	22.0	A	delL747.P753InsS	L861Q	Hetero
China	34	F	No	ADC	2	PR	6.1	A	L858R		Homo
China	61	F	No	ADC	2	PR	14.3	A	L858R		Hetero
China	49	M	Yes	ADC	1	PR	25.4	A	L858R		Homo
China	37	M	No	ADC	0	PR	15.9	A	delE746.S752InsV		Hetero
Japan	71	F	No	ADC	1	PR	14.2	A	del719G(G)C to GC		Hetero
Japan	66	F	No	ADC	0	PR	9.5	A	delE746.T751		Homo
Japan	54	F	No	ADC	2	PR	18.7	D	delE746.A750		Homo
Japan	60	F	No	ADC	3	PR	15.3	A	L718P		Hetero
Japan	50	M	No	ADC	0	PR	18.4	A	delL747.T751InsF		Hetero
Japan	52	M	Yes	ADC	1	PR	8.9	A	delE746.A750		Homo
Japan	42	F	No	ADC	2	CR	18.9	A	delE746.A750		Homo
USA	84	F	No	ADC	1	PR	11.7	D	delE746.A750		Hetero

Abbreviations: Pathol, pathologic diagnosis; ADC, adenocarcinoma; LCC, large cell carcinoma; PR, partial response; CR, complete response; A, alive; D, dead; AA, amino acid; Homo, homozygogous; Hetero, heterozygous; wt, wild type.

the PC9 cell line; one was delE746-T751; four contained an amino acid insertion (one delE746-T751insF, one delE746-T751insA, and two delE746-752insV); and one tumor contained both an amino acid insertion (delL747-P753insS) and a missense mutation L861Q in exon 21. Four tumors contained an L858R mutation in exon 21. One tumor had an L718P mutation and another had a nucleotide deletion (guanine) in codon 719, both in exon 18. This second mutation was heterozygous. The G deletion affects the reading frame 5′ downstream of this position. The protein is nonfunctional, and the new sequence has a stop codon in codon 747 (TAA instead of TTA).

Twenty-two patients (32%) achieved a partial response to gefitinib. Table 2 shows the clinical characteristics of all responders. Sixteen of the 17 patients (94.1%) carrying EGFR mutations attained a partial radiographic response in contrast with 6 (12.6%) of the 51 patients with wild-type EGFR ($P < 0.0001$) Patients with *EGFR* mutations had 17.1 times greater probability of response ($P = 0.02$). Probability of response was also increased in nonsmokers, patients receiving a greater number of prior chemotherapy regimens, Asians, and younger patients (Table 3).

In general, patients harboring *EGFR* mutations obtained dramatic responses. For example, a Japanese female with adenocarcinoma underwent three pulmonary resections between 1999 and 2002; two of the three resected tumors contained an EGFR mutation (delE746-T751). The patient developed multiple bilateral lung metastases and did not respond to several chemotherapy regimens. After 2 months of gefitinib treatment, almost complete response was attained and the patient remains in remission at the time of submitting this article (Fig. 1). A second patient, a Spanish

Table 3. Adjusted odds ratio for the joint effect on response of different covariates

	Odds ratio (95% confidence interval)	*P*
Odds ratio adjusted by covariates		
EGFR mutations	17.1 (5.1-58.8)	0.002
EGFR mutations by sex (female)	8.7 (2.2-34.6)	0.0001
EGFR mutations by smoking status (nonsmoker)	37.4 (3.1-426)	0.005
EGFR mutations by no. prior chemotherapy regimens	73.1 (7.6-462)	0.005
EGFR mutations by ethnicity	61.7 (5.9-639)	0.001
EGFR mutations by age	105.0 (11.4-981)	0.00001
Crude odds ratio		
Sex (female)	1.4 (0.6-3.4)	0.4
Smoking status (smoker)	0.6 (0.4-0.8)	0.001
No. prior chemotherapy regimens	0.7 (0.5-0.9)	0.003
Ethnicity (Asian)	4.0 (1.7-9.2)	0.001
Age	0.9 (0.98-0.99)	0.008

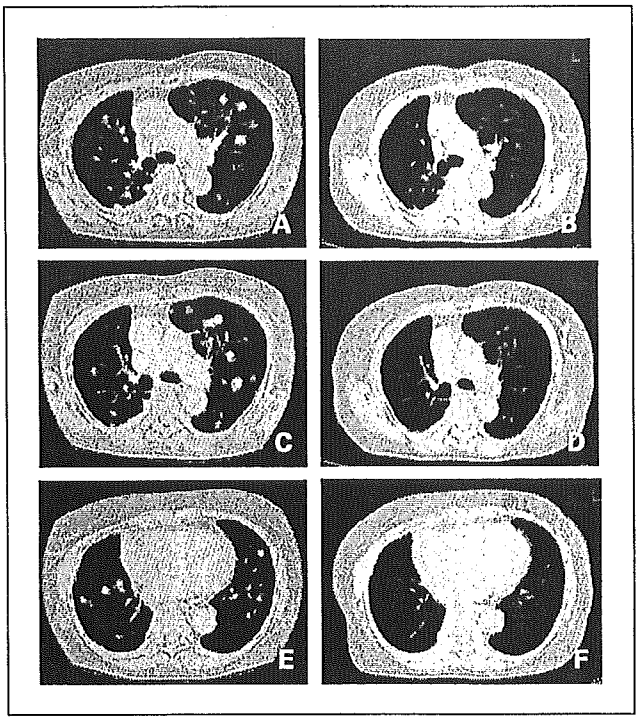


Fig. 1. Example of response to gefitinib in a representative patient with recurring NSCLC after three lines of chemotherapy. Computed tomography slices before gefitinib treatment (*A*, *C*, and *E*) and after 8 weeks of gefitinib treatment (*B*, *D*, and *F*).

female with relapsed lung adenocarcinoma underwent an upper left lobe lobectomy in 2002; the tumor contained an *EGFR* mutation (delE746-A750). One and a half years later, in 2004, the patient developed severe neurologic symptoms with impairment of walking, eating, and speaking and required a gastric feeding tube. The brain computed tomography showed multiple cystic, rim-enhancing supratentorial masses of various sizes (Fig. 2*A*). Brain biopsy was not done. Dexamethasone was given, without improvement, and brain irradiation was not indicated. One month later, gefitinib was given through the gastric feeding tube, and a rapid recovery of neurologic functions was observed, accompanied by a regression of the brain metastases (Fig. 2*B*). The patient is still in remission. A third patient, an 84-year-old North American female with lung adenocarcinoma underwent a lobectomy in 2003; the tumor contained an *EGFR* mutation (delE746-A750). The patient relapsed with bone and lung metastases; one cycle of chemotherapy was given, but she suffered a pulmonary embolism with a myocardial infarction. She recovered but did not receive additional chemotherapy. She developed a cardiac tamponade with clear evidence of progression of her lung metastases. Seven months later, in 2004, she started gefitinib treatment, and 3 weeks later she was clinically improved. New bone metastases were detected after 1 year and the patient died . Finally, a 42-year-old Japanese female with lung adenocarcinoma underwent a left upper lobectomy in 2001; the tumor contained not only a delE746-A750 mutation but also >20 *EGFR* gene copies by fluorescence *in situ* hybridization, elevated *EGFR* (47.3) and *caveolin-1* (0.9) mRNA expression, and increased number of CA repeats (20 of 21; Fig. 3). The patient developed brain metastases 9 months later, in 2002 and received stereotactic radiosurgery. Multiple lung metastases developed after 2 months, and six cycles of cisplatin/gemcitabine/vinorelbine were given. Eight months later, in 2003, the patient initiated gefitinib treatment. Before treatment, her carcinoembryonic antigen level was 257.2 ng/mL (normal level, <5 ng/mL). After 6 months of gefitinib treatment, her carcinoembryonic antigen level was 2.2 ng/mL. A complete remission of the lung metastases has been attained.

Median survival for patients carrying *EGFR* mutations was not reached, whereas it was 9.9 months (95% CI, 6.8-12.9) for those patients carrying wild-type *EGFR* ($P = 0.001$; Fig. 4).

