CME/SAM

EGFR and K-*ras* Mutations Along the Spectrum of Pulmonary Epithelial Tumors of the Lung and Elaboration of a Combined Clinicopathologic and Molecular Scoring System to Predict Clinical Responsiveness to EGFR Inhibitors

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Upon completion of this activity you will be able to:
- define the probability of response to epidermal growth factor receptor (EGFR) inhibitors in lung cancer based on a combination of clinicopathologic features as well as mutational analysis of *EGFR* and K-*ras*.
- predict the relationship between histologic type and mutations of *EGFR* and K-*ras*.

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Questions appear on p 596. Exam is located at www.ascp.org/ajcpcme.

Abstract

*We tested 418 neoplasms along the whole spectrum of primary lung tumor histotypes for epidermal growth factor receptor (*EGFR*) and K-*ras *mutations. Clinicopathologic data from 154 patients undergoing treatment with EGFR tyrosine kinase inhibitors (TKIs) were retrospectively studied. A scoring system assigning a score for each positive or negative characteristic (+1, female sex, nonsmoking status, adenocarcinoma histotype, Asian ethnicity, and* EGFR *mutation; –1, current smoker and K-*ras *mutation; and 0, male sex, ex-smoker, nonadenocarcinoma histotype, and no mutations) was elaborated and tested with EGFR-TKI response.*

Salivary gland–type, mucin-rich, and neuroendocrine tumors do not harbor EGFR *mutations. A subset of nonmucinous adenocarcinomas, not necessarily of the bronchioloalveolar type, is related to* EGFR *mutations. Three probability groups significantly correlating with response to EGFR-TKIs were identified. Of note, the addition of molecular results did not significantly change the predictive value obtained by the combination of clinicopathologic characteristics alone in this scoring system.*

*K-*ras *mutations, significantly associated with the mucin-secreting type of adenocarcinoma, consistently predict lack of response in white patients.*

Lung cancer remains the leading cause of tumor-related death in industrialized countries,[1] with adenocarcinoma the most common histotype in the United States and Europe.[2,3] However, today, the distinction between small cell lung carcinoma (SCLC) and non–small cell lung carcinoma (NSCLC) seems to be an inadequate classification scheme for management of patients with lung cancer. This inadequacy is mainly because of the advent of novel targeted therapies showing therapeutic efficacy of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) or anti–vascular endothelial growth factor antibodies in a subset of NSCLC.[4-6] In particular, it is widely accepted that some clinicopathologic characteristics (female sex, nonsmoking status, and Asian ethnicity), together with the adenocarcinoma histotype, are the main clinical positive predictive factors when using EGFR-TKIs (ie, gefitinib and erlotinib).[7-10] In addition, mutations involving *EGFR* are known to be significantly associated with the aforementioned features[11-13] and response to EGFR-TKIs and are mutually exclusive with K-*ras* mutations, which significantly predict primary resistance to EGFR-TKI.[14-16]

Among the adenocarcinoma histotypes, those showing bronchioloalveolar (BAC) or papillary features are consistently related to a high response rate to EGFR-TKIs.[17-22] Given the high costs and possible toxic effects of EGFR-TKIs, oncologists are deeply involved in identifying the subset of patients with the best chance of clinical benefit.[23,24] Among all biologic predictive parameters so far studied (including expression of

EGFR and downstream signaling molecules or *EGFR* gene copy number assessed by fluorescent in situ hybridization), coordinated mutational analysis of *EGFR* and K-*ras* seems to be the most reliable for discriminating responders, even correlating significantly with survival.[25-34] In this scenario, it seems clear that pathologists are called on to closely collaborate with oncologists to accurately identify patients having the highest chance of clinical response to EGFR-TKI treatment.

This work was planned with a 2-fold aim: First, we sought to better evaluate the relationship between *EGFR* and K-*ras* mutational status and histologic features in a nearly complete spectrum of primary lung epithelial neoplasms (418 cases), reclassified according to the recent 2004 World Health Organization (WHO) classification of pulmonary tumors.[35] Second, we attempted to elaborate retrospectively a scoring system combining the baseline clinicopathologic features and the molecular assessment for *EGFR* and K-*ras* mutations helpful in predicting clinical response of patients treated with EGFR-TKIs.

Materials and Methods

Clinical and Pathologic Information

We obtained 418 cases of primary pulmonary epithelial tumors of different histotypes along the spectrum of the new 2004 WHO classification of lung tumors[35] from the files of the sections of pathologic anatomy from hospitals in Modena, Reggio Emilia, and Mestre, Italy. The data for patients with other known primary tumors were excluded from the study. All the original histologic slides were reviewed by 3 pathologists (G.R., A.C., and B.M.), and tumors were reclassified according to the criteria set by the new WHO classification[35]; uniform consensus was obtained in all cases. The series included 319 surgically resected, routinely formalin-fixed and paraffin-embedded cases. A mean of 5 H&E-stained slides per tumor were available (range, 2-12 slides). Diagnosis in the remaining 99 cases was performed on generous biopsy specimens not precluding an appropriate histologic examination and mutational analysis. In selected cases, immunohistochemical analysis was performed using an automated immunostainer (Benchmark, Ventana, Tucson, AZ) and the following antibodies: pan-cytokeratins (MNF116, Dakopatts, Glostrup, Denmark; and AE1/AE3, Ventana), thyroid transcription factor-1 (8G7G3/1, Ventana), epithelial membrane antigen (E29, DAKO), desmin (D33, DAKO), S-100 (NeoMarkers, Fremont, CA), cytokeratin 7 (OV-TL 12/30, DAKO), chromogranin A (DAK-A3, DAKO), CD56/NCAM (123C3, NeoMarkers), p63 (4A4, NeoMarkers), synaptophysin (Ventana), estrogen receptor (6F11, Ventana), CD31 (JC/70A, Ventana), thyroglobulin (2H11/6E1, Ventana), cytokeratin 20 (Ks 20.8, DAKO), CDX2 (7C7/D4, BioGenex, San Ramon, CA), MUC2 (M53, NeoMarkers), and MUC5AC (45M1, NeoMarkers).

