EGFR and *HER2* Gene Copy Number and Response to First-Line Chemotherapy in Patients with Advanced Non-small Cell Lung Cancer (NSCLC)

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Background: A critical point in designing clinical trials comparing chemotherapy with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) in patients with non-small cell lung cancer (NSCLC) is the expected benefit with standard chemotherapy in presence of biological features indicative of TKI sensitivity. The aim of this study was to assess whether EGFR and HER2 gene copy number and Akt activation are associated with response to first-line chemotherapy.

Methods: Tumor samples from 190 patients with NSCLC were analyzed. EGFR and HER2 gene copy number were evaluated by fluorescence in situ hybridization in 185 and 184 cases, respectively. Akt activation was assessed by immunohistochemistry ($n = 176$). Additional biomarkers included EGFR DNA sequencing (n = 65), and EGFR immunohistochemistry ($n = 185$).

Results: Response rate was not associated with EGFR, HER2, and P-Akt status, irrespective of the method used for biomarker assessment. Among patients with EGFR gene mutations, response to chemotherapy was observed only in individuals with exon 19 deletion (response rate: 46.6% versus 0%, $p = 0.02$). Among the 190 patients analyzed, 123 received a treatment with a TKI as second- or third-line therapy. When assessed by fluorescence in situ hybridization or DNA sequencing, EGFR-positive patients seemed to be more sensitive to TKIs than to chemotherapy in terms of response rate and time to progression, whereas in EGFR-negative patients, response rate and time to progression favored chemotherapy.

Conclusion: This study suggested that EGFR expression and gene copy number, HER2 gene copy number, and P-Akt expression are not associated with response to first-line chemotherapy in NSCLC. Prospective phase III trials should compare standard chemotherapy with a TKI in selected NSCLC.

Key Words: epidermal growth factor receptor, HER2, Akt, Chemotherapy, Tyrosine kinase inhibitor, Non-small cell lung cancer.

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Non-small cell lung cancer (NSCLC) has been the leading cause of cancer death in the world.[1] Despite tangible progresses have been made during the past decade, prognosis of patients with this disease is still disappointing, and even with newly developed chemotherapy strategies, the median survival rarely exceeds 8 to 9 months.[2–4] Combination of cisplatin or carboplatin with third-generation agents such as gemcitabine, paclitaxel, docetaxel, and vinorelbine represents the standard of care for fit patients with advanced disease,[3–5] whereas single-agent therapy with vinorelbine or gemcitabine represents the standard approach for unfit or elderly NSCLC.[6] Because further advances with chemotherapy are unlikely, the key for improving outcomes for NSCLC patients turned to targeted therapy. In particular, agents targeting the epidermal growth factor receptor (EGFR) have had a major impact on the treatment of advanced NSCLC. Unfortunately, the dominant clinical trial strategy for patients with NSCLC has been to include a generic population of patients, with no selection based on biological criteria and, most importantly, with none or poor target assessment. Although recent clinical trials in NSCLC have demonstrated survival improvement without a defined biological endpoint,[7,8] the hazard of continuing to perform clinical trials without any patient selection carries the risk of administering the wrong drug to the wrong patient and considering ineffective a drug that could dramatically improve the outcome of some patients, even if those are few in number.

Gefitinib (ZD 1839, Iressa; AstraZeneca) and erlotinib (OSI 774, Tarceva; Genentech) are orally active, selective EGFR tyrosine kinase inhibitors (EGFR-TKIs) that demon-

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strated antitumor activity in approximately 10% of unselected NSCLC.[9,10] During the past 2 years, molecular mechanisms underlying TKI sensitivity have been identified. The main biological events demonstrated to lead to TKI sensitivity are the presence of specific EGFR gene mutations and increased copy numbers of the EGFR gene.[11–17] Additional studies showed that other mechanisms are involved in TKI sensitivity, such as increased copy numbers of HER2[18] and activation of the antiapoptotic protein Akt.[19,20]