Table 4 shows the characteristics of the 28 patients in whom we assessed CA repeats, *EGFR* and *caveolin-1* mRNA expression, and *EGFR* gene copy numbers. All patients with *EGFR* mutations also had increased numbers of CA repeats (≥19). The highly gefitinib-sensitive PC9 lung adenocarcinoma cell line, which harbored the deletion delE746-A750, also displayed increased numbers of CA repeats (20 of 20). There were no differences in median mRNA levels of *EGFR* or *caveolin-1* according to EGFR mutation status. Increased *EGFR* gene copy numbers were observed more frequently in patients with EGFR mutations. Gene amplification ranged widely from low to high levels, and in some patients,

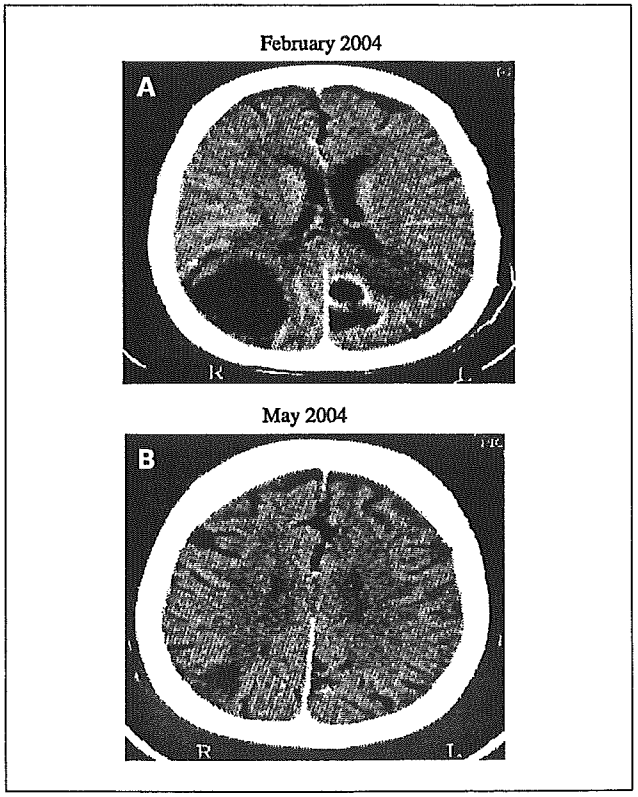


Fig. 2. Example of response to gefitinib in a lung adenocarcinoma patient with brain metastases. Computed tomography before gefitinib treatment (*A*) and after 8 weeks of gefitinib treatment (*B*). *A*, enlarged ventricles were observed in the pretreatment computed tomography. *B*, after treatment, with the disappearance of the periventricular brain metastases, ventricles were less visible.

amplification was seen in only 25% of the tumor cells examined. In this subset of 28 patients, the response rate for patients with increased gene copy numbers was 45%, in contrast with 89% for patients with *EGFR* mutations (P = 0.02). The response rate was 100% in patients with both *EGFR* mutations and gene amplification. Table 5 illustrates the levels of *EGFR* and *caveolin-1* mRNA according to *EGFR* mutation status and further broken down by gene copy numbers and number of CA repeats. The highest levels of *EGFR* mRNA were observed in the group of patients with both *EGFR* mutations and increased *EGFR* copy numbers. Patients with both *EGFR* mutations and low levels of *EGFR* or *caveolin-1* mRNA had a median survival of 13 months, whereas median survival has not been reached for those patients with *EGFR* mutations and high levels of *EGFR* or *caveolin-1* mRNA (data not shown).

Discussion

In the present study, we have observed that *EGFR* mutations are a strong predictor of gefitinib response in chemoresistant NSCLC patients. Sixteen of 17 patients (94.1%) with *EGFR* mutations attained an objective response, in contrast with only 6 of 51 patients (12.6%) with wild-type *EGFR* ($P <$ 0.0001). These results mirror accumulated data from three studies (10–12) in which 25 of 31 (81%) NSCLC patients with *EGFR* mutations attained an objective response, whereas none of 29 nonresponders had mutations. Furthermore, it has recently been shown that in 16 gefitinib-treated Taiwanese NSCLC patients, seven of nine responders had *EGFR* mutations (13). The delE746-A750 in the PC-9 cell line found in the present study has also been observed in a separate study (29), in which it also conferred hypersensitivity to gefitinib. *EGFR* mutations found in previous studies have mostly been heterozygous (10–12); however, Paez et al. (11) reported one homozygous mutation at exon 19 and Pao et al. (12) found homozygous mutations in two of seven gefitinib-treated patients, leading them to speculate that homozygosity may be the result of the selective amplification of the mutant gene or that mutations in general may be homozygous with the wild-type sequence originating from contaminating "normal" DNA. In the study by Huang et al. (13), 4 of 10 mutations were homozygous, and in the present study, 6 of 17 mutations were also homozygous. Contaminating "normal" cells with wild-type EGFR seems the most likely explanation for apparently heterozygous mutations, because even with microdissection, nonneoplastic tissue contamination cannot be completely ruled out. However, amplification of mutant *EGFR* could account for detection of only mutant sequences.

In the original studies (10, 11), only one mutation per tumor was detected. However, Pao et al. (12) found a tumor sample with two mutations, from a female never smoker with adenocarcinoma, treated with erlotinib for 13 months, and surviving 22 months. Furthermore, in the study by Huang et al. (13), two patients had two mutations in their tumors; one responded and one did not. In our study, one patient had two mutations: a 67-year-old Hong Kong Chinese female never smoker with adenocarcinoma. She attained a partial response and is still alive at 22 months (January 2005). It is not possible to draw definite conclusions from only four patients, and more data regarding the potential predictive value of two mutations in the same tumor is needed.

In the present study, 6 of 51 patients with wild-type *EGFR* attained partial response to gefitinib. There were no differences in baseline clinical characteristics between responders with EGFR mutations and responders with wild-type *EGFR* (Table 2). However, only 16% of responders with wild-type *EGFR* remain alive at the time of submitting this article, in contrast with 81% of responders harboring *EGFR* mutations. In the series reported by Lynch et al. (10), one of nine gefitinib-sensitive patients did not have *EGFR* mutations. Along the same lines, Pao et al. (12) reported that 5 of 17 patients with partial response or clinical improvement to gefitinib or erlotinib had wild-type *EGFR* in exons 18 to 24.

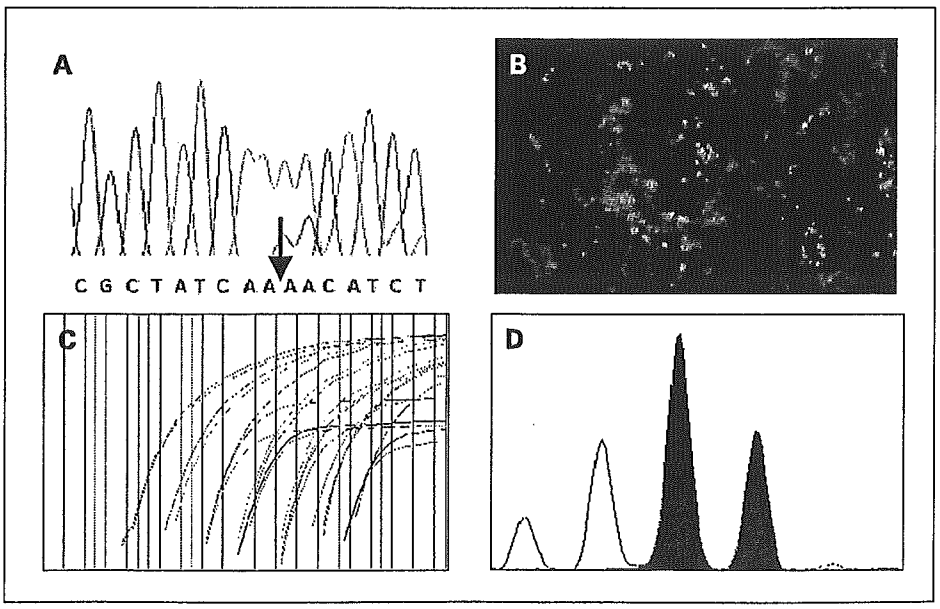
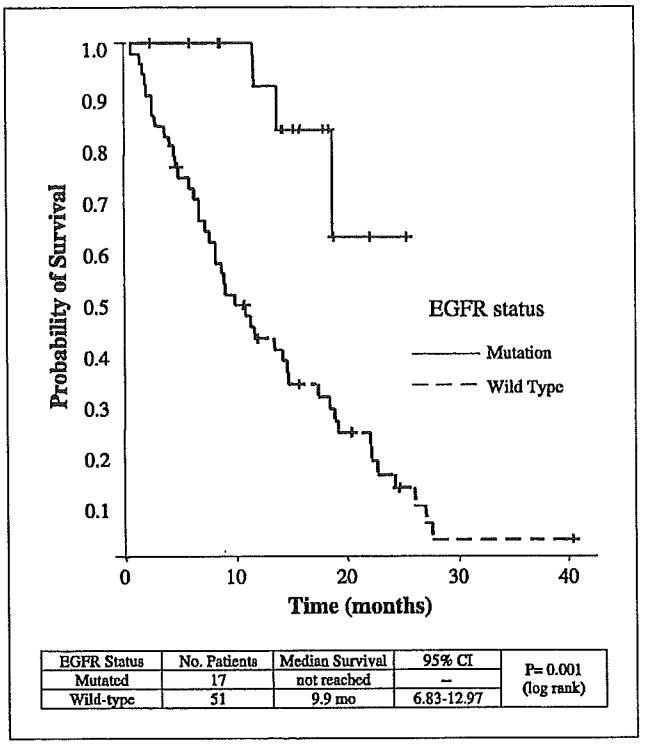