Clinical data were obtained in all cases from pathology reports, clinical charts, referring physicians, or from the patient's families. The following data were recorded: age, sex, smoking history, tumor stage and grade, presence of mucin production, foci of atypical adenomatous hyperplasia (AAH) in surgically resected cases, and clinical response to EGFR-TKIs, when applicable. Staging was evaluated according to the American Joint Committee on Cancer.[36] In particular, lung tumors were considered as mucin-producing according to the mucin-rich variants included in the WHO classification (mucinous-type BAC, colloid adenocarcinoma, signet-ring cell adenocarcinoma, mucoepidermoid carcinoma, solid with mucus type of adenocarcinoma, and mixed acinar adenocarcinoma with mucinous type BAC) or when at least 10% of the entire tumor overtly showed a mucin-producing neoplastic component by H&E staining **Image 1**.

In regard to smoking habit, patients were subdivided as never smokers (lifetime exposure of <100 cigarettes), former smokers (quit smoking >3 years before the diagnosis), and current active smokers.

Treatment with EGFR-TKIs was given with 250 mg daily of oral gefitinib in 118 patients and with 150 mg daily of oral erlotinib in 36 patients. Therapy was stopped in case of disease progression or intolerable adverse effects.

Clinical response to targeted therapy was assessed by imaging studies according to the Response Evaluation Criteria in Solid Tumors.[37] The investigators (G.S., G.R., A.C., B.M.) were blinded to patient response to EGFR-TKI therapy.

DNA Extraction and Sequencing Analysis

Molecular analysis of *EGFR* exons 18, 19, and 21 and K-*ras* exon 2 was performed by direct-sequencing polymerase chain reaction as previously described[38] and both forward and reverse directions.

Scoring System Elaboration

According to the large body of evidence accepted worldwide about predictive factors in patients treated with EGFR-TKIs, we identified some clinicopathologic (female sex, never smoker, adenocarcinoma histotype, and Asian ethnicity) and molecular features (*EGFR* mutations) as positive predictive factors of response to EGFR small molecule inhibitors and then assigned 1 point (+1) for each of these characteristics (up to +5). By contrast, 1 point less (–1) was recorded when a patient was a current smoker or the tumor showed K-*ras* mutation (up to –2). No score (0) was given for male sex, former smoker, histotype other than adenocarcinoma, ethnicity other than Asian, or a tumor with a wild-type molecular setup (lack of *EGFR* and K-*ras* mutations). Thus, the final score could range from –2 to +5, leading to 8 possible categories.

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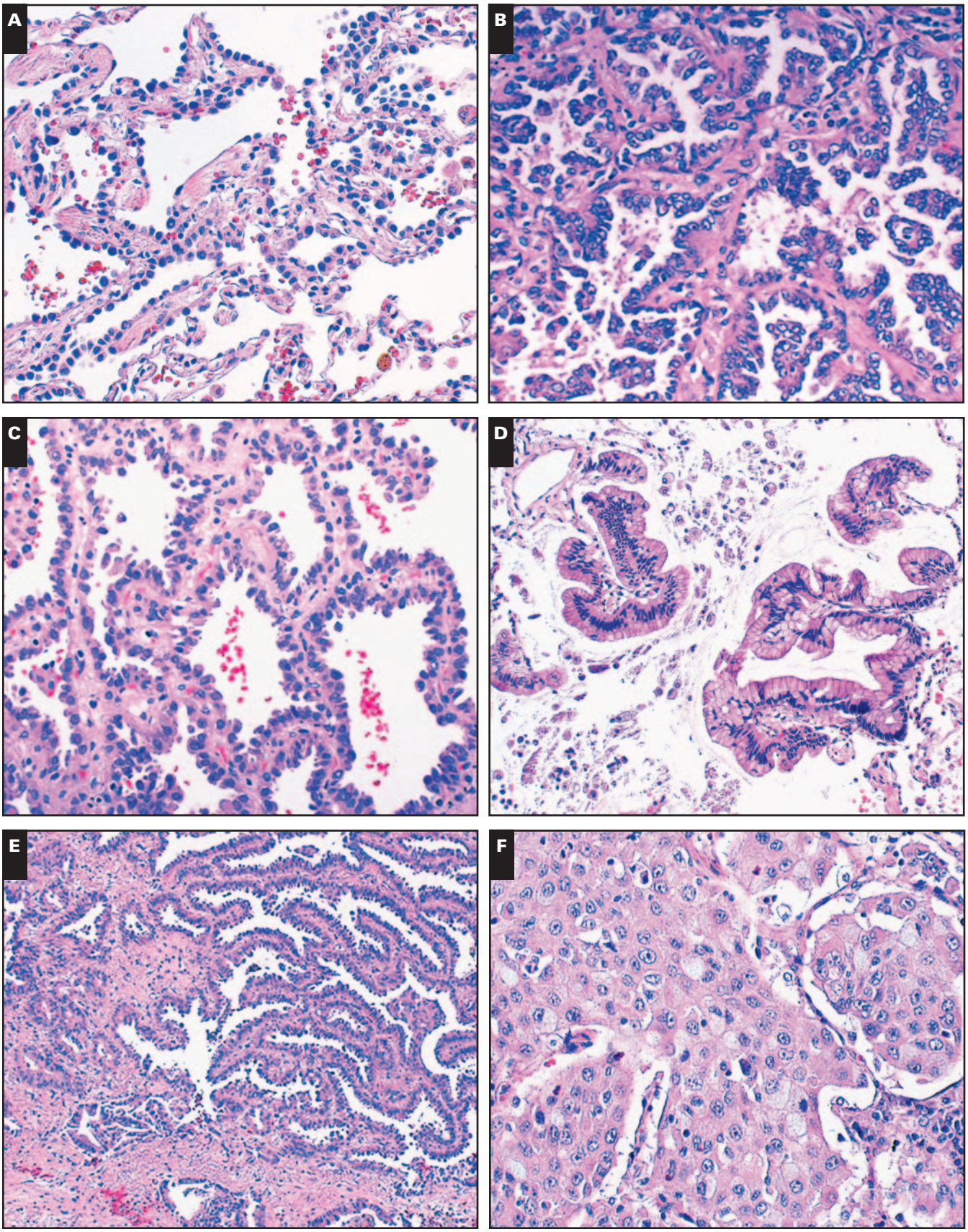

