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ORIGINAL REPORT

Genetic Abnormalities of the *EGFR* Pathway in African American Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
Previous studies in non–small-cell lung cancer (NSCLC) have demonstrated a wide variation in responsiveness to epidermal growth factor receptor (EGFR) –targeting agents and in genetic aberrancies of the *EGFR* pathway according to ethnic background, most notably a higher frequency of activating *EGFR* mutations among East-Asian patients. We investigated the frequency of *EGFR* pathway aberrancies among African American patients with NSCLC, for whom limited information presently exists.

Patients and Methods
EGFR fluorescent in situ hybridization (FISH) was performed on archived tissues from 53 African American patients. Extracted DNA was sequenced for mutational analysis of *EGFR* exons 18 to 21 and *KRAS* exon 2. Results were compared by multivariate analysis to an historical control cohort of 102 white patients with NSCLC.

Results
African Americans were significantly less likely to harbor activating mutations of *EGFR* than white patients (2% *v* 17%; $P = .022$). Only one *EGFR* mutation was identified, a novel S768N substitution. *EGFR* FISH assay was more frequently positive for African Americans than for white patients (51% *v* 32%; $P = .018$). *KRAS* mutational frequency did not differ between the groups (23% *v* 21%; $P = .409$).

Conclusion
African American patients with NSCLC are significantly less likely than white counterparts to harbor activating mutations of *EGFR*, which suggests that EGFR tyrosine kinase inhibitors (TKIs) are unlikely to yield major remissions in this population. Our findings add to a growing body of evidence that points to genetic heterogeneity of the *EGFR* pathway in NSCLC among different ethnic groups and that underscores the need for consideration of these differences in the design of future trials of agents that target the *EGFR* pathway.

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INTRODUCTION

Epidermal growth factor receptor (EGFR) is critically involved in the pathogenesis of NSCLC and recently emerged as an important target for the development of molecular therapeutics. Both small-molecule EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, and the monoclonal anti-EGFR antibody cetuximab have demonstrated significant promise. Erlotinib therapy leads to a survival benefit in the second- and third-line management of NSCLC,[1] and cetuximab in addition to chemotherapy recently demonstrated improved survival in the large, randomized, FLEX trial.[2]

Somatic EGFR mutations, most significantly exon 19 deletions and the L858R mutation, identify tumors dependent on this pathway for growth and proliferation and appear to sensitize tumors to the effects of adenosine triphosphate–mimetic, small-molecule inhibitors. These mutations occur more frequently in specific subsets of patients, such as women, never smokers, and patients with adenocarcinoma histology. Mutation frequency in NSCLC is also recognized to vary across ethnic groups, with a notably higher prevalence observed in East-Asian trials (30% to 60%), than in North American studies (10% to 20%).[3-6] The reasons for ethnic influence on mutation frequency remain poorly understood. Several reports suggest that EGFR mutations confer survival benefit independent of treatment.[7,8] More recent information also suggests that the presence of classical EGFR mutations is predictive of survival

benefit after EGFR TKI therapy. In the pivotal East-Asian, IPASS study that compared upfront carboplatin and paclitaxel with gefitinib in never smokers or light ex-smokers with advanced adenocarcinoma of the lung, gefitinib demonstrated superiority both in response rates and in progression-free survival, although benefit was restricted to the EGFR-mutant subset.[9] A major area of current research focus involves understanding the mechanisms of molecular resistance mediated by secondary *EGFR* or *MET* abnormalities that curtail the long-term efficacy of these agents.[10-12]