Fig. 3. Gefitinib responder showing, clockwise from top left: (*A*) an *EGFR* mutation (del E746-A750); (*B*) a high level of gene amplification (spots); (*C*) high *EGFR* and *caveolin-1* mRNA levels (superimposed one on the other); (*D*) and increased numbers of CA repeats. *C*, cDNAs for the gene of interest and an internal reference gene (*β-actin*) were quantified using a fluorescence-based real-time detection method. For each sample, parallel triplexTaqman PCR reactions were performed for the gene of interest and the *β-actin* reference gene to normalize for input cDNA. The expression of individual *EGFR* and *caveolin-1* was calculated using a relative quantification algorithm. In this patient, the *EGFR* mRNA level was 47 and the *caveolin-1* mRNA level was 0.98. *D*, number of CA repeats, determined by GeneScan analysis software (Applera). The number of CA repeats is determined by the mobility in the chromatogram. The shaded peaks represent the intensities of the two alleles. The left peak represents 20 CA repeats and the right peak represents 21 CA repeats. At submission, this patient has been in complete remission for 18.9 months.



EGFR Status	No. Patients	Median Survival	95% CI	P= 0.001 (log rank)
Mutated	17	not reached	–	
Wild-type	51	9.9 mo	6.83-12.97	

Fig. 4. Survival from the start of gefitinib treatment according to EGFR mutation status.

Mutations in these responders may not have been detected because they were below the detection rate of sequencing assays (30), or increased *EGFR* gene copy numbers in these responders may have conferred enhanced gefitinib sensitivity (12) in the absence of *EGFR* mutations. NSCLC cell lines with wild-type *EGFR* but with high levels of *EGFR*, *ErbB2*, or *ERbB3* mRNA have shown intermediate sensitivity to gefitinib and erlotinib (31).

The small number of patients examined in the present study limits the conclusions that can be drawn as to the role of CA repeats, *EGFR* and *caveolin-1* mRNA expression, and *EGFR* gene copy numbers. However, interethnic differences in the number of CA repeats warrant further investigation in Asian lung cancer patients, in whom increased numbers of CA repeats may be more frequently associated with the presence of *EGFR* mutations (19, 20). Amador et al. (32) found that head and neck cell lines with decreased numbers of CA repeats had higher expression of *EGFR* mRNA and were more sensitive to the inhibitory effects of erlotinib. In addition, in 19 gefitinib-treated colorectal cancer patients (32), 84% of those with decreased numbers of CA repeats developed skin toxicity, a feature related to the antitumor activity of EGFR inhibitors (33), compared with only 33% of those with increased numbers of CA repeats (P = 0.04; ref. 32).

In surgically resected NSCLC patients (13, 34), *EGFR* mutations were associated with well and moderately differentiated adenocarcinomas and smoking status but not with female gender. Dramatic clinical response to gefitinib is observed in only 10% to 19% of chemorefractory advanced NSCLC. Kris et al. (5) showed that female gender predicted response to gefitinib, whereas the number of prior chemotherapy regimens did not influence response. In our study, the number of prior chemotherapy regimens increased the probability of response in tumors containing *EGFR* mutations.

The strong correlation we observed between EGFR mutations and improved response and survival leads us to recommend the assessment of *EGFR* mutations in lung adenocarcinoma

Table 4. Patient characteristics of a subgroup of 28 patients according to *EGFR* mutation status, number of CA repeats in intron 1, *EGFR* and *caveolin-1* mRNA levels, and *EGFR* gene copy numbers

	Wild-type *EGFR*, *n* (%)	Mutated *EGFR*, *n* (%)	*P*
No. patients	19	9	
Age (y)			
<65	10 (52)	6 (66.6)	NS
≥65	9 (48)	3 (33.3)	
Sex			
Male	15 (79)	4 (45)	NS
Female	4 (21)	5 (55)	
Ethnicity			
Caucasian	8 (42)	4 (45)	NS
Asian	11 (58)	5 (55)	
Histology			
Adenocarcinoma	16 (85)	9 (100)	NS
Large cell carcinoma	1 (5)	0	
Squamous cell carcinoma	2 (10)	0	
Smoking status			
Smoker	15 (79)	3 (33.3)	0.035
Nonsmoker	4 (21)	6 (66.6)	
Response to gefitinib			
Yes	2 (11)	8 (88)	<0.0001
No	16 (84)	1 (12)	
Nonevaluable	1 (5)	—	
Duration of gefitinib response (wk)			
Median (range)	6.93 (0.2-27.6)	7.73 (1.05-15.63)	NS
CA repeats in intron 1			
<19	3 (20.5)*	0 (-)*	NS
≥19	11 (79.5)*	7 (100)*	
EGFR mRNA levels			
No. patients	15	8	0.087
Median (range)	2.61 (0.42-23.09)*	5.04 (1.79-47.37)*	
Caveolin-1 mRNA levels			
No. patients	14	8	NS
Median (range)	0.71 (0.06-2.16)*	0.55 (0.19-1.07)*	
EGFR gene copy numbers			
Increased	4 (21)	5 (55)	0.087
Normal	15 (79)	4 (45)	

Abbreviation: NS, not significant.
*Disparity between some figures is due to the lower availability of tumor tissue in some patients.

Table 5. *EGFR* and *caveolin-1* mRNA levels in patients with wild-type and mutated *EGFR*, further broken down according to number of CA repeats in intron 1 and *EGFR* gene copy numbers

	Wild-type *EGFR*				Mutated *EGFR*			
	EGFR mRNA		*Caveolin-1* mRNA		*EGFR* mRNA		*Caveolin-1* mRNA	
	No. patients	Median (range)	No. patients	Median (range)	No. patients	Median (range)	No. patients	Median (range)
CA repeats								
<19	2	14.24 (5.38-23.09)	1	2.16 (2.16-2.16)	0		0	
≥19	10	2.94 (1.54-6.44)	10	0.75 (0.19-1.47)	6	13.21 (3.0-47.37)	6	0.41 (0.19-1.07)
EGFR gene copy numbers								
Increased	4	5.12 (2.61-23.09)	3	1.23 (0.19-2.16)	5	20.31 (3.0-47.37)	5	0.61 (0.19-1.07)
Normal	11	2.04 (0.42-6.43)	11	0.57 (0.06-1.47)	3	3.01 (1.79-3.97)	3	0.46 (0.30-0.63)

patients to customize treatment. NSCLC cell lines containing *EGFR* mutations are chemoresistant but highly sensitive to gefitinib (17, 35). In the present study, we detected an unprecedented median survival in patients with *EGFR* mutations. The Spanish Lung Cancer Group is currently screening for *EGFR* mutations in metastatic lung adenocarcinomas to identify patients who could benefit from treatment with tyrosine kinase inhibitors.