Image 1 Histopathologic examples of atypical adenomatous hyperplasia (**A**, H&E, ×230), papillary-type adenocarcinoma (**B**, H&E, ×200), nonmucinous-type bronchioloalveolar carcinoma (BAC; **C**, H&E, ×200), mucinous-type BAC (**D**, H&E, ×200), mixed acinar/conventional adenocarcinoma with nonmucinous-type BAC (**E**, H&E, ×100), and adenocarcinoma, solid with mucus type (**F**, H&E, ×200).

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Table 1
Baseline Clinicopathologic Features of the Entire Series of Patients With Lung Tumors and the Subgroup of Patients Treated With EGFR-TKI*

Characteristic	All Patients (n = 418)	EGFR-TKI–Treated (n = 154)
Sex		
Male	219 (52.4)	74 (48.1)
Female	199 (47.6)	80 (51.9)
Smoking habit		
Current	268 (64.1)	103 (66.9)
Never	118 (28.2)	36 (23.4)
Former	32 (7.7)	15 (9.7)
Histotype		
Adenocarcinoma†	181 (43.3)	98 (63.6)
nmBAC	10 (2.4)	2 (1.3)
mBAC	13 (3.1)	5 (3.2)
Mixed adenocarcinoma/BAC	37 (8.9)	10 (6.5)
Colloid	9 (2.2)	0 (0)
Signet-ring cell	9 (2.2)	3 (1.9)
Papillary	14 (3.3)	5 (3.2)
Solid with mucus production	9 (2.2)	5 (3.2)
Fetal type	2 (0.5)	0 (0)
Adenosquamous	4 (1.0)	2 (1.3)
Squamous cell	31 (7.4)	13 (8.4)
Large cell‡	6 (1.4)	1 (0.6)
Small cell	6 (1.4)	1 (0.6)
LCNEC	20 (4.8)	2 (1.3)
Typical carcinoid	20 (4.8)	0 (0)
Atypical carcinoid	5 (1.2)	0 (0)
Sarcomatoid	13 (3.1)	5 (3.2)
Carcinosarcoma	4 (1.0)	1 (0.6)
Adult blastoma	2 (0.5)	0 (0)
Cystic blastoma	1 (0.2)	0 (0)
Mucoepidermoid	5 (1.2)	1 (0.6)
Adenoid cystic	3 (0.7)	0 (0)
Sclerosing hemangioma	14 (3.3)	0 (0)
AAH§		
Present	48/319 (15.0)	15/70 (21)
Absent	271/319 (85.0)	55/70 (79)
Mucin production		
Present	57 (13.6)	16 (10.4)
Absent	361 (86.4)	138 (89.6)
Stage		
I A + B	180 (43.1)	33 (21.4)
II A + B	39 (9.3)	15 (9.7)
III A + B	15 (3.6)	8 (5.2)
IV	135 (32.3)	98 (63.6)
NA	49 (11.7)	—
Grade		
I	84 (20.1)	18 (11.7)
II	63 (15.1)	22 (14.3)
III + IV	246 (58.9)	104 (67.5)
NA	25 (6.0)	10 (6.5)
EGFR mutation		
Wild-type	367 (87.8)	121 (78.6)
Exon 18	1 (0.2)	0 (0)
Exon 19	27 (6.5)	18 (11.7)
Exon 21	23 (5.5)	15 (9.7)
K-*ras* mutation		
Wild-type	316 (75.6)	99 (64.3)
Exon 2	102 (24.4)	55 (35.7)

AAH, atypical adenomatous hyperplasia; BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; LCNEC, large cell neuroendocrine carcinoma; mBAC, mucinous BAC; NA, not available; nmBAC, nonmucinous BAC; TKI, tyrosine kinase inhibitor.
* Data are given as number (percentage) or number/total (percentage).
† Predominantly acinar/conventional adenocarcinoma.
‡ Including 2 basaloid and 2 clear cell types.
§ Considering only the surgically resected cases.

However, because all the patients included in the study were white, the score ranged from –2 to +4. Scores were combined into 3 groups, as follows: low probability of response to treatment (–2 to –1), intermediate probability (0 to +1), and high probability (+2 to +4).

Statistical Analysis

Correlation between clinicopathologic and molecular factors was determined by using the χ^2 and Fisher exact tests. Univariate correlation between all analyzed parameters was performed by using the Pearson correlation coefficient using the Statistical Package for the Social Sciences, version 13.0 (SPSS, Chicago, IL). Significance was determined when *P* values were less than .05. All tests were 2-sided.