The encouraging results in terms of response rate (RR), time to disease progression (TTP), and survival observed in recently completed phase II trials of TKIs in selected NSCLC[21–23] strongly support further phase III studies comparing a TKI with standard chemotherapy. A critical point in designing such trials is the potential benefit from standard chemotherapy in patients with biological features indicative of TKI sensitivity. Large phase III randomized trials comparing standard chemotherapy plus placebo or a TKI[24–27] showed no overall benefit for patients receiving chemotherapy plus TKI, raising concern that both modalities target the same patient population. Although preclinical data showed that EGFR or HER2 are implicated in the development of cancer cells resistant to cytotoxic drugs,[28] only few clinical data are available in lung cancer and are confined to EGFR.[29] In the retrospective study performed by Dziadziuszko et al.[29] EGFR gene copy number or EGFR expression were not associated with TTP or to survival of NSCLC patients treated with chemotherapy. No clinical data exist on HER2, and the role of Akt activation has been evaluated only in preclinical models. In the study performed by Brogard et al.,[30] Akt resulted constitutively active in NSCLC cell lines and promoted resistance to chemotherapy and radiation therapy. The aim of this retrospective study was to evaluate whether response to first-line chemotherapy is associated to EGFR and HER2 gene copy numbers and to Akt activation.

METHODS

Patient Population

This retrospective study was conducted in a cohort of 190 NSCLC patients followed at the Bellaria-Maggiore Hospital in Bologna (Italy) between January of 2001 and December of 2005. This cohort included 46 patients analyzed in previously published studies.[13,31] The primary endpoint was response to chemotherapy according to EGFR and HER2 gene copy numbers and to Akt status, and secondary endpoints were response to chemotherapy according to presence of EGFR mutations, TTP, and survival. Patients included into the analysis were selected based on the following criteria: histologically confirmed diagnosis of NSCLC; availability of tumor tissue and full clinical data; presence of at least one measurable lesion according to the Response Evaluation Criteria In Solid Tumors[32]; metastatic or locally advanced (stage III) NSCLC treated with standard first-line chemotherapy and not treated with concomitant radiotherapy. Patients were classified as never smoker (<100 cigarettes per lifetime), former smoker (quit smoking >6 months before starting chemotherapy therapy), or current smoker (quit smoking <6 months before starting chemotherapy or active smokers). Written informed consent for study biomarker analyses was obtained from each patient entering the study. The study was conducted in accordance with ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines on good clinical practice, whichever represented the greater protection of the individual.

Tissue Preparation and Biomarker Analyses

Sections from paraffin-embedded tissue blocks containing representative malignant cells and obtained before any cancer therapy was used for all analysis. Histopathological classification was determined on hematoxylin-eosin–stained sections based on the World Health Organization criteria.[33] Gene copy number per cell was investigated by fluorescence in situ hybridization (FISH) using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Molecular) and the PathVysion DNA probe Kit (Vysis, Abbott Molecular), which includes the LSI HER-2 SpectrumOrange and the CEP 17 SpectrumGreen probes. Assays and analyses were performed as described elsewhere.[13,16,18] Tumors were classified as FISH positive when carrying four or more copies of the gene in 40% or more of cells or gene amplification; and FISH negative when carrying four or more copies of the gene in less than 40% of cells.

Paraffin-embedded tissue sections were stained with antibodies against phospho-Akt (P-Akt), purchased from Cell Signaling Technology (Beverly, MA). Staining was performed according to the protocol described in the manufacturer's guide. Sections were placed on glass slides and deparaffinized. The antigen was unmasked by heating samples in 1X Microstain Unmasker Buffer (pH8) (Ventana) for 40 minutes at 98°C. The reaction was quenched using 1% hydrogen peroxide. Nonspecific binding sites were blocked with 5% goat serum in phosphate-buffered saline for 1 hour at room temperature. Samples were then immunostained using P-Akt (Ser 473) rabbit polyclonal antibody (1:50). The staining technique used is a two-step method with goat polyvalent and streptavidin peroxidase reagent (Lab Vision). Immunohistochemically stained slides were interpreted blindly and independently by two pathologists (C.L., E.M.), using a four-tiered grading system based on staining pattern and intensity. We considered as P-Akt positive all cases with moderate or strong staining (2+ or 3+) in at least 10% of tumor cell nuclei, as previously reported.[19]

EGFR protein expression was evaluated by immunohistochemistry (IHC) using methods and criteria described elsewhere.[13,34] Specimens were stained with monoclonal antibody to EGFR (Zymed Laboratories, Inc., San Francisco, CA).

EGFR mutation analysis was performed according to methods previously reported.[13]

Statistical Analysis

Response to chemotherapy according to EGFR, HER2, and P-Akt status was assessed by χ^2 test or Fisher's exact test. TTP, overall survival (OS), and the 95% confidence intervals were evaluated by the Kaplan-Meier method,[35] comparing the groups by log-rank test. Test for proportions was used to compare RR with chemotherapy and with EGFR-TKIs.