References

1. Massarelli E, Andre F, Liu DD, et al. A retrospective analysis of the outcome of patients who have received two prior chemotherapy regimens including platinum and docetaxel for recurrent non-small-cell lung cancer. Lung Cancer 2003;39:55–61.
2. Hirata A, Ogawa S, Kometani T, et al. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. Cancer Res 2002;62:2554–60.
3. Gazdar AF, Shigematsu H, Herz J, Minna JD. Mutations and addiction to EGFR: the Achilles "heal" of lung cancers? Trends Mol Med 2004;10:481–6.
4. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 2003;21:2237–46.
5. Kris M, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer. JAMA 2003;290:2149–58.
6. Wakeling AE, Guy SP, Woodburn JR, et al. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res 2002;62:5749–54.
7. Suzuki T, Nakagawa T, Endo H, et al. The sensitivity of lung cancer cell lines to the EGFR-selective tyrosine kinase inhibitor ZD1839 ("Iressa") is not related to the expression of EGFR or HER-2 or to K-ras gene status. Lung Cancer 2003;42:35–41.
8. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol 2003;21:2658–63.
9. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ("Iressa", ZD1839) in non-small-cell lung cancer. Br J Cancer 2004;91:108–12.
10. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
11. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
12. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.
13. Huang SF, Liu HP, Li LH, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small-cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 2004;10:8195–203.
14. Lee JW, Soung YH, Kim SY, et al. Absence of EGFR mutation in the kinase domain in common human cancers besides non-small cell lung cancer. Int J Cancer 2005;113:510–1.
15. Barber TD, Vogelstein B, Kinzler KW, Velculescu VE. Somatic mutations of EGFR in colorectal cancers and glioblastomas. N ENgl J Med 2004;351:2883.
16. Nagahira H, Mimori K, Ohta M, et al. Somatic mutations of epidermal growth factor receptor in colorectal carcinoma. Clin Cancer Res 2005;11:1368–71.
17. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163–7.
18. Buerger H, Gebhardt F, Schmidt H, et al. Length and loss of heterozygosity of an intron 1 polymorphic sequence of *egfr* is related to cytogenetic alterations and epithelial growth factor receptor expression. Cancer Res 2000;60:854–7.
19. Tidow N, Boecker A, Schmidt H, et al. Distinct amplification of an untranslated regulatory sequence in the *egfr* gene contributes to early steps in breast cancer development. Cancer Res 2003;63:1172–8.
20. Buerger H, Packeisen J, Boecker A, et al. Allelic length of a CA dinucleotide repeat in the *egfr* gene correlates with the frequency of amplifications of this sequence: first results of an inter-ethnic breast cancer study. J Pathol 2004;203:545–50.
21. Eckert KA, Yan G, Hile SE. Mutation rate and specificity analysis of tetranucleotide microsatellite DNA alleles in somatic human cells. Mol Carcinog 2002;34:140–50.
22. Mineo C, Gill GN, Anderson GW. Regulated migration of epidermal growth factor receptor from caveolae. J Biol Chem 1999;274:30636–43.
23. Lavie Y, Fiucci G, Liscovitch M. Up-regulation of caveolae and caveolar constituents in multidrug-resistant cancer cells. J Biol Chem 1998;273:32380–3.
24. Yang CPH, Galbiati F, Volonte D, Horwitz SB, Lisanti MP. Upregulation of caveolin-1 and caveolae organelles in Taxol-resistant A549 cells. FEBS Lett 1998;439:368–72.
25. Sunaga N, Miyajima K, Suzuki M, et al. Different roles for caveolin-1 in the development of non-small-cell lung cancer versus small cell lung cancer. Cancer Res 2004;64:4277–85.
26. Ono M, Hirata A, Kometani T, et al. Sensitivity to gefitinib (Iressa, ZD1839) in non-small cell lung cancer cell lines correlates with dependence on the epidermal growth factor (EGF) receptor/extracellular signal-regulated kinase 1/2 and EGF receptor/Akt pathway for proliferation. Mol Cancer Ther 2004;3:465–72.
27. Wei Q, Cheng L, Amos CI, et al. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. J Natl Cancer Inst 2000;92:1764–72.
28. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
29. Arao T, Fukumoto H, Takeda M, Tamura T, Saijo N, Nishio K. Small in-frame deletion in the epidermal growth factor receptor as a target for ZD6474. Cancer Res 2004;64:9101–4.
30. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 2005;23:857–65.
31. Amann J, Kalyankrishna S, Massion PP, et al. Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. Cancer Res 2005;65:226–35.
32. Amador ML, Oppenheimer D, Perea S, et al. An epidermal growth factor receptor intron 1 polymorphism mediates response to epidermal growth factor receptor inhibitors. Cancer Res 2004;64:9139–43.
33. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. J Clin Oncol 2004;22:3238–47.
34. Kosaka T, Yatabe Y, Endoh HH, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the *epidermal growth factor receptor* gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–23.
35. Tracy S, Mukohara T, Hansen M, Meyerson M, Johnson BE, Jänne PA. Gefitinib induces apoptosis in the EGFR ^{L858R} non-small-cell lung cancer cell line H3255. Cancer Res 2004;64:7241–4.

Jpn J Clin Oncol 2006;36(1)12–16
doi:10.1093/jjco/hyi217

Phase I Study of Amrubicin Hydrochloride and Cisplatin in Patients Previously Treated for Advanced Non-small Cell Lung Cancer

Jiro Ikeda, Riichiroh Maruyama, Tatsuro Okamoto, Fumihiro Shoji, Hiroshi Wataya and Yukito Ichinose

Department of Thoracic Oncology, National Kyushu Cancer Center, Fukuoka, Japan

Received July 21, 2005; accepted December 1, 2005; published online January 17, 2006

Objective: A single-center phase I trial was designed to determine both the dose-limiting toxicities and the maximum tolerated dose (MTD) for amrubicin hydrochloride in combination therapy with cisplatin for advanced non-small cell lung cancer (NSCLC) patients with prior chemotherapy.

Methods: Eligible patients received amrubicin and cisplatin on days 1 through 3 every 3 or 4 weeks. Cisplatin was administered at a fixed dosage of 20 mg/m^2 while the administered dose of amrubicin was started at 20 mg/m^2. Each group comprised 3 or 6 patients. When dose limiting toxicities were noted in three or more of six patients at a particular level, that level was estimated to be the MTD.

Results: Fifteen patients were enrolled in this study, including 5 males and 10 females, with a median age of 57. The dose limiting toxicities included grade 4 neutropenia which lasted 4 or more days and febrile neutropenia. The non-hematologic toxicities were well managed and rarely severe. The MTD of amrubicin in this combination regimen was estimated to be 30 mg/m^2. A partial response was observed in 4 of 15 patients (27%).

Conclusions: The recommended dose was thus determined to be 25 mg/m^2 amrubicin with 20 mg/m^2 cisplatin for 3 consecutive days. A phase II study is currently underway.

Key words: amrubicin hydrochloride – cisplatin – non-small cell lung cancer – phase I study – prior chemotherapy

INTRODUCTION

Platinum-based combination chemotherapy has been the standard first line treatment for advanced non-small cell lung cancer (NSCLC) (1). The median survival time (MST), however, ranges from 7.4 to 8.1 months in patients treated with platinum-based combination chemotherapy and this treatment still remains unsatisfactory regarding its overall clinical effectiveness (2,3). On the other hand, docetaxel monotherapy has shown an approximately 7% response rate, thus leading to an average increased survival time of 3 months and a better quality of life when used as a second-line therapy in patients with advanced NSCLC, in comparison to the best supportive care (4,5). Based on these results, docetaxel monotherapy is widely regarded as a standard second-line treatment (6,7). Recently, pemetrexed has been shown to have the same effect as docetaxel in terms of the response rate and survival (8). In addition, epidermal growth factor receptor-tyrosine kinase inhibiting drugs such as gefitinib and erlotinib have also been approved for the treatment of NSCLC patients with prior chemotherapy (9,10). Although we now have several choices for the treatment of patients with a progressive disease either during or after undergoing other types of chemotherapy, the number of effective drugs for such patients still remains limited.

Amrubicin hydrochloride, which is a totally synthetic 9-aminoantracycline, is metabolically activated by a liver enzyme to amrubicinol. Amrubicin is reported to have either an equivalent or a stronger anti-tumor effects in comparison with doxorubicin in nude mice transplanted with human tumor cells (11–13). The anti-tumor mechanism of amrubicin itself and its active form, amrubicinol, is due to the break-down of DNA strands during the stabilization process of DNA-topoisomerase II cleavable complex (14). In a phase I trial of the intravenous administration of amrubicin for 3 consecutive days at 3-week intervals in patients without prior chemotherapy, the maximum tolerated dose (MTD) and the recommended dose were estimated to be 50 mg/m^2 and 45/m^2, respectively. The major dose limiting toxicity (DLT) was myelosuppression (15). A subsequent phase II trial in patients with advanced NSCLC without prior chemotherapy demonstrated a response rate of 23% with a MST of 9.4 months (16).

For reprints and all correspondence: Yukito Ichinose, Department of Thoracic Oncology, National Kyushu Cancer Center, 3-1-1, Notame, Minami-ku, Fukuoka 811-1395, Japan. E-mail: yichinos@nk-cc.go.jp

These findings suggest that amrubicin may be a promising anti-tumor agent for treatment of NSCLC.

To our knowledge, no clinical trials using amrubicin in previously treated advanced NSCLC patients have yet been conducted. Since the main toxicity of amrubicin is myelotoxicity, it is expected that the administration of a full dose of amrubicin will not be tolerable for previously treated NSCLC patients. A synergistic or additive anti-tumor effect between cisplatin and amrubicin has been reported (17,18). Since the major toxicity of cisplatin is not only non-hematologic but it can also be reduced by dividing up the administered doses, the concurrent combination of amrubicin and cisplatin of a low dose may possibly augment the anti-tumor activity of amrubicin without any severe myelotoxicity, even in patients who have already received other types of platinum-based chemotherapy as prior treatment (19,20). Based on this hypothesis, we conducted a phase I trial to find the MTD of amrubicin which was concurrently administered with a low dose of cisplatin, 20 mg/m^2, for 3 consecutive days, in advanced NSCLC patients with a history of prior chemotherapy.