Results

Clinicopathologic Characteristics of the Entire Series

Baseline characteristics of the patients analyzed in this study are summarized in **Table 1**. Briefly, the case series included 219 men and 199 women (268 current, 32 former, and 118 never smokers), with a mean age of 63.7 years (66 years for men and 61.2 years for women; range, 2-93 years). Histologic examination revealed that the tumors mainly consisted of adenocarcinoma (286 cases [68.4%]), including 57 cases (13.6%) with mucin production. Among the other histotypes, the series comprised adenosquamous carcinomas (4 cases); squamous cell carcinomas (31 cases); neuroendocrine tumors (51 cases) from low- (typical carcinoids), intermediate- (atypical carcinoids) to high-grade (SCLC and large cell neuroendocrine carcinoma) types; large cell carcinomas (6 cases); salivary-type malignancies (5 mucoepidermoid and 3 adenoid cystic carcinomas); sarcomatoid carcinomas (19 cases); and sclerosing hemangiomas (14 cases). AAH was identified in 48 (15.0%) of 319 surgically resected cases.

Overall, we identified 51 mutations (12.2%) in *EGFR* and 102 mutations (24.4%) in K-*ras*. In particular, *EGFR* mutations consisted of 27 frame deletions in exon 19 (E746_A750del in 16 cases; L747_P753del in 8; and E746_T751del in 3), 23 amino acid substitutions in exon 21 (L858R in 22 cases and a new missense mutation at codon 829, E829Q, in 1 adenosquamous carcinoma), and 1 missense mutation in exon 18 (G719C). All K-*ras* exon 2 mutations were missense mutations (G12C in 58 cases; G12V in 20; G12D in 9; G12S in 5; G12A in 4; G13C in 5; and G12-G13insG in 1).

Clinicopathologic Characteristics of Patients Treated With EGFR-TKI

As shown in Table 1, the subset of 154 EGFR-TKI–treated patients was representative of the entire series of patients. There were 80 women and 74 men with a mean age of 65.4 years at diagnosis (66.3 years for men and 64.6 years for

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women; range, 36-93 years), including 103 current, 36 never, and 15 former smokers.

Adenocarcinoma was the most common histotype (129 cases [83.8%]), including pure BAC (2 nonmucinous and 5 mucinous) and mixed adenocarcinoma-BAC (10 cases). Sixteen tumors (10.4%) showed mucin production, and tumor-associated AAH foci were observed in 15 surgically resected cases (21%).

More than half of the patients had advanced disease at diagnosis, and 67.5% of tumors were high-grade (grade III-IV). *EGFR* mutations were observed in 33 cases (21.4%), 18 on exon 19 and 15 on exon 21. Mutations on K-*ras* exon 2 were identified in 55 cases (35.7%).

Overall, the response rate was 18.8% (29 cases). Stable disease was observed in 48 cases (31.2%) and progression of disease in 77 cases (50%).

Predictive scores ranged from –2 (6.5%) to +4 (12.3%), but the majority of patients had a score of –1 (28.6%) or 0 (24.0%). Low (–2 or –1), intermediate (0 or +1), and high (+2, +3, and +4) probability of response was observed in 54 (35.1%), 59 (38.3%), and 41 (26.6%) of cases, respectively.

Statistical Correlations in the Entire Series

Female sex was significantly associated with nonsmoking status ($P < .0001$), adenocarcinoma histotype with BAC features or pure nonmucinous type of BAC ($P = .040$), and lower tumor grade ($P < .0001$) and stage ($P = .040$). Female sex was also significantly associated with the occurrence of *EGFR* mutations (42 of 51 *EGFR* mutations; $P < .0001$), whereas K-*ras* mutations were significantly more frequent in men (30.6% vs 17.1%; $P = .001$) **Table 2**.

An active smoking habit was significantly correlated with male sex (81.2% vs 45.2%; $P < .0001$), mucin-producing adenocarcinoma (76.2% vs 60.4%; $P = .003$) and mucinous-type BAC ($P < .001$), higher tumor grade ($P < .0001$) and stage ($P = .014$), and K-*ras* mutations (33.2% vs 2.5% in never smokers; $P < .0001$). Overall, 73% of patients with mucin-producing tumors were heavy smokers.

Among tumor histotypes, adenocarcinoma (including all variants) was significantly associated with the presence of AAH in resected cases (47/48 cases [98%] of AAH in patients with adenocarcinoma; $P < .0001$), higher tumor stage (39.1% of adenocarcinomas vs 17.5% of other histotypes; $P = .001$) and grade (56.3% of adenocarcinomas vs 36.6% of other histotypes; $P < .0001$), and *EGFR* (49/51; $P < .0001$) and K-*ras* mutations (87/102; $P < .0001$). The numbers and percentages of *EGFR* and K-*ras* mutations for each tumor type are shown in Table 2. Of note, salivary gland–derived tumors (mucoepidermoid and adenoid cystic carcinomas), carcinoid tumors (typical and atypical), blastomas, fetal-type adenocarcinomas, and sclerosing hemangiomas did not show mutations in *EGFR* or K-*ras* genes.

The presence of mucin production was significantly correlated with higher tumor grade, lack of AAH and *EGFR* mutations (none detected in mucin-producing tumors) ($P < .0001$), and the presence of K-*ras* mutations (47% vs 8.3% in non–mucin-producing tumors; $P < .0001$).

