The Prognostic Value of Chromosome 7 Polysomy in Non-small Cell Lung Cancer Patients Treated with Gefitinib

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Introduction: Specific subpopulations of non-small cell lung cancer (NSCLC) patients defined by clinical features and molecular profiles seem to derive greater benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, but no general consensus on molecular testing to optimize treatment has emerged. The objective of this study was to evaluate chromosome 7 polysomy and other potential indicators of gefitinib efficacy in advanced NSCLC patients.

Methods: Paraffin-embedded tumors from 82 patients treated with gefitinib were analyzed by immunohistochemistry for expression of *EGFR* and other markers, and by fluorescence in situ hybridization for EGFR gene or chromosome copy number. Mutational status was assessed by single-strand conformational polymorphism, sequence-specific polymerase chain reaction, and direct sequencing. Molecular and clinical characteristics were evaluated in relation to objective response (OR), progression-free survival (PFS), and overall survival (OS).

Results: EGFR mutational status ($p = 0.002$), never smoking ($p = 0.052$), and chromosome 7 polysomy ($p = 0.029$) were significant indicators of OR. *EGFR* mutation, pAKT or PTEN expression, and chromosome 7 polysomy were associated with longer OS. There was a significant difference in OS between the chromosome 7 polysomy groups ($p = 0.015$) and the groups with both chromosome 7 polysomy and $pAkt^+$ ($p = 0.002$) and both chromosome7 polysomy and $PTEN^+$ ($p = 0.04$). In a stepwise proportional hazards analysis, chromosome 7 polysomy and $PTEN^+$ expression were both significantly associated with longer OS ($p = 0.004$ and 0.017 respectively).

Conclusion: These results suggest that further study of chromosome 7 polysomy and of pAKT and PTEN expression in patients treated with EGFR tyrosine kinase inhibitors is warranted in developing a clinical test for selecting patients for gefitinib therapy.

Key Words: Non-small cell, Targeted therapy, Molecular biomarkers, Aneusomy, *EGFR* mutations.

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In phase II trials, gefitinib (Iressa, AstraZeneca), a small molecular tyrosine kinase inhibitor (TKI),[1] demonstrated objective remissions, but in phase III trials, only a nonsignificant trend in survival was observed compared with placebo. Women, Asians, never-smokers, and patients with bronchoalveolar tumors had more favorable results.[2–4] Subsequent work has shown that these associations arise from molecular differences related to epidermal growth factor receptor (EGFR) in the tumors in these subpopulations. Specifically, *EGFR*-activating mutations, high *EGFR* gene copy number, and alterations in proteins involved in EGFR signaling may identify patients with the best outcomes from TKI treatment.[5]

Nonrandom structural and numerical abnormalities in chromosome 7, where *EGFR* is located, may also be important in this context, because they have been observed in several types of malignancy including malignant mesothelioma and lung cancer. Polysomy 7 was found exclusively in malignant mesothelioma compared with benign mesothelial cells.[6] In one study on non-small cell lung cancer (NSCLC), polysomy 7 has been reported to have no significant influence on prognosis,[7] whereas another study has reported significant association of increased *EGFR* gene and chromosome 7 copy number with poor differentiation.[8]

The relationship of molecular and cytogenetic abnormalities to response to TKI is complex and controversial. Correlating *EGFR* status with response to therapy and survival is complicated by the potentially important role of tumor-related changes in downstream factors in the *EGFR* signaling pathways, such as phosphatase and tensin homolog (PTEN) and phosphorylated v-AKT murine thymoma viral oncogene homolog 1 (pAKT).[9–11] Methods used to assess these features can be subjective, technically challenging, and difficult to interpret. Alternatively, detection of polysomy by fluorescence in situ hybridization (FISH) is a straightforward analysis that is performed in many molecular and cytogenetic

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Part of this work has previously been presented in abstract form.

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laboratories. To better define the relationship between chromosome 7 polysomy and other molecular biomarkers, such as *EGFR* amplification, *EGFR* mutations, EGFR expression, and expression of related proteins, with efficacy of TKI, this study evaluated chromosome 7 polysomy with a panel of putative potential predictors of gefitinib efficacy in NSCLC. Using several different FISH parameters to describe polysomy and gene status, we evaluated chromosome 7 polysomy and EGFR gene status to determine which method of genomic copy number assessment was most effective. The data suggest that chromosome 7 polysomy, which can be measured easily and unambiguously, may be important in selecting NSCLC patients for TKI therapy.

MATERIALS AND METHODS

Patients and Clinical Assessment

Specimens from 82 expanded access trial NSCLC patients treated for more than 1 week with gefitinib were obtained from the pathology department of Rush University Medical Center and the University of Chicago (Chicago, IL). Chart review and study analyses were approved by the Rush University Medical Center and the University of Chicago institutional review boards. The diagnosis of NSCLC in the archival material was obtained from pathology reports and confirmed by histologic evaluation before further analysis.