An increase in *EGFR* gene copy number, either via high polysomy or true amplification, as determined by fluorescence in situ hybridization (FISH), is observed in 30% to 50% of patients with NSCLC, and higher frequency appears associated with advanced stage.[13] FISH positivity, defined by the Colorado classification,[14] has been shown repeatedly to be a significant predictor of treatment response, time to progression, and survival in NSCLC. The predictive role of FISH positivity for EGFR TKI therapy was demonstrated first by Cappuzzo et al[15] in a multicenter study that involved 102 patients who received gefitinib. In this study—from which we have drawn the comparator cohort of white patients for this analysis—a third of tumors demonstrated high polysomy or amplification by *EGFR* FISH analysis. FISH positivity correlated with higher response rates and longer median survival, and patients with *EGFR* amplification had higher response rates than patients with high polysomy. However, FISH positivity was not predictive of significant treatment benefit in either the INTEREST study[16] that compared salvage gefitinib with docetaxel monotherapy or in the INVITE study[17] that compared gefitinib with vinorelbine in elderly, chemotherapy-naïve patients with NSCLC. Hirsch et al,[18] in a retrospective analysis that drew from the SWOG 0342 trial, recently have provided evidence to support a role for *EGFR* FISH in predicting clinical benefit from the addition of cetuximab to chemotherapy in patients with advanced NSCLC. It is important to note that significant overlap is observed between FISH-positive and EGFR-mutant NSCLC, which may confound interpretation of results in these studies. In the study of Takano et al,[19] 56% of patients with *EGFR* mutations also had high copy numbers by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). In the series of Cappuzzo et al,[15] 64% of patients with *EGFR* mutations were positive by FISH, and two thirds of these had true amplification.

KRAS mutations are present in roughly 25% of NSCLC tumors, principally adenocarcinomas, but the overall impact of these mutations on clinical outcome in NSCLC remains unclear. The presence of mutations of the *KRAS* gene (mainly codons 12 and 13 of exon 2) appear mutually exclusive of *EGFR* mutations. Evidence suggests that *KRAS* mutations confer resistance to upstream targeting of *EGFR* in a manner similar to colorectal cancer.[7,20] In the TRIBUTE study, which compared carboplatin and paclitaxel with or without erlotinib, Eberhard et al[7] found that the presence of *KRAS* mutations was associated with poorer outcomes in patients treated in the combined arm. However, as recently reported by Zhu et al,[21] biomarker reanalysis that involved 240 of 328 specimens from the BR.21 trial, which compared erlotinib with placebo in chemotherapy-refractory, advanced NSCLC, revealed in multivariate analysis that only EGFR FISH positivity was predictive of survival benefit from erlotinib (10.5 *v* 3.1 months; $P = .005$) and of poorer median survival with placebo (3.1 *v* 4.7 months; $P = .025$). Neither *KRAS* mutation nor *EGFR* mutation status were predictive or prognostic.

African American patients with lung cancer have significantly poorer 5-year survival than their white counterparts across all stages. Moreover, the incidence of lung cancer is higher among the African American population.[22] The reasons for these disparities remain debatable. As previously observed in the Surveillance, Epidemiology, and End Results (SEER) database by Gadgeel et al[23] African Americans were more likely to present with advanced stage at diagnosis. The Multiethnic Cohort Study[24] identified a uniquely increased smoking-related risk of lung cancer for African Americans restricted to moderate smokers (< one pack per day; $P < .001$) but not seen for heavy smokers (> 30 cigarettes per day) or never smokers. Despite a large number of studies that assessed the frequency of *EGFR* mutations in a wide-range of populations, only one study to our knowledge has included a reasonable number of African American patients with NSCLC. Yang et al[25] sequenced tumors from 219 patients with NSCLC and identified activating mutations in 14.1% (25 of 177) of white patients and only 2.4% (1 of 41) of African American patients (del19 E746-A751). Data regarding *EGFR* gene copy number changes among African American patients with NSCLC have not been reported previously. The relative frequency of *KRAS* mutations in African American patients with NSCLC is at least as high, if not higher, than in white patients.[26] Information on the frequency of these abnormalities will contribute to the design of clinical studies and may help determine optimal use of EGFR-targeted agents in this population. In this study, we characterized the frequency of genetic abnormalities that involved the *EGFR* pathway by FISH and by *EGFR* and *KRAS* mutational analysis in a representative population of African American patients with NSCLC.