Statistical Correlations in Patients Treated With EGFR-TKI

Correlations between baseline clinicopathologic characteristics and *EGFR* and K-*ras* gene mutations are shown in **Table 3**. Female sex, nonsmoking status, presence of *EGFR* mutation, and lack of K-*ras* mutations were all significantly ($P < .0001$) associated with a positive clinical response (partial and complete responses). Also the adenocarcinoma histotype and the lack of mucin production ($P < .0001$) were significantly related to response rate. In addition, a history of never smoking was significantly correlated with female sex ($P < .0001$), adenocarcinoma histotype ($P = .013$), lower tumor grade ($P < .0001$), nonmucinous tumors ($P = .012$) and presence of *EGFR* mutations ($P < .0001$).

Mutations of *EGFR* and K-*ras* were directly and inversely, respectively, correlated with the response rate in a highly significant manner ($P < .0001$). Thus, as expected, a significant association between the scoring system including all of these parameters and clinical responsiveness was observed in the subset of EGFR-TKI–treated patients **Table 4**. A higher score (+2 to +4) was statistically correlated with female sex (36 vs 5 patients), never smoking status (32 never vs 3 current smokers), and nonmucinous adenocarcinomas (41 nonmucinous vs 0 mucinous adenocarcinomas), but not with nonmucinous-type BAC, presence of AAH, or tumor grade. It is noteworthy that if the results of molecular analysis for *EGFR* and K-*ras* mutations were excluded as determining factors in the scoring system, female sex, never smoking status, and nonmucinous adenocarcinoma histotype nevertheless showed a highly statistical relationship with a positive clinical response ($P < .0001$) **Table 5**.

Most important, when the probability group based on the scoring system was applied, patients in the low probability group (score –2 to –1) had no clinical response to EGFR-TKIs. Patients in the intermediate probability group (score 0 to +1) showed 2 partial and 2 complete (globally, 7%) responses, whereas 25 (61%) of 41 patients in the high probability group (score +2 to +4) experienced a positive clinical response (22 partial and 3 complete responses). Stable disease was observed in 12 (22%) of 54, 21 (36%) of 59, and 15 (37%) of 41 patients and progressive disease in 42 (77%) of 54, 34 (58%) of 59, and 1 (2%) of patients in the low, intermediate, and high probability groups, respectively. Not surprisingly, this 3-group stratification showed highly significant correlation with clinical response ($P < .0001$) **Table 6**.

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Table 2
Statistical Correlations Between Baseline Characteristics of the Entire Series (n = 418) of Lung Tumors and of *EGFR* and K-*ras* Mutations*

Characteristic	*EGFR*	*P*	K-*ras*	*P*
Sex		<.0001		.001
Male (n = 219)	9 (4.1)		67 (30.6)	
Female (n = 199)	42 (21.1)		34 (17.1)	
Smoking habit		<.0001		<.0001
Current (n = 268)	4 (1.5)		89 (33.2)	
Never (n = 118)	42 (35.6)		3 (2.5)	
Former (n = 32)	5 (16)		10 (31)	
Histotype 1				
Adenocarcinoma (n = 181)†	33 (18.2)		50 (27.6)	
nmBAC (n = 10)	2 (20)		2 (20)	
mBAC (n = 13)	0 (0)		10 (77)	
Mixed adenocarcinoma/BAC (n = 37)	12 (32)		8 (212)	
Colloid (n = 9)	0 (0)		4 (44)	
Signet-ring cell (n = 9)	0 (0)		4 (44)	
Papillary (n = 14)	2 (14)		6 (43)	
Solid with mucus (n = 9)	0 (0)		3 (33)	
Fetal type (n = 2)	0 (0)		0 (0)	
Adenosquamous (n = 4)	3 (75)		0 (0)	
Squamous cell (n = 31)	0 (0)		1 (3)	
Large cell (n = 6)‡	0 (0)		3 (50)	
Small cell (n = 6)	0 (0)		1 (17)	
LCNEC (n = 20)	0 (0)		3 (15)	
Typical carcinoid (n = 20)	0 (0)		0 (0)	
Atypical carcinoid (n = 5)	0 (0)		0 (0)	
Sarcomatoid (n = 13)§	0 (0)		7 (37)	
Cystic blastoma (n = 1)	0 (0)		0 (0)	
Mucoepidermoid (n = 5)	0 (0)		0 (0)	
Adenoid cystic (n = 3)	0 (0)		0 (0)	
Sclerosing hemangioma (n = 14)	0 (0)		0 (0)	
Histotype 2		<.0001		<.0001
Adenocarcinoma (n = 286)	49 (17.1)		87 (30.4)	
Others (n = 132)	2 (1.5)		15 (11.3)	
AAH‖		<.0001		NS
Present (n = 48)	16 (33)		9 (19)	
Absent (n = 271)	15 (5.5)		60 (22.1)	
Mucin production		<.0001		<.0001
Present (n = 57)	0 (0)		27 (47)	
Absent (n = 361)	51 (14.1)		30 (8.3)	
Stage		NS		NS
I A + B (n = 180)	19 (10.6)		47 (26.1)	
II A + B (n = 39)	6 (15)		9 (23)	
III A + B (n = 15)	0 (0)		6 (40)	
IV (n = 135)	22 (16.3)		32 (23.7)	
NA (n = 49)	4 (8)		8 (16)	
Grade		.018		NS
I (n = 84)	12 (14)		19 (23)	
II (n = 63)	15 (24)		14 (22)	
III + IV (n = 246)	20 (8.1)		67 (27.2)	
NA (n = 25)	4 (16)		2 (8)	

AAH, atypical adenomatous hyperplasia; BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; LCNEC, large cell neuroendocrine carcinoma; mBAC, mucinous BAC; NA, not available; nmBAC, nonmucinous BAC; NS, not significant.
* Data are given as number (percentage).
† Predominantly acinar/conventional adenocarcinoma.
‡ Including 2 basaloid and 2 clear cell types.
§ Including 4 carcinosarcomas and 2 adult blastomas.
‖ Considering only the 319 surgically resected cases.