RESULTS

Patient Characteristics

A total of 190 patients were included in this study. Patient clinical and biological characteristics are shown in Tables 1 and 2. Median age was 63.5 years (range, 33–80), the majority were males (67.4%), and with good performance status (0–1: 98.4%). Adenocarcinoma was the most frequent histology (48.9%), followed by squamous cell carcinoma (25.3%), undifferentiated carcinoma (14.7%), bronchioloalveolar carcinoma or adenocarcinoma with bronchioloalveolar features (10%), and large cell carcinoma (1.1%). The majority of patients were current (37.4%) or former smokers (46.8%), and standard platinum-based doublets were offered to 80% of individuals. Thirty-eight patients received single-agent chemotherapy with gemcitabine or vinorelbine because of age (27 patients) or the presence of comorbidity contraindicating platinum-based chemotherapy (11 cases). Patients received standard doses of platinum (cisplatinum 75–80 mg/m^2, carboplatin area under the curve 5–6) in combination with gemcitabine (1000–1250 mg/m^2 on days 1 and 8) in 107 cases, in combination with taxanes (paclitaxel 200–225 mg/m^2, docetaxel 75 mg/m^2) in 23 cases, or in combination with vinorelbine (25–30 mg/m^2 on days 1 and 8) in 22 cases. Chemotherapy cycles were repeated every 21 days. Patients with locally advanced disease (25.8%) received sequential radiotherapy and/or surgery with curative intent.

TABLE 1. Clinical and Demographic Characteristics of the Patient Population

Characteristic	No.	%
Total patients	190	100
Median age, yr	63.5	
Range	33–80	
Sex		
Male	128	67.4
Female	62	32.6
Stage		
III	60	31.6
IV	130	68.4
Histology		
Adenocarcinoma	93	48.9
Squamous cell carcinoma	48	25.3
BAC/adeno-BAC	19	10.0
Large cell	2	1.1
Undifferentiated	28	14.7
ECOG performance status		
0	156	82.1
1	31	16.3
2	3	1.6
Smoking history		
Never	30	15.8
Former	89	46.8
Current	71	37.4
Therapy		
Total treated with platinum-based chemotherapy	152	80.0
Induction platinum-based chemotherapy followed by RT ± surgery	49	25.8
First-line chemotherapy not followed by any local therapy	103	54.2
Total treated with nonplatinum compounds	38	20.0
Patients treated with EGFR-TKI therapy as second or third-line		
Total treated	123	64.7
Gefitinib	108	56.8
Erlotinib	15	13.8

BAC, bronchioloalveolar carcinoma; adeno, adenocarcinoma; ECOG, Eastern Cooperative Oncology Group; RT, radiation therapy; EGFR-TKI, epidermal growth factor receptor–tyrosine kinase inhibitor.

TABLE 2. Biological Characteristics of NSCLC

Biomarker	No.	%
EGFR FISH		
Total	185	100
Positive	47	25.4
Negative	138	74.6
HER2 FISH		
Total	184	100
Positive	53	28.8
Negative	131	71.2
EGFR IHC		
Total	185	100
Positive	91	49.2
Negative	94	50.8
P-Akt IHC		
Total	176	100
Positive	82	46.6
Negative	94	53.4
EGFR mutation		
Total	65	100
Exon 19	15	23.1
Exon 20	2	3.0
Exon 21	7	10.8
Wild type	41	63.1

NCSLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

EGFR FISH analysis was successfully performed in 185 cases, and 47 (25.4%) were positive. HER2 was evaluated by FISH in 184 patients, and 53 (28.8%) were positive. EGFR and P-Akt were successfully evaluated by IHC in 185 and 176 cases, respectively; EGFR was positive in 91 patients (49.2%), and P-Akt was positive in 82 (46.6%).

Response to Chemotherapy and Biological Characteristics

In the whole population, RR to chemotherapy was 34.1%. As expected, in patients treated with platinum-based chemotherapy, RR was 38.9%, higher than in patients treated with a single agent (13.9%). As summarized in Table 3, RR was not significantly associated with any biological characteristic. Response to chemotherapy was 37.0% and 32.8% in EGFR FISH positive and negative, respectively ($p = 0.6$). No