PATIENTS AND METHODS

ELIGIBILITY

Patients with either cytologically or histologically confirmed advanced NSCLC who demonstrated disease progression either during or after a prior chemotherapy were eligible. The eligibility criteria also included the following factors: an age ranging from 20 to 75 years; an Eastern Cooperative Oncology Group performance status $\leq$1; a life-expectancy of $\geq$3 months; no chemotherapy or radiation therapy within 4 weeks of treatment; an adequate hematopoietic status [absolute neutrophil count (ANC) $\geq$2000/μl, hemoglobin level $\geq$10 g/dl, platelet count $\geq$100 000/μl], hepatic (transaminases $\leq$2 $\times$ institutional normal upper limit, total bilirubin $\leq$1.5 $\times$ institutional normal upper limit) and renal (creatinine $\leq$ institutional normal upper limit) functions; either measurable or evaluable but nonmeasurable disease such as numerous small sized pulmonary metastases, $PaO_2 \geq 60$ torr; and left ventricle ejection fraction of 60% or more based on ultrasound cardiogram. Patients gave their written informed consent before treatment. The protocol was approved by the institutional review board of the National Kyushu Cancer Center.

DOSAGE AND DRUG ADMINISTRATION

Amrubicin and cisplatin were administered on days 1 through 3 of each 3 or 4-week cycle. Cisplatin was administered at a fixed dosage of 20 mg/m^2/day for 3 consecutive days while amrubicin was started at 20 mg/m^2/day for 3 consecutive days and then increased by 5 mg/m^2/day until reaching the MTD. Amrubicin was dissolved in either 20 ml of a 5% glucose solution or saline for the intravenous injections. Following the administration of amrubicin, cisplatin was administered intravenously together with 1500 ml of hydration. At least, two cycles of this combination chemotherapy were administered every 3 or 4 weeks unless either a disease progression or unacceptable toxicity occurred. The MTD was defined as the lowest dose at which three or more of six patients experienced DLT during the first course of the treatment. At least three patients were treated at each dose level that did not result in DLT. If one or two of the initial three patients developed DLT, then three additional patients were entered at the same dose level. DLT was defined as follows; grade 4 leukopenia or neutropenia lasting for 4 days or more; an ANC of $\leq$1000/μl associated with fever ($\geq$38.5°C), a platelet count of $\leq$20 000/μl; and a grade 3 or greater non-hematological toxicity (excluding anorexia, nausea and vomiting). The prophylactic use of growth factors was not permitted. However, at the discretion of the treating physician, granulocyte colony stimulating factor was used for the treatment of febrile neutropenia and grade 4 neutropenia. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria Version 2.0.

PRETREATMENT AND FOLLOW-UP STUDIES

The performance status, the interval toxicities, concurrent medications, physical examinations, complete blood counts, electrolytes and chemistries were evaluated before treatment and weekly thereafter. Pretreatment studies also included chest radiography, computed tomography scans to evaluate all sites of disease, an electrocardiogram and ultrasound cardiogram. Computed tomography scans were repeated every 4 weeks. A complete response was scored if there was a disappearance of all active disease on two measurements separated by a minimum period of 4 weeks, and a partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all lesions documented separated by at least 4 weeks. Any concurrent increase in the size of any lesion by $\geq$25% or the appearance of any new lesion was considered to indicate progressive disease. No change was defined as the absence of a partial or complete response without progressive disease being observed for at least 4 weeks after the start of the treatment.

RESULTS

GENERAL

Between July 2003 and March 2004, 15 patients were enrolled in this study. Table 1 shows the patient characteristics. The study included 5 males and 10 females with a median age of 57 years ranging from 51 to 72. Five and 10 patients had a performance status of 0 and 1, respectively, and most patients histologically had adenocarcinoma. Concerning previous treatments other than chemotherapy, four patients each underwent either surgery or thoracic radiotherapy. Regarding prior chemotherapy, platinum-based chemotherapy was performed in 14 patients while one patient received chemotherapy using gemcitabine plus vinorelbine. The

Table 1. Patient Characteristics

	No. of patients
Sex	
Male	5
Female	10
Age (years)	
Median	57
Range	51–72
Performance status (ECOG)	
0	5
1	10
Histology	
Adenocarcinoma	13
Others	2
Previous treatment	
Chemotherapy only	7
Chemotherapy and radiotherapy	4
Chemotherapy and surgery	4
No. of prior chemotherapy regimen	
Median	2
Range	1–5

chemotherapeutic regimens most frequently used as a prior treatment were cisplatin plus gemcitabine plus vinorelbine in nine patients, cisplatin plus docetaxel in six and carboplatin plus paclitaxel in five. The number of prior chemotherapy regimens ranged from 1 to 5, with a median number of 2.

At an amrubicin dosage of 20 mg/m^2, no DLT was observed. At an amrubicin dosage of 25 mg/m^2, grade 4 neutropenia lasting 4 days or more was observed in one of the first three patients. Therefore, another three patients were treated at the same dose. Since these patients did not show any additional DLT, the dosage was then escalated to 30 mg/m^2. Grade 4 neutropenia lasting 4 days or more was observed in two of the first three patients. Therefore, another three patients were assigned to receive the treatment at the same dose. Out of those three patients, one patient developed febrile neutropenia. Therefore, DLT was observed in three of six patients at an amrubicin dosage of 30 mg/m^2. As a result, amrubicin 30 mg/m^2 was determined to be the MTD. A total of 11 and 17 cycles of the treatment were performed at a dose of 20 and 25 mg/m^2, respectively. DLT was observed in 0 of 11 cycles at 20 mg/m^2 and in 1 of 17 cycles at 25 mg/m^2.

Toxicity

Myelosuppression, especially neutropenia, was the principal toxicity of this combination chemotherapy as shown in Table 2. At the MTD (amrubicin 30 mg/m^2), all three DLTs were related to neutropenia. Two patients had grade 4 neutropenia which persisted for 4 days or more. The third patient with grade 4 neutropenia had a fever associated with neutropenia that lasted for 4 days. The other three patients at the MTD also had grade 4 neutropenia which persisted <4 days. All six patients were treated with G-CSF after grade 4 neutropenia had been found. Grade 3 neutropenia was observed in one patient at dose of 20 mg/m^2 and grades 3/4 neutropenia in two at a dose of 25 mg/m^2. The onset of neutropenia (ANC $\leq$ 1500/μl) occurred between days 9 and 21: the median time to nadir and the recovery of neutrophil count (ANC $\geq$ 1500/μl) from the nadir was 16 days (range 3–23) and 5 days (range 1–24), respectively. No grade 3 or greater thrombocytopenia was observed.

Table 2. Toxicities occurring during the first cycle

Amrubicin (mg/m^2)	20		25		30	
Cisplatin (mg/m^2)	20		20		20	
	(n = 3)		(n = 6)		(n = 6)	
NCI-CTC grade	1/2	3/4	1/2	3/4	1/2	3/4
Leukopenia	1/1	1/0	2/3	0/0	0/2	1/3
Neutropenia	0/1	1/0	0/3	1/1	0/0	0/6
Febrile neutropenia	–/–	0/0	–/–	0/0	–/–	1/0
Thrombocytopenia	1/0	0/0	2/0	0/0	1/1	0/0
Hemoglobin	1/2	0/0	2/3	0/0	2/4	0/0
Fatigue	0/0	0/0	0/0	0/0	0/3	0/0
Vomiting	1/1	0/0	2/1	0/0	4/1	0/0
AST/ALT	0/1	0/0	3/1	0/0	3/1	0/0
Creatinine	1/0	0/0	1/0	0/0	0/0	0/0

The non-hematologic toxicity was mild. The toxicities mainly included nausea/vomiting, an elevation of AST/ALT and fatigue while all of them were rated as grade 2 or less and manageable.

Antitumor Activity

A partial response was observed in four patients, no change in eight and progressive disease in three. Therefore, the overall response rate was 27% (95% confidence interval: 4–49%). Responding patients were observed at every dose level of amrubicin: 1/3 at 20 mg/m^2, 2/6 at 25 mg/m^2 and 1/6 at 30 mg/m^2. Out of four patients with a partial response, two and two patients received three cycles and four cycles of amrubicin plus cisplatin chemotherapy, respectively. The responding duration of the four patients was 62, 65, 70 and 174 days.

DISCUSSION

The present study was designed to determine the recommended dose of amrubicin by estimating the MTD for combination

chemotherapy using amrubicin and cisplatin in previously treated NSCLC patients. The MTD was estimated to be 30 mg/m^2 when cisplatin at a dose of 20 mg/m^2 was administered concurrently for 3 consecutive days. At the MTD (30 mg/m^2), all six patients had grade 4 neutropenia which resulted in DLT in a half of these patients. Based on the MTD, the recommended dose was thus determined to be 25mg/m^2. The observed hematological toxicities, except for neutropenia and non-hematological toxicities, were not severe and thus were manageable. Although this study included four patients who had previously undergone thoracic radiotherapy, neither an aggravation of radiation pneumonitis nor any acute pulmonary disorders occurred.