The presence of mucin production was significantly associated with the low probability group (P = .035). None of the 16 mucin-producing adenocarcinomas had a high score.

Discussion

Although it is too early to definitively conclude whether molecular targeted therapies will significantly modify the survival of patients with advanced NSCLC, the recent findings of the key role of *EGFR* in lung cancer and adenocarcinomagenesis, in particular, coupled with the remarkable clinical responses from EGFR-TKIs (ie, gefitinib and erlotinib) in a subset of patients have greatly increased our knowledge of and hope for success with lung cancer molecular mechanisms and therapy. In previous trials, never smoking status, female

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Table 3
Correlations Between Baseline Characteristics of 154 EGFR-TKI–Treated Patients and *EGFR* and *K-ras* Mutations*

Characteristic	*EGFR*	*P*	K-*ras*	*P*
Sex		<.0001		<.0001
Male (n = 74)	5 (7)		37 (50)	
Female (n = 80)	28 (35)		18 (23)	
Smoking habit		<.0001		<.0001
Current (n = 103)	4 (3.9)		47 (45.6)	
Never (n = 36)	25 (69)		2 (6)	
Former (n = 15)	4 (27)		6 (40)	
Histotype		.02		NS
Adenocarcinoma (n = 129)	32 (25)		49 (38)	
Others (n = 25)	1 (4)		6 (24)	
AAH†		.01		NS
Present (n = 15)	6 (40)		6 (40)	
Absent (n = 55)	11 (20)		21 (38)	
Mucin production		.001		NS
Present (n = 16)			8 (50)	
Absent (n = 138)	33 (23.9)		47 (34.1)	
Stage		NS		NS
I A + B (n = 33)	8 (24)		14 (42)	
II A + B (n = 15)	3 (20)		7 (47)	
III A + B (n = 8)			4 (50)	
IV (n = 98)	22 (22)		30 (31)	
Grade		.01		.003
I (n = 18)	6 (33)		8 (44)	
II (n = 22)	9 (41)		3 (14)	
III + IV (n = 104)	14 (13.5)		42 (40.4)	
NA (n = 10)	4 (40)		2 (20)	
Response type		<.0001		<.0001
Progression (n = 77)	1 (1)		45 (58)	
Stable (n = 48)	8 (17)		10 (21)	
Partial (n = 24)	21 (88)			
Complete (n = 5)	3 (60)			
Score‡		<.0001		<.0001
–2 (n = 10)			10 (100)	
–1 (n = 44)			31 (70)	
0 (n = 37)	1 (2)		11 (30)	
+1 (n = 22)			2 (9)	
+2 (n = 6)	2 (33)		1 (17)	
+3 (n = 16)	11 (6)			
+4 (n = 19)	19 (100)			
Probability groups		<.0001		<.0001
Low (n = 54)			41 (76)	
Intermediate (n = 59)	1 (2)		13 (22)	
High (n = 41)	32 (78)		1 (2)	

AAH, atypical adenomatous hyperplasia; EGFR, epidermal growth factor receptor; NA, not available; NS, not significant; TKI, tyrosine kinase inhibitor.
* Data are given as number (percentage).
† Considering only surgically resected cases.
‡ Scoring was as follows: +1 for female sex, nonsmoking status, adenocarcinoma histotype, Asian ethnicity, and *EGFR* mutation; –1 for current smoker and K-*ras* mutation; and 0 for male sex, ex-smoker, nonadenocarcinoma histotype, and no mutations. Scores were combined into 3 groups for probability of response to treatment as follows: low, –2 to –1; intermediate, 0 to +1; and high, +2 to +4.

sex (especially of Asian ethnicity), and adenocarcinoma histotype (mainly with BAC features) were shown to be the best clinicopathologic characteristics associated with a positive clinical response to EGFR-TKIs.[7-10]

Given the high costs of this molecular treatment, which is not without toxic effects, there is an urgent need for better predictive factors for accurately predicting tumor sensitivity to this molecular therapy. Several biologic predictive markers (eg, immunohistochemical expression of EGFR and downstream molecule *EGFR* gene copy number by fluorescence in situ hybridization) have been reported in the literature with somewhat inconsistent results, and mutational analysis of the *EGFR* and K-*ras* genes, although not perfect,[39,40] remains the most reliable factor for predicting clinical response and survival of patients treated with EGFR-TKIs.[24-34,41-47] In particular, a lack of response has been observed in K-*ras*–mutated tumors (negative predictive marker),[14-16] whereas the great majority of tumors with the *EGFR* mutation on exons 19 and 21 are significantly inhibited (positive predictive marker) by gefitinib or erlotinib.[45] Of note, previous works have evidenced significant correlations between *EGFR* and K-*ras* mutations and with some histologic variants of lung adenocarcinoma.[17-22,48-57] Thus, K-*ras* mutations have been usually associated with mucin-producing adenocarcinomas

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Table 4
EGFR-TKI Responsiveness and Predictive Score Values in 154 Patients With Lung Cancer*

	Score						
Response Type (No. [%])†	**–2**	**–1**	**0**	**+1**	**+2**	**+3**	**+4**
Progression (77 [50.0%])	9	33	24	10	1	0	0
Stable (48 [31.2%])	1	11	12	9	2	11	2
Partial (24 [15.6%])	0	0	0	2	3	3	16
Complete (5 [3.2%])	0	0	1	1	0	2	1
Total No. (%)	10 (6.5)	44 (28.6)	37 (24.0)	22 (14.3)	6 (3.9)	16 (10.4)	19 (12.3)

EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.
* Scoring was as follows: +1 for female sex, nonsmoking status, adenocarcinoma histotype, Asian ethnicity, and *EGFR* mutation; –1 for current smoker and K-*ras* mutation; and 0 for male sex, ex-smoker, nonadenocarcinoma histotype, and no mutations.
† $P < .0001$.