Eligibility criteria for the gefitinib expanded access trial included histologic or cytologic confirmation of stage IIIb or IV NSCLC; at least one previous course of chemotherapy, or determination by an investigator that a patient was not suitable for chemotherapy or radiotherapy; and written informed consent. Exclusion criteria included another active malignancy, concurrent radiotherapy or systemic anticancer therapy, incomplete healing from previous surgery, other significant clinical disorders or laboratory findings, pregnancy, and breast feeding.

Clinical data were established from chart review. Nonsmoking status was defined by lifetime consumption of fewer than 100 cigarettes. Objective response (OR) was assessed according to Response Evaluation Criteria in Solid Tumors. Progression was determined by radiologic studies. Patients who progressed within 70 days were classified as having progressive disease for their best response. Patients who didn't meet Response Evaluation Criteria in Solid Tumors for partial or complete remission and who had not progressed sooner than 70 days from starting gefitinib were classified as stable disease for their best response. Progression-free survival (PFS) and overall survival (OS) were measured in months from the start of gefitinib treatment to the time of disease progression or death.

Immunohistochemistry

Immunohistochemistry (IHC) and FISH specimens were 5.0-μm sections of formalin-fixed paraffin-embedded tumor tissue or sections from cytology cell blocks. FISH and EGFR expression data were obtained from 81 of the 82 patients, respectively. PTEN and pAKT expression data were obtained from 74 patients, and mutation and CA repeat data were obtained from 58 patients. One patient with mutation data was not assessable by FISH.

Immunostaining methods and reagents were described previously.[12] Staining frequency and intensity of all tumor cells on each slide were estimated on a scale of 0 to 4, without knowledge of clinical patient data. A positive tumor cell count of less than 1% was scored as 0, 1% to 10% as 1, 11% to 35% as 2, 36% to 70% as 3, and over 70% as 4. Only cell membrane–associated staining was considered for EGFR. Before analysis, IHC expression was dichotomized into two levels: detected (+1 to +4) and undetected (0).

In Situ Hybridization

EGFR and centromere 7 (CEN7) probes were used to examine *EGFR*/cell, CEN7/cell, and *EGFR*/CEN7. Specimen slides were hybridized with two-color FISH probe solutions (Vysis SpectrumOrange LSI and EGFR/SpectrumGreen CEP 7; Abbott Molecular Inc., Des Plaines, IL). Paraffin pretreatments II and III were performed essentially according to the kit package inserts. FISH slides were evaluated under a Zeiss Axioscope microscope (Carl Zeiss, Thornwood, NY) with a DAPI single–band-pass filter to visualize nuclei, an orange single–band-pass filter set to visualize EGFR probe, and a green single–band-pass filter set to visualize CEP 7 (all filter sets from Abbott Molecular, Inc.). Only nuclei with morphology characteristic of malignant cells were counted (Figure 1).

Typically, 30 to 90 (median = 80) cells were enumerated in each specimen. The mean number of EGFR or CEN7 signals per cell (EGFR/cell or CEN7/cell, respectively) was calculated by dividing the total number of signals by the number of cells counted. Mean *EGFR* probe signals per cell were divided by the mean CEP 7 signals per cell to yield *EGFR*/CEN7 ratios. EGFR percent gain or CEN7 percent gain was calculated as the percentage of cells with more than two *EGFR* or CEP 7 signals, respectively. EGFR/CEN7 percent gain was the percentage of cells that showed more *EGFR* signals than CEP 7 signals. Optimal cutpoints for defining high ratios or high percent gains were selected by first generating contingency tables for OR and survival ($<$1 year versus $\geq$1 year) for a wide range of cutpoints. Cutpoints yielding the lowest chi-square probabilities were selected for further analysis. *EGFR* high polysomy (at least four copies of *EGFR* in $\geq$40% of the cells), and amplification (*EGFR* gene:CEN7 ratio $\geq$2, or $\geq$15 copies of *EGFR* gene per cell in $\geq$10% of the cells), according to criteria published by Cappuzzo et al.[9] (referred to here as UC FISH$^+$ status) were also evaluated. Nevertheless, the presence of EGFR signal clusters was not included in the analysis, because evaluation of specimens occurred before publication of the study by Cappuzzo et al.[9] It is likely that most signal clusters would have been enumerated to provide a result of high polysomy or amplification.

Molecular Genetics

DNA was extracted from paraffin-embedded specimens by manual microdissection and proteinase K digestion. *EGFR* gene mutation status was assessed using single-strand conformation polymorphism, sequence-specific polymerase chain reaction (PCR), and direct sequencing. Polymorphic