In lung cancer patients with no prior treatment, the recommended dose of amrubicin (days 1 to 3) has been reported to be 45 mg/m^2 while it is 40 mg/m^2 when cisplatin 60 mg/m^2 is administered on day 1 (15,21). The main toxicity of amrubicin monotherapy and the combination chemotherapy tends to be myelosuppression, especially, neutropenia. Grade 3/4 neutropenia was observed in 75% of all patients with amrubicin monotherapy and in 95% of those with the combination chemotherapy (15,16,21). Therefore, the treatment regimens described above are considered to be intolerable in patients with prior chemotherapy whose bone marrow function has been, more or less, damaged.

In the present study, the concurrent administration of amrubicin and cisplatin of low dose for 3 consecutive days was performed based on the hypothesis that the addition of cisplatin to amrubicin may augment the anti-tumor activity of amrubicin. In an *in vitro* study using lung cancer cell lines, the addition of cisplatin to amrubicinol, which is an active metabolite of amrubicin , has been reported to not only enhance the inhibitory activity of topoisomerase II by amrubicinol but also to increase the formation of the DNA intestrand cross by cisplatin (18). In addition, the combination treatment with cisplatin has been shown not to alter the pharmacokinetics of either amrubicin or amrubicinol (21). These observations suggest that the concurrent administration of amrubicin and cisplatin may have an excellent anti-tumor activity without any unexpected severe toxicity, even in patients who had previously been administered platinum-based chemotherapy.

In conclusion, the MTD and recommended dose of amrubicin were determined to be 30 mg/m^2 and 25 mg/m^2, respectively, when cisplatin 20 mg/m^2 was administered concurrently for 3 consecutive days, in NSCLC patients who had received prior chemotherapy. The DLTs included neutropenia lasting 4 days or more, and febrile neutropenia. The overall response rate was 27%. A Phase II study is currently underway in these patients.

Acknowledgments

We would like to thank Mr Brian Quinn for his critical review and Ms Yumiko Oshima for her help in preparing the manuscript.

References

1. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, Baker S, Jr, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004;22:330–53.
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92–8.
3. Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. *BMJ* 1995;311:899–909.
4. Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* 2000;18: 2095–103.
5. Fossella FV, DeVore R, Kerr RN, Crawford J, Natale RR, Dunphy F, et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. *J Clin Oncol* 2000;18:2354–62.
6. Shepherd FA, Fossella FV, Lynch T, Armand JP, Rigas JR, Kris MG. Docetaxel (Taxotere) shows survival and quality-of-life benefits in the second-line treatment of non-small cell lung cancer: a review of two phase III trials. *Semin Oncol* 2001;28:4–9.
7. Fossella FV, Lynch T, Shepherd FA. Second line chemotherapy for NSCLC: establishing a gold standard. *Lung Cancer* 2002;38 (Suppl 4): 5–12.
8. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* 2004;22:1589–97.
9. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 2003;21: 2237–46.
10. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–32.
11. Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y. Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. *Jpn J Cancer Res* 1998;89:1067–73.
12. Morisada S, Yanagi Y, Noguchi T, Kashiwazaki Y, Fukui M. Antitumor activities of a novel 9-aminoanthracycline (SM-5887) against mouse experimental tumors and human tumor xenografts. *Jpn J Cancer Res* 1989;80:69–76.
13. Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y. In vivo efficacy and tumor-selective metabolism of amrubicin to its active metabolite. *Jpn J Cancer Res* 1998;89:1055–60.
14. Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T, Yamaoka T. A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. *Jpn J Cancer Res* 1998;89:1229–38.
15. Sugiura T, Ariyoshi Y, Negoro S, Nakamura S, Ikegami H, Takada M, et al. Phase I/II study of amrubicin, a novel 9-aminoanthracycline, in patients with advanced non-small-cell lung cancer. *Invest New Drugs* 2005;23:331–7.
16. Furuse K, Ikegami H, Ariyoshi Y. Two phase II studies of amrubicin (SM-5887), a novel 9-amino-anthracycline, in patients with advanced non-small cell lung cancer (NSCLC): West Japan Lung Cancer Group Trials. *Ann Oncol* 1998;9(Suppl 4):88 (abstract 422).
17. Takigawa N, Shibayama T, Tada A, Aoe K, Tabata M, Kiura K, et al. Rational combinations of amurubicin with cisplatin and irinotecan in small-cell lung cancer cell line. *Jpn J Lung Cancer* 2004;44:(abstract 11–62).
18. Yamauchi S, Kudoh S, Kimura T, Hirata K, Yoshikawa J. Additive effects of amrubicin with cisplatin on human lung cancer cell lines. *Osaka City Med J* 2002;48:69–76.

19. Chiba Lung Cancer Study Group. Comparative study on vindesine plus cisplatin treatment for advanced non small cell lung cancer (three divided doses and single doses of cisplatin) (Chairman: Yutaka Yamaguchi). *Jpn J Cancer Chemother* 1991;18:425–30.
20. Belliveau JF, Posner MR, Ferrari L, Crabtree GW, Cummings FJ, Wiemann MC, et al. Cisplatin administered as a continuous 5-day infusion: plasma platinum levels and urine platinum excretion. *Cancer Treat Rep* 1986;70:1215–7.
21. Ohe Y, Negoro S, Matsui K, Nakagawa K, Sugiura T, Takada Y, et al. Phase I-II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer. *Ann Oncol* 2005; 16:430–6.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

A Prospective Japanese Study of the Association between Personality and the Progression of Lung Cancer

Jun Nagano[1], Yukito Ichinose[2], Hiroshi Asoh[2], Jiro Ikeda[2], Akira Ohshima[3], Nobuyuki Sudo[4] and Chiharu Kubo[4]

Abstract

Objective To examine predictive values for the effect of the "Type 1" (hopeless and emotion-suppressive, cancer prone), "Type 4" (autonomous, healthy), and "Type 5" (rational/antiemotional, cancer prone) personalities proposed by Grossarth-Maticek on the prognosis of lung cancer patients.

Methods 68 lung cancer patients were scored on the Types 1, 4, and 5 personality scales of the Short Interpersonal Reactions Inventory and were followed until the date of death or were censored at a maximum of 5.7 years after entry.

Results The stage at diagnosis tended to be higher in patients with a high Type 1 or a low Type 4 score. A univariate Cox proportional hazards model showed that a high tendency toward Type 1 or Type 5 was related to an increased hazard of death. Adjustment for age, performance status, and stage, however, attenuated the relation to Type 1, leaving only Type 5 as a significantly related personality factor.

Conclusion A high Type 5 tendency may predict poor survival in lung cancer patients, whereas Types 1 and 4 may not be independent predictors.

Key words: prospective study, lung cancer, personality, stress, survival

(DOI: 10.2169/internalmedicine.45.1453)

INTRODUCTION

Lung cancer is one of the most common cancers worldwide (1). In Japan, the mortality rate from lung cancer has constantly increased from the late 1900s, and now is the leading cause of cancer death for men and the second leading cause for women (2). The cure rate of lung cancer is poor and has little improved in the past two decades, especially for patients with advanced disease (3).

Several psychosocial factors have been linked to the onset and progression of cancer (4-6). Hopelessness/helplessness and suppression of negative emotions are personality factors that have been associated with cancer in previous prospective studies, especially studies examining their effects on cancer progression, although much more research will be necessary before a definite conclusion can be made (4). The above factors have been linked to cancer progression mainly in studies of breast cancer (7-11) or cancer of mixed sites (12, 13). However, little has been reported on lung cancer, except for recent studies of a personality relevant to emotional suppression (14) and of optimism, which may be the opposite of hopelessness/helplessness (15).

Grossarth-Maticek and colleagues conceptualized a disease-prone/healthy personality theory including a notion of six personality types, "Type 1" to "Type 6" (see Appendix) (16-19). This theory began with four types, Types 1 to 4, with the other two types added later (17). Types 1, 4, and 5, which include either one or both emotional suppression and hopelessness/helplessness as elements, have been linked to cancer. Type 1 is an "object dependent" personality that has a highly valued object (person or condition) through which well-being is chronically swayed toward hopelessness/helplessness and depression by withdrawal of the ob-

[1] Institute of Health Science, Kyushu University, Fukuoka, [2] Department of Thoracic Oncology, National Kyushu Cancer Center, Fukuoka, [3] Department of Psycho-oncology, National Kyushu Cancer Center, Fukuoka and [4] Department of Psychosomatic Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka

Received for publication May 8, 2005; Accepted for publication October 22, 2005

Reprint requests should be addressed to Jun Nagano, Institute of Health, Science, Kyushu University, 6-1 Kasuga-koen, Kasuga, Fukuoka 816-8580

DOI: 10.2169/internalmedicine.45.1453

ject. It is also characterized by altruistic behaviors and inhibition when expressing negative emotions and personal needs. Type 4 is an "autonomous" personality, the opposite of object dependence, and expresses personal needs in appropriate ways to obtain wellbeing. Type 5 is characterized by an extreme tendency toward "rational and antiemotional" reactions to stress, which also is an aspect of suppression of negative emotions. In cohort studies that began in Yugoslavia in the 1960s and in West Germany in the 1970s, Type 1 was shown to be prone to cancer, Type 4 was the most healthy and resistant to diseases, including cancer and cardiovascular disease (16), and Type 5 (17), as is its original concept of the rationality/antiemotionality (R/A) personality (20) was also prone to cancer. Not many studies have so far independently addressed the relation between these personalities and cancer risk (21-25), and no study has explored their possible relations with cancer progression.