TABLE 3. Response to Chemotherapy According to the Biological Characteristics

	Whole Study Population			Platinum-Based Chemotherapy			Nonplatinum-Based Chemotherapy		
Status	**Total**	**RR (%)**	***p***	**Total**	**RR (%)**	***p***	**Total**	**RR (%)**	***p***
EGFR FISH+	46	37.0	0.6	36	38.9	1.0	10	30.0	0.1
EGFR FISH−	134	32.8		108	38.9		26	7.7	
HER2 FISH+	51	37.3	0.5	40	42.5	0.5	11	18.2	0.6
HER2 FISH−	128	32.8		104	37.5		24	12.5	
EGFR IHC+	89	36.0	0.6	70	41.4	0.6	19	15.8	1.0
EGFR IHC−	91	33.0		75	37.3		16	12.5	
P-Akt+ IHC+	81	32.1	0.5	63	38.1	0.8	18	11.1	0.6
P-Akt- IHC−	90	36.7		75	40.0		15	20.0	
EGFR mutated	24	29.1	0.6	22	31.8	0.6	2	50.0	NA
EGFR wild type	41	36.5		41	36.5		0	0	

EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NA, not assessable.

difference in response was also observed between HER2 FISH positive and negative (37.3% versus 32.8%, $p = 0.5$), between EGFR IHC positive and negative (36.0% versus 33.0%, $p = 0.6$), and between P-Akt positive and negative (32.1% versus 36.7%, $p = 0.5$). No differences were observed when the analysis was restricted to patients treated with platinum-based chemotherapy or to individuals receiving a single-agent treatment.

TTP and Survival

In the study cohort, median TTP was 6.6 months, and median OS was 20.9 months. These two variables were not associated with any biological characteristic evaluated in the present study (Table 4). In patients treated with nonplatinum-based chemotherapy ($n = 38$), EGFR FISH positive had longer TTP (8.1 versus 4.1 months, $p = 0.1$) and longer OS (28.5 versus 14.8 months, $p = 0.07$) than EGFR FISH negative, although these differences were not statistically significant. Conversely, HER2 FISH positive had a significantly longer TTP (8.4 versus 5.0 months, $p = 0.01$) and OS (50.8 versus 14.8 months, $p = 0.01$) than HER2 FISH negative. All patients treated with nonplatinum-based chemotherapy had stage IIIB or IV not suitable for local therapy with curative intent, and 29 patients received a TKI at disease progression. No different survival results were observed when patients not treated with a TKI were excluded.

TTP and OS did not differ according to EGFR IHC status or P-Akt status. In the subgroup of patients treated with platinum-based chemotherapy ($n = 152$), differences in TTP and OS were not different irrespective of EGFR (FISH and IHC), HER2, or P-Akt status.

Because of the possible confounding effects of radiotherapy or surgery in patients with stage III disease, we further evaluated TTP and OS in patients who did not receive subsequent locoregional treatments (stage IIIB with effusion or stage IV), as illustrated in Table 5. In patients treated with platinum-based chemotherapy ($n = 104$), TTP and OS were not different in patients positive or negative for EGFR (FISH or IHC), HER2, or P-Akt.

EGFR Mutation Analysis

Due to the relevance of EGFR gene mutations for response to TKI,[11–14] we further evaluated the association of exons 19 through 21 EGFR gene mutations and response to

TABLE 4. Time to Disease Progression (TTP) (in Months) and Median Overall Survival (OS) (in Months) According to the Biological Characteristics

	Whole Study Population					Platinum-Based Chemotherapy					Nonplatinum-Based Chemotherapy				
Status	**Total**	**TTP**	***p***	**OS**	***p***	**Total**	**TTP**	***p***	**OS**	***p***	**Total**	**TTP**	***p***	**OS**	***p***
EGFR FISH+	46	6.5	0.62	28.0	0.3	36	6.5	0.8	31.1	0.4	10	8.1	0.1	28.5	0.07
EGFR FISH−	134	6.4		20.0		108	7.3		20.3		26	4.1		14.8	
HER2 FISH+	51	6.7	0.91	36.6	0.1	40	6.5	0.2	29.6	0.5	11	8.4	0.02	50.8	0.01
HER2 FISH−	128	6.6		18.9		104	7.4		20.3		24	5.0		14.8	
EGFR IHC+	89	6.5	0.72	26.2	0.3	70	6.6	0.7	31.4	0.1	19	5.0	0.6	28.5	0.3
EGFR IHC−	91	7.4		17.9		75	7.6		18.9		16	5.2		21.6	
P-Akt+ IHC+	81	6.7	0.27	20.3	0.2	63	6.8	0.4	31.1	0.9	18	5.0	0.4	16.7	0.3
P-Akt− IHC−	90	6.9		25.3		75	6.9		20.3		15	6.1		21.7	

EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

TABLE 5. Time to Disease progression (TTP) and Median Overall Survival (OS) According to Biological Characteristics in Stage IIIB and IV Patients Who Received Platinum-based Chemotherapy

Biomarker	Total	TTP (mo)	*p*	OS (mo)	*p*
EGFR FISH+	29	5.9	0.9	17.4	0.9
EGFR FISH−	71	4.8		20.3	
HER2 FISH+	33	6.2	0.3	25.3	0.1
HER2 FISH−	68	5.2		17.9	
EGFR IHC+	50	5.8	0.8	25.3	0.8
EGFR IHC−	50	5.7		17.5	
P-Akt+ IHC+	46	6.3	0.7	20.3	0.6
P-Akt− IHC−	51	4.8		25.3	
EGFR mutation +	19	5.2	0.4	36.6	0.5
EGFR wild type	28	4.9		20.3	

EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

chemotherapy. The analysis was only conducted in a fraction of patients (65 cases, 34.2%) including 46 cases previously reported[13] because of the scarce amount of tumor tissue available. Mutations in the EGFR gene were detected in 24 cases, including deletion in exon 19 in 15 cases, point mutation in exon 20 in two cases, and point mutation in exon 21 in seven cases. All but two patients received a platinum-based chemotherapy. Response to chemotherapy was achieved 36.5% in patients with wild-type EGFR and in 29.1% patients with EGFR mutation. Noteworthy, all EGFR mutation–positive patients responding to chemotherapy had an exon 19 deletion. Response to chemotherapy was 46.6% in patients with exon 19 deletion, and 0% in patients with other EGFR mutations ($p = 0.02$).

Biological Characteristics and Sensitivity to TKIs

Among the 190 patients analyzed, 123 received a treatment with a TKI as second- or third-line therapy. Gefitinib was offered in 108 cases, and erlotinib in additional 15 patients. Patients positive for EGFR (FISH, mutation, or IHC), HER2 FISH, or P-Akt had a significantly higher RR, TTP, and OS (data not shown). In Table 6, we report the results in terms of RR and TTP observed according to different biomarkers in patients treated with chemotherapy and further treated with a TKI. To avoid any confounding effect of radiotherapy and/or surgery, TTP analysis was confined to patients who have not received any locoregional therapy. RR was higher and TTP was longer for EGFR FISH– or EGFR mutation–positive patients when treated with a TKI than with chemotherapy, whereas RR and TTP favored chemotherapy in EGFR-negative patients. Chemotherapy produced higher RR and longer TTP than TKIs in patients negative for HER2 FISH, EGFR IHC, and P-Akt.

DISCUSSION

The aim of this study was to assess whether biological determinants for TKI sensitivity influenced sensitivity to first-line chemotherapy in NSCLC, and we observed that the outcome of patients was not dependent on EGFR, HER2, or P-Akt status.

During the past 2 years, several retrospective studies showed that presence of activating EGFR gene mutations and an increased copy number of the EGFR gene were strongly associated with TKI sensitivity.[11–17] Additional data from preclinical and clinical experience showed that other biomarkers were relevant for TKI sensitivity, including HER2[18] and P-Akt.[19,20] Recent prospective studies confirmed the central role of EGFR in TKI sensitivity.[21–23] In these trials, patients with EGFR mutations[21–23] or EGFR increased copy number[22] treated with a TKI had a higher RR and a longer OS than reported with standard chemotherapy,[3] supporting randomized phase III studies comparing chemotherapy with an EGFR-TKI in selected NSCLC. In designing these trials, it is critical to know the expected benefit of standard chemotherapy because it is possible that patients sensitive to TKIs are the same patients who are also sensitive to chemotherapy. In a recent retrospective study, Dziadziuszko et al..[29] found no

TABLE 6. Response Rate (RR) and Time to Disease Progression (TTP) According to Different Biomarkers in Patients Treated with Chemotherapy and Further Treated with a TKI

Biomarker	No.	RR to Chemotherapy (%)	RR to EGFR-TKIs (%)	*p*	No.	TTP with Chemotherapy (mo)	TTP with EGFR-TKIs (mo)
EGFR FISH+	36	31.4	52.9	0.1	34	6.4	7.1
EGFR FISH-	85	25.9	3.8	0.0002	67	4.1	2.1
HER2 FISH+	36	31.4	46.9	0.3	35	5.8	7.4
HER2 FISH−	82	25.6	7.7	0.005	64	4.1	2.4
EGFR IHC+	60	28.8	31.5	0.9	52	5.2	4.5
EGFR IHC−	58	27.6	5.4	0.003	46	4.8	2.1
P-Akt+	53	25.0	24.0	1.0	46	5.2	3.6
P-Akt−	58	31.0	9.4	0.01	47	4.8	2.1
EGFR mutation+	24	30.4	60.9	0.07	21	5.7	9.2
EGFR wild type	41	36.6	2.4	<0.001	28	4.9	2.2