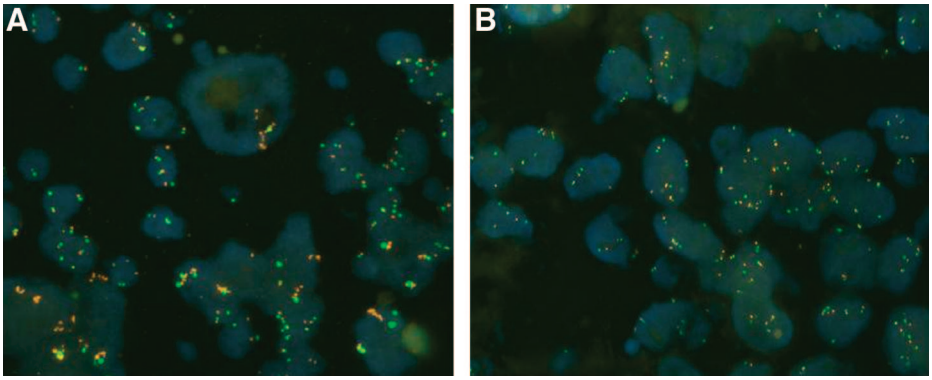

FIGURE 1. FISH images of paraffin-embedded lung tumor sections stained with SpectrumOrange LSI *EGFR* probe and SpectrumGreen CEP 7 centromere probe showing (*A*) high *EGFR* gene copy number (>6/cell; orange signals), and (*B*) chromosome 7 polysomy (≥3.6/cell; green signals). FISH slides were evaluated under a Zeiss Axioscope epifluorescence microscope (Carl Zeiss, Thornwood, NY) with a DAPI single–band-pass filter set to visualize nuclei, an orange single–band-pass filter set to visualize EGFR probe, and a green single–band-pass filter set to visualize CEP 7 (Abbott Molecular filter sets). Magnification is ×40.

numbers of repeats of the cytosine-adenine (CA) dinucleotide sequence in intron 1 of the *EGFR* gene were analyzed by PCR amplification of the CA repeat region followed by capillary electrophoresis of the PCR products. The size of the peaks in base pairs was converted to CA repeat number based on GenBank accession sequence M38425.1. The median peak was used to define each allele. The sum of alleles is the total of median CA repeats from both chromosomes. Alleles of 16 CA repeats and the sum of alleles of 34 CA repeats were midpoints in the range of alleles in this patient group. Sequences of primers used to amplify and sequence exons 18, 19, and 21 and intron 1 CA repeat polymorphisms of the *EGFR* gene are available on request.

Statistical Analysis

The associations between response to gefitinib (yes/no) and categorical covariates were tabulated, and Fisher's exact test was used to measure their significance. The Kaplan–Meier method was used to estimate the probability of survival as a function of time. Survival differences among comparator groups were analyzed by the log-rank test. Logistic regression and Cox proportional hazards models were used to select and model the effects of molecular markers and other variables on OR and PFS, respectively. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC), and all reported p values are two sided.

RESULTS

Patient Characteristics and Response to Gefitinib

Table 1 shows patient characteristics and clinical response to gefitinib treatment. Median age was 67 years, and the OR rate was 15%; two patients had complete response, 10 had partial response, 36 (44%) had stable disease, and the progression rate was 41%. Nonsmoking status was significantly associated with OR ($p < 0.001$).

Median PFS for all patients was 3.3 months, with a 95% confidence interval (CI) of 2.3 to 4.2. PFS was significantly longer for nonsmokers than for smokers (median 11.7 and 2.9, respectively, $p = 0.013$). Other patient characteristics were not significantly associated with OR or PFS.

Median OS was 7.1 months (CI 5.6–9.4). OS was significantly longer for nonsmokers than for smokers (median 21.1 and 6.3 months, respectively, $p = 0.028$). Patients with performance status (PS) 0 to 1 survived significantly longer than patients with PS 2 to 3 (median 9.0 and 5.5, $p = 0.035$).

TABLE 1. Patient Characteristics and Clinical Response to Gefitinib Treatment

Characteristic	Number of Patients (%)	Objective Response (%)
Total	82 (100)	12 (15)
Age (yr)		
≥60	62 (77)	8 (13)
<60	20 (23)	4 (20)
Gender		
Male	37 (46)	5 (14)
Female	44 (54)	7 (16)
Smoking status*		
Yes	70 (85)	5 (7)
Never smoked	12 (15)	7 (58)
Histopathological subtype		
Adenocarcinoma/bronchoalveolar	56 (69)	10 (18)
Other	26 (32)	2 (8)
Performance status		
0–1	46 (57)	6 (13)
2–4	34 (43)	6 (17)
Previous chemotherapy		
None	14 (17)	2 (14)
One	39 (49)	7 (18)
Two or more	28 (34)	3 (11)

*$p < 0.001$.

Molecular Markers and Clinical Response to Gefitinib

The following genotypic and phenotypic markers were analyzed for patients with assessable tumor specimens: chromosome 7 polysomy (CEN7/cell ≥3.6 and CEN7/cell ≥4) *EGFR* gene amplification (measured as *EGFR*/cell, *EGFR* percent gain, *EGFR*/CEN7, *EGFR*/CEN7 percent gain), *EGFR*- and *K-ras*-activating mutations, *EGFR* intron 1 CA repeat status, and EGFR, pAKT, and PTEN protein expression. High polysomy 7 (CEN7/cell ≥4) was significantly associated with OR ($p = 0.029$). *EGFR* mutation ($p < 0.001$), *EGFR* copy number ≥6.0/cell ($p = 0.009$), and *EGFR* percent gain ≥75 ($p = 0.035$) were also significantly associated with response to gefitinib.