In 1998, lung cancer patients hospitalized in a regional cancer center in Japan were asked to complete a Japanese version of the Short Interpersonal Reactions Inventory (SIRI) (26). The SIRI is a self-report questionnaire that consists of six scales corresponding to Types 1 to 6 (17). This data was used as part of a case-control study to examine the associations between Types 1 to 6 and the risks of lung cancer and myocardial infarction (24). The results failed to support the hypothesis that Types 1 and 5 were positively associated and Type 4 was inversely associated with lung cancer risk. These findings, however, did not exclude the possibility that Types 1 and 5 increase, while Type 4 decreases, the hazard of death in lung cancer patients. In view of the paucity of data on personality and lung cancer progression (14, 15), we studied the relationships of Types 1 to 6 personalities and the survival of lung cancer patients over a follow-up period of up to 5.7 years.

Table 1. Demographic, Behavioral, and Clinical Characteristics of the Subjects (N=68)

Characteristics		%
Gender	Male	74
	Female	26
Age	<40 years	3
	40-49 years	18
	50-59 years	41
	60+ years	38
Education	Junior high school	22
	High school	46
	Junior college or equivalent	10
	College or higher	22
Marital status	Married	75
	Not married	25
Smoking	Never	26
	Past	3
	Current	71
Time since diagnosis	0-4 weeks	49
	5-8 weeks	34
	9-12 weeks	7
	13+ weeks	10
Histology	Small cell carcinoma	15
	Non-small cell carcinoma	85
Performance status	0	74
	1	21
	2	6
Stage	I	29
	II	4
	IIIA	16
	IIIB	19
	IV	31

METHODS

Subjects

Eligible lung cancer patients in a consecutive series were asked to participate in the study during their admission to the National Kyushu Cancer Center in Japan from February to December 1998. Criteria for enrollment were age of 70 years or under and not too severely ill to complete a self-administered questionnaire without assistance. Participating patients were asked to complete a set of questionnaires including the SIRI. The results of a case-control study using the baseline data of these subjects were reported previously (24). Follow-up was terminated in January 2004, and data was censored at that time. Demographic and clinical data were obtained from medical records.

The Short Interpersonal Reactions Inventory (SIRI)

The SIRI is a self-administered questionnaire developed to measure the 6 Grossarth-Maticek personality types (17). All the participants completed a Japanese version of the SIRI, for which psychometrical reliability and validity were reported elsewhere (26). The SIRI contains 70 items with a dichotomous answer, "yes" or "no", of which 10 items correspond to each of the six types, except for Type 4 which is represented by 20 items, including 10 reverse items. The score for each type is the number of positive responses, except for the Type 4 score which is divided by two so that all types reflect a score between 0 and 10.

Analysis

Survival was measured from the date of recruitment through the date of death or was censored at the last contact date for surviving patients. Demographic, behavioral, and clinical factors including sex, age (continuous), marital status (married, not married), education years (12 years or less, 13 years or over), smoking status (current smoker, never/past smoker), time since diagnosis (0-4 weeks, 5+ weeks), histology (small cell carcinoma, other types), and stage (ordinal) were considered as known or potential risk factors for death in the lung cancer patients. Associations

DOI: 10.2169/internalmedicine.45.1453

Table 2. **Association Between Peraonality Factors and Demographic, Behavioral, and Clinical factors**

Characteristics	Grossarth-Maticek personality											
	Type 1				Type 4				Type 5			
Score	0-3	4-5	6+		0-6.5	7-8	8.5+		0-5	6-7	8+	
N	27	20	21	p[a]	21	28	19	p[a]	24	26	18	p[a]
Male (%)	56	85	86	0.0243	86	71	63	0.26	58	81	83	0.11
Age, mean (years)	54.4	59.5	56.1	0.13[b]	54.5	56.0	59.2	0.21[b]	52.2	57.1	61.2	0.0021[b]
Marital status: married (%)	70	75	81	0.70	62	79	84	0.23	71	85	67	0.34
Education years 13+ (%)	33	20	43	0.29	38	36	21	0.46	42	19	39	0.19
Current smoker (%)	56	85	76	0.07	76	68	68	0.79	63	77	72	0.53
Time since diagnosis, mean (weeks)	7.4	7.4	9.5	0.55[c]	10.1	7.4	6.6	0.39[c]	10.1	7.4	6.3	0.49[c]
Histology: small cell carcinoma (%)	7	10	24	0.22	14	7	21	0.38	13	12	17	0.88
Performance status 1-2 (%)	26	15	38	0.24	29	29	21	0.82	21	19	44	0.13
Early stage disease (stage I) (%)	41	45	0	0.0004[d]	10	36	42	0.0449[d]	29	27	33	0.94[d]

[a]P-values were based on the chi-square test, [b]analysis of variance, [c]Kruskal-Wallis test, or [d]Fisher's exact test.

between these factors and the SIRI scores were examined by either the student's t-test or one-way analysis of variance, as appropriate. A univariate or multivariate Cox proportional hazards model was used to examine the associations between a factor(s) of interest and survival. Of these demographic and clinical variables, those found to be associated with survival with a p-value <0.1 on univariate analysis were adjusted for each other in a multivariate model. Factors related to survival with a p-value <0.1 in the multivariate model were then retained as variables to be considered in exploring the relationship of the SIRI scores with survival. According to the SIRI scores, the subjects were categorized into tertiles. Hazard ratios of death with a 95% confidence interval for the intermediate and highest score categories were estimated in comparison with the lowest category, and a linear trend of association was tested, with or without adjustment for covariates as determined above. Computations were done using the SAS software (UNIX version release 8.2, SAS Institute Inc.). Reported p-values were two-sided, and p-values less than 0.05 were regarded as statistically significant.

RESULTS

Of the 101 eligible patients, 95 agreed to participate in the study and signed a written consent form. For the present longitudinal analyses, 25 patients who at entry were admitted for a second or later admission for the treatment of relapse or re-growth of the disease were excluded. Data on survival status were obtained for the remaining 70 patients who were in their first admission. Also included were two patients who had been transferred from another hospital for the purpose of radiotherapy. Of these 70, two were excluded who had at first been strongly suspected of having primary lung carcinoma, but the diagnosis was not confirmed on further examinations. All patients had been informed of their diagnosis before the time of recruitment.

Demographic, behavioral, and clinical characteristics of the subjects and their associations with the personality variables at baseline

Table 1 summarizes demographic, behavioral, and clinical characteristics of the studied patients. Nearly 80% of the patients were 50 years or older at entry, and 74% were male. Approximately 30% had an education of junior college or higher, 13+ years, 75% were married and living with their spouse, and 71% were current smokers. Time from the cancer diagnosis through recruitment was 12 weeks or less for most (90%) patients. Most (85%) patients had a histological diagnosis of non-small cell carcinoma, 95% were at the Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, and fewer than 30% had an early stage disease according to the International Staging System (27). During the follow-up period, 40 patients died and 28 were censored. The median time of survival was 0.8 (range 0.2-5.4) years for diseased subjects, and the median follow-up was 5.1 (range 4.7-5.7) years for survivors.