Table 5
EGFR-TKI Responsiveness and Predictive Score Values in 154 Patients With Lung Cancer Excluding Mutational Results of the *EGFR* and K-*ras* Genes*

	Score				
Response type (No. [%])†	**–1**	**0**	**+1**	**+2**	**+3**
Progression (77 [50.0%])	17	37	21	2	0
Stable (48 [31.2%])	6	15	11	14	2
Partial (24 [15.6%])	0	0	4	5	15
Complete (5 [3.2%])	0	1	1	2	1
Total No. (%)	23 (14.9)	53 (34.4)	37 (24.0)	23 (14.9)	18 (11.7)

EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.
* Scoring was as follows: +1 for female sex, nonsmoking status, adenocarcinoma histotype, Asian ethnicity, and *EGFR* mutation; –1 for current smoker and K-*ras* mutation; and 0 for male sex, ex-smoker, nonadenocarcinoma histotype, and no mutations.
† $P < .0001$.

Table 6
Statistical Correlation Between the Score-Based Categories of Probability and Response Rate in 154 Patients Treated With EGFR-TKI

	Probability Category		
Response Type*	**Low (35.1%)**	**Intermediate (38.3%)**	**High (26.6%)**
Progression	42	34	1
Stable	12	21	15
Partial	0	2	22
Complete	0	2	3

EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.
* $P < .0001$.

(mainly mucinous-type BAC),[49-52] whereas *EGFR* mutations seem to characterize nonmucinous-type BAC and papillary adenocarcinoma.[17-22,48,52-56]

In our study, almost all histologic variants included in the 2004 WHO classification of lung tumors were analyzed for *EGFR* (exons 18, 19, and 21) and K-*ras* (exon 2) mutations by direct-sequencing polymerase chain reaction, and the results obtained were correlated with clinical responsiveness to EGFR-TKI. Because only a few cases of unusual tumor variants were included in the series, no unequivocal data could have been achieved for these histotypes.

To our knowledge, our study is the first to suggest that *EGFR* and K-*ras* mutations might not have an important role in the development of specific subtypes of lung cancers, such as neuroendocrine tumors and salivary gland–derived tumors. Of note, few K-*ras* mutations were detected in high-grade neuroendocrine tumors (3/20 [15%] of large cell neuroendocrine carcinomas and 1 [17%] of 6 SCLCs), and none were found in carcinoid tumors. Besides the clear-cut predilection for the adenocarcinoma histotype, as previously evidenced,[58,59] K-*ras* gene mutations also seem to characterize undifferentiated lung tumors such as large cell carcinomas (3/6 [50%]) and sarcomatoid carcinomas (41%).

For the first time, our results also show that not only mucinous-type BACs but also mucin-producing lung adenocarcinomas in general show a significant correlation with K-*ras* mutations. In fact, more than half of the mucin-producing adenocarcinomas (including 10 of 13 mucinous-type BACs, 4 of 9 each of colloid and signet-ring cell adenocarcinomas, 3 of 9 solid adenocarcinomas with mucus production, and 1 mixed adenocarcinoma with mucinous-type BAC) displayed K-*ras* mutations. By contrast, we failed to find K-*ras* mutations in 5 mucoepidermoid carcinomas (another mucin-producing salivary gland–derived tumor). This apparent controversy could be likely related to the frequent occurrence of mucin-

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producing adenocarcinomas in heavy smokers (73%), while mucoepidermoid carcinoma often arises in children or never smoking subjects, as in our series.

Mucin production (particularly MUC5AC) seems to be regulated by EGFR activation in normal airways and in patients with asthma or in smokers with chronic bronchitis.[60-62] Recent works demonstrated a significant and rapid effect of gefitinib in improving bronchorrhea in patients with BAC-like adenocarcinoma and in lung cancer cell lines (A549 adenocarcinoma and NCI-H292 mucoepidermoid carcinoma) in vitro,[63-67] possibly through up-regulation of MAPK and Akt downstream signaling pathways promoted by shedding of EGFR proligands and EGFR phosphorylation. Previous works have suggested that BAC and papillary adenocarcinomas are strongly associated with *EGFR* mutations and a high rate of clinical response to EGFR-TKIs.[17-22] Assuming that with the term *BAC* oncologists would mean pure nonmucinous-type BAC (this statement is almost never clearly specified in previous published works), there are some consistent arguments against this conviction.

First, clinical trials using EGFR-TKIs included patients with advanced stage disease (stage IIIB or IV)[7-10,18] who generally had cytologic testing or biopsy to prove lung cancer, but a diagnosis of BAC according to the 2004 WHO classification of lung tumors should be posed only on surgical resections. So, it seems that published studies mainly referred to BAC as a clinicoradiologic (diffuse, bilateral lung consolidations/pneumonia-like pattern with bronchorrhea) rather than a well-defined histologic tumor entity,[68-70] nonmucinous-type BAC basically resulting in the in situ lesion of the adenocarcinoma histotype according to the WHO criteria.[35]

Second, pure BAC is rare, and several works have demonstrated *EGFR* mutations also in invasive mixed adenocarcinoma with a nonmucinous BAC component, not only in pure nonmucinous BAC.[57,71,72] According to Yatabe et al,[53] Yatabe,[56] and Yatabe and Mitsudomi,[72] *EGFR* mutations seem to characterize a peculiar type of adenocarcinoma, namely terminal respiratory unit-type (TRU-type) adenocarcinoma, that occurs more frequently in nonsmoking women.[73] TRU-type adenocarcinoma is more akin to an invasive mixed adenocarcinoma with nonmucinous type BAC and papillary features that retains immunohistochemical markers of peripheral airways, such as thyroid transcription factor-1 and surfactant apoproteins.[53,56,72] In agreement with this view, we found *EGFR* mutations in 20% of pure nonmucinous-type BACs, 32% of mixed adenocarcinomas with a BAC component, and 18.2% of predominantly acinar/conventional adenocarcinomas.