The effect of expression of downstream signal transduction factors pAkt and PTEN on response was also assessed. All 11 gefitinib-responsive tumors expressed either EGFR or pAKT protein, whereas 18/63 (29%) of the nonresponders expressed neither protein ($p = 0.056$). Combining pAKT expression and *EGFR* mutation data revealed that patients with pAKT expression and *EGFR* mutations had a significantly higher response rate (6/9; 67%) than "any negative" patients (5/47; 10%, $p < 0.001$). With regard to PTEN expression, patients with PTEN expression and *EGFR* mutations had a significantly higher OR (7/12; 58%) than "any negative" patients (4/42; 10%, $p = 0.001$). None of the 16 double-negative (*EGFR* mutation$^-$ and PTEN$^-$) patients responded to gefitinib, compared with a 30% OR (11/37) for patients with "any positive" value ($p = 0.023$).

Potential Molecular Predictors of PFS and OS

Kaplan–Meier PFS and OS analyses are summarized in Table 2. OS was significantly longer for patients with chromosome 7 polysomy (≥3.6 CEN7/cell; median of 16.2 months, 95% CI 6.7–31.3) than for those without (median 6 months, 95% CI 4.9 to 8.8; $p = 0.015$; Figure 2A). Nevertheless, the association between PFS and chromosome 7 polysomy was found to be not significant. Both OS and PFS were significantly longer for patients with *EGFR* mutations (median 23.8 and 13.6 months, respectively, 95% CI 9.4 to 36.7 and 4.5 to 17.5) than for patients without mutations (median 7.3 and 3.3 months, 95% CI 5.5–11.4 and 1.9–3.9, $p = 0.046$ and 0.001, Figure 2B). Patients with IHC expression of pAKT or PTEN survived somewhat longer than patients lacking expression of these markers, but these comparisons were only marginally significant ($p \approx 0.10$). Patients with *EGFR* gene amplification (*EGFR*/CEN7 percent gain ≥34; median 4.5 months, 95% CI 3.2–6.0) had longer PFS than patients without (median 2.4 months, 95% CI 1.9–3.5, $p = 0.012$). PFS was significantly longer for patients with amplification measured as *EGFR*/CEN7 >1.0 ($p = 0.022$). IHC expression of EGFR ($p = 0.077$) resulted in marginally statistically significant longer PFS.

Table 3 shows the association between combined markers and PFS or OS. Patients whose tumors expressed either EGFR or pAKT protein (any positive) had marginally longer PFS ($p = 0.059$) and significantly longer OS ($p = 0.030$; PFS = 3.7 months, 95% CI 2.6–5.0; OS = 8.4 months, 95% CI 5.8–12.5) than those expressing neither protein (PFS = 2.4 months, 95% CI 1.8–3.5; OS = 4.7 months, 95% CI 3.9–6.6). Patients with tumors that expressed either EGFR or PTEN protein (any positive) had significantly longer PFS (4.0 months, 95% CI 2.2–4.9) than patients whose tumor expressed neither protein (2.9 months, 95% CI 1.8–3.7, $p = 0.033$).

Chromosome 7 polysomy (CEN 7/cell >3.6) combined with either pAKT or PTEN expression by IHC was associated with significantly longer OS. Patients with polysomy 7$^+$ pAKT$^+$ tumors (24.5 months, 95% CI 12.5 lower bound) survived significantly longer than "any negative" patients (5.9 months, 95% CI 4.9 to 8.7; $p = 0.002$; Figure 2C). Similarly, patients with polysomy 7$^+$ PTEN$^+$ tumors (24.5 months, 95% CI 4.1 lower bound) survived longer than "any negative" patients (7.1 months, 95% CI 5.5–9.6; $p = 0.04$; Figure 2D).

Combining pAKT expression and *EGFR* mutation data showed that mutation$^+$ pAKT$^+$ patients had longer PFS ($p = 0.043$) and OS, ($p = 0.040$; PFS = 16.3 months, 95% CI 9.8–17.5; OS = 24.5 months, 95% CI 17.4–39.5) than patients with "any negative" value (PFS = 3.4 months, 95% CI 2.0–4.3; OS = 7.6 months, 95% CI 5.5–11.4; Figure 2E). Similar findings were observed when PTEN expression was combined with *EGFR* mutation data. Mutation$^+$ PTEN$^+$ patients had significantly longer PFS ($p = 0.002$) and OS ($p = 0.023$; PFS = 16.6 months, 95% CI 9.0–19.3; OS = 29.8 months, 95% CI 11.5–39.5) than "any negative" patients (PFS = 3.3 months, 95% CI 1.9–4.7; OS = 8.3 months, 95% CI 5.5–12.5; Figure 2F). Mutation$^-$ PTEN$^-$ patients also had significantly shorter PFS (3.8 months, 95% CI 2.0–4.9) than "any positive" patients (4.3 months, 95% CI 2.6–9.8, $p = 0.049$).