Table 2 shows the personality scores in relation to demographic and clinical characteristics at baseline. The percentage of men in the lowest Type 1 category was lower than that of the higher two categories. Age tended to increase linearly as the Type 5 score increased. Marital status, education level, smoking status, time since diagnosis, histology, and PS were not associated with any of the three personality types. Regarding the disease stage, there were notable associations. None of the patients in the high Type 1 category were diagnosed as having an early stage disease (stage I), while more than 40% were in stage I in the lower two Type 1 categories. Conversely, the low Type 4 category included a smaller percentage of early-stage patients than the two higher Type 4 categories. These associations of disease stage with Types 1 and 4 were statistically significant. Type 5 was

DOI: 10.2169/internalmedicine.45.1453

Table 3. Association Between Personality and the Hazard of Death of Lung Cancer Patients

			Unadjusted (crude) HR		Adjusted HR[a]	
Scale	Score	N	HR (95%CI)	p trend	HR (95%CI)	p trend
Type 1	0-3	27	1.00 (ref.)		1.00 (ref.)	
	4-5	20	0.74 (0.31-1.79)		0.51 (0.20-1.26)	
	6+	21	3.26 (1.59-6.69)	0.0021	1.73 (0.80-3.73)	0.16
Type 2	0-1	34	1.00 (ref.)		1.00 (ref.)	
	2-3	18	1.18 (0.60-2.34)		0.98 (0.48-2.00)	
	4+	16	1.27 (0.87-1.86)	0.18	0.97 (0.64-1.48)	0.90
Type 3	0-1	18	1.00 (ref.)		1.00 (ref.)	
	2-3	25	0.63 (0.28-1.42)		0.86 (0.38-1.95)	
	4+	25	0.95 (0.44-2.06)	0.96	1.08 (0.48-2.40)	0.83
Type 4	0-6.5	21	1.00 (ref.)		1.00 (ref.)	
	7-8	28	0.70 (0.35-1.42)		0.63 (0.29-1.35)	
	8.5+	19	0.57 (0.25-1.31)	0.18	0.65 (0.27-1.55)	0.29
Type 5	0-5	24	1.00 (ref.)		1.00 (ref.)	
	6-7	26	1.75 (0.79-3.86)		2.20 (0.96-5.03)	
	8+	18	3.21 (1.41-7.30)	0.0054	2.79 (1.13-6.86)	0.0221
Type 6	0	24	1.00 (ref.)		1.00 (ref.)	
	1	22	0.94 (0.43-2.08)		0.75 (0.32-1.74)	
	2+	22	1.36 (0.65-2.81)	0.42	1.07 (0.49-2.34)	0.85

HR: hazard ratio; CI: confidence interval. [a]Adjusted for age, performance status, and stage.

not related to the stage at baseline. None of the other scores of Types 2, 3, and 6 were appreciably associated with any demographic or clinical characteristic at baseline (data not shown).

To search for reasons that would explain the relation between stage and Types 1 and 4, we studied the charts for any two factors that may represent health-care behaviors that had led to diagnosis. First, we classified the cues to diagnosis into three categories, "asymptomatic and found by check-up", "found by symptoms such as cough and chest pain", and "incidental, e.g., found when patient saw a doctor for reasons unrelated to cancer". Second, we calculated the time that it took from the cue to the time of diagnosis. These factors were examined in relation to the Type 1 and 4 categories. However, no two factors were significantly associated with those personalities (data not shown).

The personality factors and survival at follow-up

Of the demographic, behavioral, and clinical factors, gender ($p=0.0287$), age ($p=0.088$), PS ($p<0.0001$), and stage ($p<0.0001$) were related to survival by univariate analyses, while education level, marital status, smoking status, time since diagnosis, and histology were not. A multivariate Cox proportional hazards model including gender, age, PS, and stage revealed that increasing age ($p=0.0226$), poorer PS ($p<0.0018$), and a more advanced stage ($p<0.0001$) were associated with a higher hazard of death, whereas gender was no longer predictive of prognosis ($p=0.11$). Therefore, age, PS, and stage were adjusted for in the subsequent analyses of the personality factors.

Table 3 shows the associations of the personality factors with the survival of lung cancer patients. In the univariate analyses, the highest Type 1 category was significantly associated with an increased hazard of death when compared with the lowest category. The crude hazard of death significantly increased as the score of Type 5 increased. When age, PS, and stage were adjusted, however, the association with Type 1 personality became unclear, and only the positive association of Type 5 to survival remained significant. The adjusted hazard ratio of death for the patients in the high Type 5 category was approximately 2.8 as compared to that of the low category. Types 2, 3, and 6 were not materially associated with survival with or without adjustment for the covariates. In Fig. 1, the Kaplan-Meier survival curves are shown according to the three categories of Type 5 scores.

DISCUSSION

This study addressed the question of whether or not the personalities proposed by Grossarth-Maticek, especially the Type 1, 4, and 5 personalities which have been associated with cancer risk in healthy populations (16, 17, 20), can be predictive of the prognosis of lung cancer patients. Univariate analyses found a high tendency for Types 1 and 5 to be related to an increased hazard of death, but adjustment for other risk factors attenuated the relation to Type 1, leaving

DOI: 10.2169/internalmedicine.45.1453

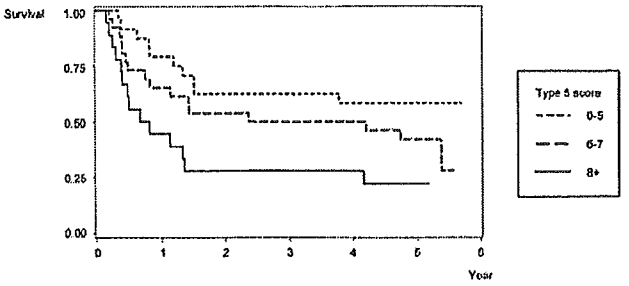


Figure 1. Survival curvers of lung cancer patients according to low, medium, or high Type 5 scores

only Type 5 as a significant, independent predictor of survival for lung cancer patients.

The Type 5 personality, characterized by "rational and antiemotional" reactions to stress, was formerly called the R/A personality and later re-conceptualized into the six type personalities. The R/A represents an extreme tendency to rationalize conflicts or frustrations and to suppress emotional reactions as represented by the SIRI item "when people make emotional demands on me, I usually react only rationally, never emotionally", and can be considered a form of the suppression of negative emotions. Few studies have independently examined this unique personality construct in association with cancer risk. A cohort study in the Netherlands examined the rationality and the antiemotionality factors separately, and found that a high antiemotionality score was associated with an increased risk of breast cancer (28). A cohort study in Japan, however, found that the risk of mortality from cancer of men scoring in the middle range of the R/A scale was lower than for those scoring low on the scale (25). To our knowledge, no previous data is available on the association between the Type 5 and R/A personalities and cancer progression.

Regarding the suppression/expression of negative emotions as a more common construct, several studies have examined this personality factor in relation to cancer progression. Most focused on breast cancer, and some (7, 10, 11), albeit not all (8, 9), agreed that suppressing or controlling negative emotions worsened the prognosis of breast cancer patients. To the contrary, "expression of negative affect" (7) and "expressing emotions" (10) were associated with a better prognosis in breast cancer. As for lung cancer, Nakahara et al found that a better survival was associated with a personality characterized by high "Free Child" and low "Adapted Child" scores (14).

Significant associations were found between the stage at baseline and the scores of Types 1 and 4. None of the patients with an early stage disease were categorized into the high Type 1 category, and only a few into the low Type 4 category. It is possible that people with a high Type 1 or a low Type 4 tendency may be much more negative than others toward taking a lung cancer screening, or that these people may be more hesitant to visit a clinic for further examination after preliminary tests indicating lung cancer. Supplemental analyses, however, did not yield evidence to support these hypotheses, which may have accounted for the associations between baseline stage and Types 1 and 4. Other hypotheses may include the possibility that the chances of cancer progression from an early to an advanced stage are higher in people with a high Type 1 or low Type 4 tendency, or that the cancer progression is more rapid, thereby creating a lower chance of having a disease discovered at an earlier stage.

It should be noted that all of the present study participants had been informed of their diagnosis before they completed the SIRI. In a population-based prospective study in the Netherlands, Bleiker et al examined the possible differences in personality traits between before and after a diagnosis of breast cancer. They found a significant decrease in the "rationality", "emotional expression-out", and "emotional-control" scores from before to after the diagnosis (29). In the present sample, although the scores for the personalities were not related to the time since diagnosis to entry, it is impossible to preclude the possibility that the patients' personalities as measured by the SIRI had changed between before and just after the diagnosis. The present associations of Type 5 with survival and Types 1 and 4 with stage at baseline may not be able to be generalized to lung cancer patients for whom a diagnosis has not yet been made or who are unaware of the diagnosis.

The present study has several limitations. The small sample size did not allow analyses with stratification by factors such as stage, gender, and histology (small cell carcinoma or non-small cell carcinoma), although the association between the Type 5 personality and survival may differ according to these factors. In addition, the mechanisms which may explain the observed association with the Type 5 personality were not clarified. Patients with different personalities may differently decide on the choice of treatments, or respond differently to a certain treatment regimen, and such diversity may affect the clinical course. The present study could not consider the possible effects of such complex treatment factors. Also, it did not examine immunological parameters that might link personality factors with survival of lung cancer patients (30-32). Nevertheless, this is the first report specifically addressing if Types 1, 4, and 5 can be of prognostic value for persons suffering from a malignancy, and would confer evidence to the notion that personality factors are related to the prognosis of lung cancer.

In summary, the current prospective data suggested that a stronger Type 5 tendency may increase the hazard of death of lung cancer patients. Types 1 and 4 personalities were not an independent risk factor for death, although they might be associated with the stage at diagnosis. The current findings should be confirmed in future studies of a larger scale that examine behavioral and biological factors that would explain the association between the Type 5 personality and cancer progression.