Papillary adenocarcinoma was significantly associated with clinical response to gefitinib in a study by Kim et al.[21] In their study, the 17 patients with a clinical response included 10 women, 11 nonsmokers, and 6 men who smoked. Patients with papillary adenocarcinoma had significantly better survival than patients with nonpapillary adenocarcinoma, and papillary adenocarcinoma, not sex or smoking status, was the only parameter significantly related to response rate. Molecular analysis for *EGFR* and K-*ras* mutations was not done.

In our series, 6 of 14 papillary adenocarcinomas had K-*ras* mutations, whereas *EGFR* mutations were observed in only 2 of these tumors. Nine patients were smokers, and 5 were women. Five patients were treated with EGFR-TKI, with progressive disease in 3 patients (all smokers with K-*ras* mutations), stable disease in 1 patient, and partial response in 1 patient (a never-smoking woman with an *EGFR*-mutated tumor). Rather than papillary variant per se as observed in Japanese subjects, it seems that response to EGFR-TKI in a white population with papillary adenocarcinoma might be related to conventional clinicopathologic criteria and results of *EGFR/*K-*ras* mutational analyses. These results need, however, to be confirmed in larger series of cases.

Moreover, a papillary growth pattern may be observed in acinar adenocarcinoma, nonmucinous BAC, and mixed forms,[74] mainly depending on how extensively the tumor is sampled by the pathologist. However, to our eyes, the key message for pathologists and oncologists is that there is a highly distinctive subset of adenocarcinomas, occurring mainly in nonsmoking and female patients, with peculiar histologic features (mixed adenocarcinoma with nonmucinous BAC and papillary features) and *EGFR* mutations (the TRU-type adenocarcinoma) associated with a high response rate to EGFR-TKIs.[53,56,71,72]

Practically speaking and with only sporadic exceptions,[39] it is widely accepted that in Asian and white populations, female sex, an adenocarcinoma histotype, and nonsmoking status are the best clinical parameters positively predicting response to EGFR-TKIs, whereas many controversies remain on the choice and use, but not the role, of predictive biologic factors. Provided that there is a distinct prevalence and inverse relationship between the occurrence of *EGFR* (48% vs 12%) and K-*ras* mutations (5%-10% vs 20%-40%) in adenocarcinoma in Asian and white patients,[23-34,41-45,57-59] it is of great interest that mutations of *EGFR* and K-*ras* are significantly related to dramatic response to EGFR-TKIs with better survival and drug resistance to EGFR-TKIs with poor prognosis, respectively.

We first attempted to elaborate and validate a simple stratification scheme based on a scoring system obtained by combining clinicopathologic (histotype, smoking habit, and sex) and molecular (*EGFR* and K-*ras* mutations) features predictive of response to EGFR-TKIs. This scoring system proved to be significantly associated with clinical responsiveness to EGFR-TKIs and is proposed as a reliable and useful stratification system to predict benefit from EGFR-TKI treatment in clinical settings and that warrants furthers validation in

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a larger series of patients with lung cancer. If the results of our studies are confirmed, it is of interest that female sex, never smoking status, and a nonmucinous adenocarcinoma histotype (not taking into account genetic analyses) also showed a highly significant statistical relationship with a positive clinical response ($P < .0001$), thus confirming their usefulness as predictors of benefit from EGFR-TKI treatment.

Similar to our results, Han et al[75] found that response rate to gefitinib in a population of 120 Korean patients with advanced lung cancer increased as the number of clinicopathologic predictive criteria increased among subjects with wild-type *EGFR*. By contrast, the authors underlined the superiority of *EGFR* mutational status compared with clinicopathologic parameters for predicting response rate, time to progression, and overall survival. We partly agree with the authors that molecular analysis for *EGFR* mutations should be done whenever possible, but particularly in Asian patients or in patients selected to receive first-line EGFR-TKI treatment, given the excellent response in patients with tumors with *EGFR* mutations in exon 19 or 21.[76-79]

Correlation between *EGFR* and K-*ras* mutations and histotype of primary pulmonary neoplasms included in the 2004 WHO classification arising in a homogeneous white population demonstrates that *EGFR* mutations are not restricted to pure BAC or papillary-type adenocarcinoma but are significantly associated with nonsmoking women, presence of AAH (in surgical resection specimens), lack of tumor mucin production, and better differentiated adenocarcinoma. K-*ras* mutations have a major role in this population and are significantly related to smoking habit, male sex, and high-grade mucin-producing malignancies.

We developed a simple scoring system combining clinicopathologic and molecular features predictive of response in a subgroup of patients treated with EGFR-TKIs and then identified 3 probability groups significantly associated with clinical response. We showed that molecular studies for *EGFR* mutations are not a prerequisite in daily practice for therapy with EGFR-TKI. A combination of clinicopathologic features alone is significantly related to response rate. This scoring system, although not perfect in predicting efficacy of EGFR-TKIs, can be a practical and satisfactory compromise when oncologists, without the possibility of performing molecular analysis or testing other biologic surrogates, are asked to identify patients more likely to benefit from EGFR-TKI therapy.

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