Associations between Molecular Variables

Chromosome 7 polysomy was observed more frequently in tumors with *EGFR*/CEN7 percent gain ≥34 ($p = 0.008$), UC FISH$^+$ status ($p < 0.001$), and expression of EGFR ($p = 0.059$). *EGFR* mutations were detected more frequently in tumors with *EGFR* percent gain ≥75 ($p < 0.001$), *EGFR*/CEN7 percent gain ≥34 ($p = 0.007$), *EGFR* copy number >6.0 ($p = 0.005$), and *EGFR*/CEN7 >1.0 ($p = 0.006$). Expression of EGFR protein was significantly associated with UC FISH$^+$ status ($p = 0.014$), *EGFR* percent gain ≥75 ($p = 0.0486$), *EGFR*/CEN7 percent gain ≥34 ($p = 0.007$), *EGFR* copy number >6.0 ($p = 0.002$), and *EGFR* FISH/CEN7 ≥1.5 ($p = 0.034$).

Expression of pAKT protein was significantly associated with *EGFR* gene copy number >6.0 ($p = 0.038$), polysomy 7 ($p = 0.018$), and UC FISH$^+$ status ($p = 0.036$). All six tumors with low trisomy FISH as defined by Cappuzzo et al.[9] had positive PTEN expression compared with 54% (36/67) with expression in the absence of low trisomy ($p = 0.035$).

Multivariate Analysis

A stepwise selection method within the logistic regression model was used to select potential predictors of OR to gefitinib. This analysis yielded *EGFR* mutation ($p = 0.002$,

TABLE 2. Potential Molecular Prognostic Indicators of Disease Progression and Survival

Variable	*n*	Progression-free Survival (mo)	Log-rank *p*	Median Survival (mo)	Log-rank *p*
EGFR mutation			0.001		0.046
No mutation	41	3.3		7.3	
Mutation	17	13.6		23.8	
EGFR expression			0.076		0.748
Not detected	35	2.5		8.5	
Present	47	4.2		6.9	
UC FISH status			0.961		0.545
Negative	45	2.6		7.3	
Positive	36	3.9		7.5	
EGFR/CEN7 % gain			0.012		0.078
<34	41	2.4		6.0	
≥34	40	4.5		10.3	
CEN7 polysomy			0.346		0.015
<3.6/cell	63	2.9		6.0	
≥3.6/cell	18	4.2		16.2	
EGFR/CEN7			0.022		0.153
≤1	22	2.0		6.0	
>1	59	4.1		7.6	
Median intron 1 CA*			0.544		0.435
<16	25	4.3		9.0	
≥16	33	3.9		8.0	
CA repeat SOA*			0.150		0.109
<34	30	3.3		6.3	
≥34	28	4.8		12.7	
pAkt expression			0.758		0.094
0	39	3.2		5.8	
+1 to +4	35	3.3		9.6	
PTEN expression			0.127		0.091
0	31	3.3		5.9	
+1 to +4	43	3.4		9.0	

EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; UC FISH, see Materials and Methods section; CEN7, centromere 7.

*Median *EGFR* intron 1 CA repeat allele and sum of alleles (SOA).

odds ratio 16.995, 95% CI 2.794–103.386) and tobacco use ($p = 0.052$, odds ratio 0.162, 95% CI 0.024–0.986) as two important indicators.

A stepwise selection approach was used within the Cox proportional hazards model to select potential covariates of OS. The initial model included molecular- and patient-level covariates. The stepwise selection method yielded chromosome 7 polysomy (CEN7/cell ≥3.6) [$p = 0.004$, hazard ratio (HR) 0.397, 95% CI 0.212–0.744]) and PTEN expression ($p = 0.017$, HR 0.543, 95% CI 0.329–0.898) as the two important covariates for OS. We note here that because the cutpoints of the chromosome 7 polysomy were selected on the basis of an exploratory analysis, its selection in combination with predefined criteria should be interpreted with caution. A similar stepwise Cox proportional hazards approach applied to PFS selected *EGFR* mutations ($p = 0.002$, HR 0.389, 95% CI 0.212–0.712) as an important covariate.

DISCUSSION

The EGFR TKI, gefitinib, demonstrated objective remissions of 18.4% and 11.8% in phase II trials,[13,14] and