For time to disease progression, *p* value is not assessable. TKI, tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RR, response rate.

difference in TTP or in survival according to EGFR gene copy number or EGFR expression. In this study, in which the RR was the primary endpoint, response to chemotherapy was not different in EGFR-positive and -negative patients, irrespective of the method used for EGFR assessment. The retrospective and noncomparative nature of the present study, together with the fact that chemotherapy and TKIs were used sequentially, does not allow us to directly compare chemotherapy with EGFR-TKIs. Despite this limitation, we observed that RR was higher and TTP was longer with TKIs than with chemotherapy in patients with EGFR gene mutations or with EGFR increased gene copy number, suggesting that EGFR-TKIs could be more effective than chemotherapy in such patients. Conversely, patient outcome was better with chemotherapy in EGFR-negative patients, suggesting that, in absence of the target, a targeted therapy is ineffective and a less specific treatment, such as chemotherapy, could give better results. Recently, Lilenbaum et al.[36] presented the results of a phase II randomized study comparing the standard combination of carboplatin plus paclitaxel versus erlotinib in untreated NSCLC patients. This study, conducted in unselected NSCLC patients with an Eastern Cooperative Oncology Group performance status of 2 showed higher RR and longer TTP and OS for patients receiving chemotherapy, supporting the importance of patient selection.

Retrospective analyses of large phase III trials comparing standard chemotherapy and the same chemotherapy regimen plus erlotinib or gefitinib[24–27] showed that presence of EGFR gene mutations was associated with longer survival independently of the treatment and supporting the hypothesis that EGFR mutations are positive prognostic factors.[37,38] Nevertheless, results of studies on NSCLC patients not exposed to TKI[39] and NSCLC patients treated with erlotinib or gefitinib[40–42] have shown that there are differences in patient outcome based on EGFR genotype. Patients with EGFR exon 19 deletion seems to be more sensitive to TKIs than patients with other EGFR mutations, including exon 21 mutations.[40–42] Although EGFR mutation analysis was not the primary endpoint in our study, which was conducted with a limited number of patients, it is intriguing that all EGFR mutation–positive patients responding to chemotherapy had an exon 19 deletion. This aspect has not been previously reported and should be considered in designing clinical trials, especially in studies comparing TKIs with chemotherapy.

In this study, the EGFR mutation rate was 34%, higher than reported in whites.[39] Considering the limited number of patients evaluated for mutations, it is likely that patients analyzed for EGFR mutations were not representative of the entire study cohort.

In breast cancer, several studies showed that HER2 amplification was associated with responsiveness to taxane- and anthracycline-containing regimens.[43,44] To the best of our knowledge, our study provides for the first time evidence that increased HER2 copy number does not influence chemotherapy sensitivity in NSCLC. The longer TTP observed in EGFR-positive or HER2-positive patients treated with single-agent chemotherapy is surprising and should be investigated in a larger cohort of patients. Conversely, the survival benefit observed in this small subgroup of patients is not surprising considering that the majority of cases received a TKI.

The clinical relevance of Akt activation on chemotherapy sensitivity in lung cancer has been explored only marginally. Akt activation has been shown in experimental models to confer chemoresistance.[30] Recently, preclinical data suggested that Akt confers resistance by modulating the direct action of p53 on the caspase-dependent mitochondrial death pathway.[45] In our study, no difference in chemotherapy outcome dependent on Akt status was observed, whereas Akt phosphorylation resulted in better TKI outcome, as previously reported.[19]

The median survival of these patients was longer than expected considering that the majority of them had stage IV disease. Although no prognostic factor was taken into account for patient selection, the high percentage of patients with good performance status (98.4%) and the effects of additional therapies (radiotherapy for patients with stage III disease and second- and third-line systemic treatments for individuals with stage IV) could have favorably influenced survival.

In conclusion, these data suggest that EGFR expression and gene copy number, HER2 gene copy number, and P-Akt expression are not predictors of response to chemotherapy in NSCLC. Prospective phase III trials should compare standard chemotherapy with a TKI in selected NSCLC.

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