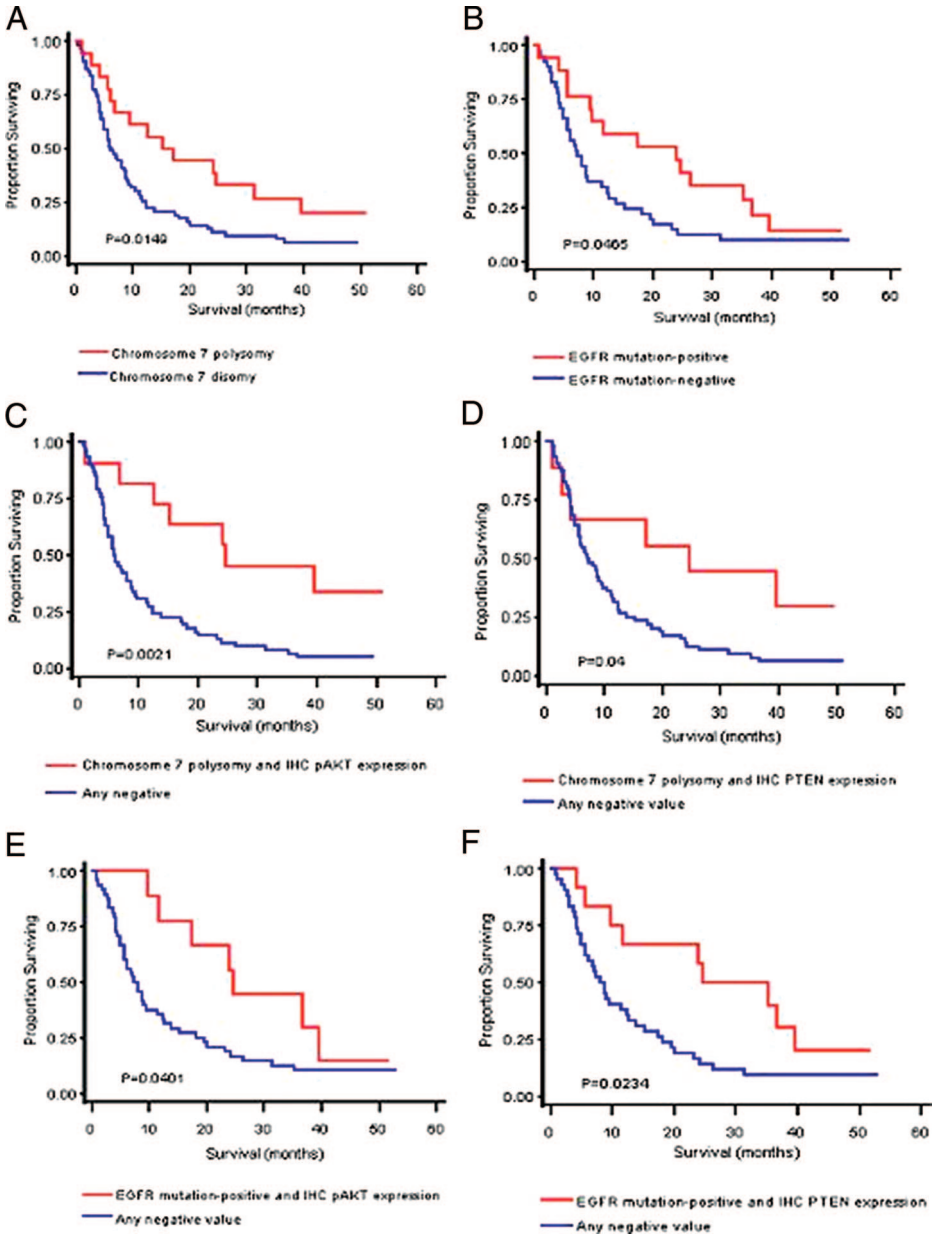

FIGURE 2. Kaplan–Meier curves comparing survival in patients with and without tumors exhibiting (*A*) chromosome 7 polysomy, (*B*) *EGFR*-activating mutations, (*C*) chromosome 7 polysomy with pAkt staining, (*D*) chromosome 7 polysomy with PTEN staining, (*E*) *EGFR*-activating mutations with pAkt staining, and (*F*) *EGFR*-activating mutations with PTEN staining.

another TKI, erlotinib (Tarceva, OSI Pharmaceuticals),[15] produced a response rate of 12.3% in previously treated patients.[16] In subsequent phase III trials in which EGFR TKIs were compared with placebos, gefitinib demonstrated a nonsignificant trend for longer survival,[7] whereas erlotinib was associated with a moderate but significant prolongation of survival.[2]

Recent reports describe the possible prognostic and predictive value of *EGFR*-activating mutations (exons 18, 19, and 21), high *EGFR* copy number, and perturbations of downstream proteins in the *EGFR* pathway in NSCLC patients treated with *EGFR* TKIs.[9,17–25] In the present study, all of these molecular markers were analyzed in a new patient set along with polysomy for chromosome 7, the location of the *EGFR* gene. Chromosome 7 polysomy can be defined in a number of ways, including average chromosome 7 copies per cell and percentages of cells with various chromosome 7 copy numbers. Different definitions of chromosome 7 polysomy were examined in the present study to determine which definition provided the best prognostic value. Similar definitions of *EGFR* gene status, in addition to gene amplification, were also evaluated.

Similar to results observed in trials conducted in Asian countries[20–22] and Spain,[23,24] significantly higher response ($p < 0.001$), longer PFS ($p = 0.001$), and longer OS ($p = 0.046$) were observed in our patients whose tumors harbored *EGFR*-activating mutations. Two groups have reported that *EGFR* mutations did not predict survival. In one study, *EGFR* mutations were associated with significantly higher response and longer PFS, but survival analysis showed a nonsignificant

TABLE 3. Epidermal Growth Factor Receptor (EGFR) Expression, Activating Mutations, and Chromosome 7 Polysomy, Combined with pAkt or PTEN Expression as Potential Prognostic Indicators of Survival

Variable	*n*	Progression-free Survival (mo)	Log-rank *p*	Median Survival (mo)	Log-rank *p*
EGFR expression, pAkt expression			0.059		0.030
EGFR$^-$, pAKT$^-$	18	2.4		4.7	
Any positive	56	3.7		8.4	
EGFR expression, PTEN expression			0.033		0.304
EGFR$^-$, PTEN$^-$	15	2.9		5.8	
Any positive	59	4.0		8.0	
UC FISH status,* pAkt expression			0.555		0.058
EGFR$^+$, pAkt$^+$	20	4.9		12.0	
Any negative	53	3.0		6.0	
EGFR % gain, pAkt expression			0.173		0.057
EGFR ≥75, pAkt$^+$	14	9.0		15.0	
Any negative	59	2.9		6.0	
EGFR mutation, pAkt expression			0.043		0.040
EGFR mut$^+$, pAkt$^+$	9	16.3		24.5	
Any negative	48	3.4		7.6	
EGFR mutation, PTEN expression					
EGFR mut$^+$, PTEN$^+$	12	16.6	0.002	29.8	0.023
Any negative	42	3.3		8.3	
EGFR mut$^-$, PTEN$^-$	16	3.8	0.049	8.0	0.360
Any positive	38	4.3		10.6	
EGFR/CEN7 % gain, PTEN expression			0.003		0.004
EGFR ≥34, PTEN$^+$	21	7.5		19.5	
Any negative	52	2.6		5.9	
CEN 7 polysomy, pAkt expression			0.332		0.002
CEN 7 ≥3.6/cell, pAkt$^+$	11	5.5		24.5	
Any negative	62	3.0		5.9	
CEN 7 polysomy, PTEN IHC			0.404		0.040
CEN 7 ≥3.6/cell, PTEN$^+$	9	4.1		24.5	
Any negative	64	3.2		7.1	

EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; CEN7, centromere 7.
**EGFR* high polysomy or gene amplification as defined by Cappuzzo et al.[9] UC FISH status.

trend for longer OS.[9] *EGFR* mutations were associated with neither response nor OS in the other study, but more than half of the *EGFR* mutations were outside exons 18, 19, and 21.[25] Although controversy persists, our observations add to the growing evidence that *EGFR* mutations are important predictors, not only for response to TKIs but also for survival.

High *EGFR* gene copy number also has been identified as a positive indicator for TKI efficacy. Cappuzzo et al.[9] defined tumors as FISH$^+$ if they had at least four copies of *EGFR* in at least 40% of the cells, if *EGFR*/chromosome 7 was at least 2, or if at least 15 copies of *EGFR* per cell were present in at least 10 % of the cells (UC FISH$^+$). FISH$^+$ tumors were associated with significantly better response, PFS, and OS. Similar FISH criteria were applied to patients in a phase III Canadian trial[25] in which FISH$^+$ tumors had significantly longer survival over placebo in univariate analyses, but not in multivariate analyses. Our analyses did not detect a significant survival benefit in patients with UC FISH$^+$ status. These discrepancies may be attributable to the smaller sample size in the current study or to differences in specimen evaluation (e.g., see clusters evaluation in the Methods section).

EGFR gene copy number has been correlated with protein expression in NSCLC.[26] EGFR protein expression has been evaluated directly by several investigators using different anti-EGFR antibodies and various definitions of "positive." Defining positive EGFR expression as tumors containing ≥1% of cells with EGFR-positive staining or according to criteria described by Cappuzzo et al.[9] or Tsao et al.,[25] our analyses failed to show that EGFR protein expression was significantly related to response, PFS, or OS.

Cappuzzo et al.[9] report that expression of pAKT by IHC in conjunction with *EGFR* FISH positivity, *EGFR* mutation, or EGFR protein expression was associated with significantly higher OR, longer PFS, and longer OS. Similarly, we observed significantly longer survival in patients with $pAKT^+$ tumors (staining in ≥1% of cells) and any of the following: $EGFR^+$ by IHC, UC $FISH^+$ status, or the presence of *EGFR* mutation. Nevertheless, in multivariate analyses, PTEN expression in combination with high chromosome 7 polysomy, and not pAKT, was most prognostic for survival. The association with OS, but not PFS, may reflect complications of secondary treatments or a more indolent course of disease in some patient subgroups. Our results are consistent with those of other investigators[27] who did not find a positive association between response and PTEN expression alone.

In the current study, multivariate analyses revealed that increased chromosome 7 copy number (≥3.6/cell) was the strongest prognostic indicator of OS ($p = 0.004$). The potential predictive value of chromosome 7 polysomy has not been previously described. The relative simplicity of determining chromosome 7 polysomy, compared with *EGFR* mutation analysis or demonstrating that a tumor is FISH-positive,[9] suggests that additional study of this marker is warranted. A clinically applicable test for selecting NSCLC patients for TKI therapy must be sufficiently simple and reproducible for use in clinical laboratories.

Polysomy 7 is a frequent event in NSCLC, and genes found in amplified regions of chromosome 7 include several potential effectors of tumorigenesis.[28–30] Moreover, gain of chromosome 7 in selected cytogenetic backgrounds may define cell populations with particular phenotypic properties.[12,31] On the basis of the association with OS in the present study, chromosome 7 polysomy may be a direct or indirect marker for distinct biology that renders tumors sensitive to *EGFR* inhibition.

In summary, chromosome 7 polysomy (≥3.6/cell) was associated with significantly improved survival after gefitinib treatment in NSCLC patients. Regarding previously studied biomarkers, the current results are consistent with reports that *EGFR* mutations have prognostic value in NSCLC patients treated with EGFR TKIs. Although our study did not show a significant association between FISH positivity as defined by Cappuzzo et al.[9] and survival in this small patient group, there was a significant association between FISH positivity and other prognostic biomarkers. Furthermore, the effects of PTEN and pAKT expression suggest that the state of these markers may influence the prognostic value of polysomy 7 or other *EGFR* determinants. These observations also raise possibilities of combining other agents with EGFR TKIs. Further studies with placebo arms are required to determine the true predictive value of this biomarker.

REFERENCES

1. Cohen MH, Williams G, Sridhara R, et al. United States Food and Drug Administration Drug Approval summary: gefitinib (ZD1839; Iressa) tablets. *Clin Cancer Res* 2004;10:1212–1218.
2. Buter J, Giacone G. EGFR inhibitors in lung cancer. *Oncology* 2005; 19:1707–1711.
3. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353: 123–132.
4. Hirsch FR, Bunn PA. Epidermal growth factor receptor inhibitors in lung cancer: smaller or larger molecules, selected or unselected populations? *J Clin Oncol* 2005;23:9044–9047.
5. Endoh E, Yatabe Y, Kosaka T, et al. PTEN and PIK3CA expression is associated with prolonged survival after gefitinib treatment in EGFR-mutated lung cancer patients. *J Thorac Oncol* 2006;1:629–634.
6. Shin H, Shin DM, Tarco E, et al. Detection of numerical aberrations of chromosomes 7 and 9 in cytologic specimens of pleural malignant mesothelioma. *Cancer* 2003;99:233–239.
7. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: results from a randomized, placebo-controlled, multicentre study (Iressa survival evaluation in lung cancer). *Lancet* 2005;366:1527–1537.
8. Dacic S, Flanagan M, Cieply K, et al. Significance of EGFR protein expression and gene amplification in non-small cell lung carcinoma. *Am J Clin Pathol* 2006;125:860–865.
9. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–655.
10. Han SW, Kim TY, Jeon YK, et al. Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. *Clin Cancer Res* 2006;12:2538–2544.
11. Conde E, Angulo B, Tang M, et al. Molecular context of the EGFR mutations: evidence for the activation of mTOR/S6K signaling. *Clin Cancer Res* 2006;12:710–717.
12. Morrison LE, Jacobson KK, Friedman M, et al. Aberrant EGFR and chromosome 7 associate with outcome in laryngeal cancer. *Laryngoscope* 2005;115:1212–1218.
13. 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 (the IDEAL 1 trial). *J Clin Oncol* 2003;21: 2237–2246.
14. Kris MG, 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: a randomized trial. *JAMA* 2003;290:2149–2158.
15. Perez-Soler R. The role of erlotinib (Tarceva, OSI 774) in the treatment of non-small cell lung cancer. *Clin Cancer Res* 2004;10:4238s–4240s.
16. 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;22:3238–3247.
17. 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;350:2129–2139.
18. Paez J, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497–1500.
19. 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;101:13306–13311.
20. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;23:2493–2501.
21. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefiftinib trteatment in patients with non-small cell lung cancerwith post-operative recurrence. *J Clin Oncol* 2005;23:2513–2520.

22. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;23:6829–6837.
23. Cortes-Funes H, Gomez C, Rosell R, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. *Ann Oncol* 2005;16:1081–1086.
24. Paz-Ares L, Sanchez JM, Garcia-Velasco A, et al. A prospective phase II trial of erlotinib in advanced non-small cell lung cancer (NSCLC) patients with mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR). *Proc Annu Meet Am Assoc Cancer Res* 2006;24:369s.
25. Tsao M, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005; 353:133–144.
26. Hirsch FR, Varella-Garcia M, Bunn PA, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003;21:3798–3807.
27. Bell D, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081–8092.
28. Garnis C, Lockwood WW, Vucic E, et al. High resolution analysis of non-small cell lung cancer cell lines by whole genome tiling path array CGH. *Int J Cancer* 2006;118:1556–1564.
29. Coe B, Lee EH, Chi B, et al. Gain of a region on 7p22.3, containing MAD1L1, is the most frequent event in small-cell lung cancer cell lines. *Genes Chromosomes Cancer* 2006;45:11–19.
30. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;117:927–939.
31. Tai A, Sham JS, Xie D, et al. Co-overexpression of fibroblast growth factor 3 and epidermal growth factor receptor is correlated with the development of nonsmall cell lung carcinoma. *Cancer* 2006;106:146–155.