PATIENTS AND METHODS

Patients and Tissues

Archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks from diagnostic and/or therapeutic procedures performed between 2002 and 2007 at University Hospitals Case Medical Center were reviewed under an institutional review board–approved protocol (ie, CASE 1506-CC094). Fifty-three African American patients with NSCLC were identified by chart review, and there was an additional requirement that uninvolved, paired tissue (typically lymph nodes) was available for analysis. Slides were reviewed by a pulmonary pathologist to assure greater than 70% tumor content as suitability for DNA extraction. Cappuzzo et al[15,27] have previously published results for an exclusively white cohort of 102 patients with NSCLC, who were accrued from three Italian centers (in Bologna, Milan, and Perugia) and who were treated between 2000 and 2004 with gefitinib, either through a prospective trial (n = 80) or an expanded access protocol (n = 22). Eligible patients had stages III or IV NSCLC and had experienced chemotherapy failure or were considered ineligible for chemotherapy. FFPE archival tissues were assayed for *EGFR* FISH and *EGFR* and *KRAS* mutation. This data set served as a white comparator cohort in this study. The FISH assay was conducted according to identical protocols in the same central laboratory for both groups; therefore, this cohort was considered optimal for comparison.

Power Analysis

Sample size was based on a power calculation that in order to have greater than 80% power to detect a difference in *EGFR* mutation frequency of 10% (ie, hypothesized frequency of 5% *v* average population frequency of 15%), a single-arm study compared with historical controls would require 53 samples by using a one-sided exact test for single population and a significance level of .05.

Mutational Analysis of EGFR *and* KRAS

After xylene deparaffinization and DNA isolation by standard phenol/chloroform extraction, exons 18 to 21 of *EGFR* and exon 2 of *KRAS* were

amplified by nested PCR with primers and conditions that have been described previously.[3,20] Sequencing was performed with the BigDye Terminator Cycle Sequencing Kit (version 3.1; Applied Biosystems, Foster City, CA) and an ABI Prism 3730 DNA Analyzer (Applied Biosystems). Bidirectional sequence data were analyzed by using DNAStar and Geospiza software (version 1.4.0; Geospiza, Seattle, WA), and analysis was followed by manual review. Positive findings were confirmed by repeat amplification/sequencing and by consensus among at least two investigators. Somatic nature of *EGFR* mutants was confirmed by sequencing of uninvolved paired tissue by identical methods.

EGFR *Gene Copy Number by FISH*

Three slides with FFPE sections of lung tumor were evaluated by dual-color FISH assay with the EGFR/CEP7 probes (Abbott Molecular, Abbott Park, IL) and were scored according to the six categories of the Colorado classification, as previously described.[14,15] Scores of five (ie, high polysomy) and six (ie, amplification) were considered FISH positive.

Statistical Analysis

The incidences of *EGFR* mutation, *EGFR* FISH positivity (defined as FISH score of five or six), and *KRAS* mutation status along with the corresponding confidence intervals were estimated by Wilson's approach.[28] The association between categoric variables, including ethnicity, was examined by χ^2 test. The important predictors, including ethnicity, on incidence of *EGFR* mutation, *EGFR* FISH positivity, and *KRAS* mutation were identified additionally by logistic regression with a forward-model selection procedure. The factors (in addition to ethnicity) included in the model selection were age, sex, histology, and stage. The difference of continuous measurement between two groups was examined by *t* test. All tests were two-sided, and a *P* value $\leq .05$ was considered statistically significant. Statistical analyses were carried out by using SAS version 8.1 (SAS Institute, Cary, NC).

RESULTS

Biomarker analysis by *EGFR* FISH and *EGFR* and *KRAS* sequencing of FFPE tissue blocks from 53 African American patients with NSCLC was compared with a historical cohort of 102 white patients with NSCLC who were treated with gefitinib by protocol for stages III and IV disease. Patient characteristics for the two cohorts are listed in Table 1. Significant differences between the African American and white cohorts were observed for sex and tumor stage, the latter as a result of differences in eligibility.

Classical *EGFR* mutations, notably L858R and exon 19 deletions, which together account for roughly 90% of activating mutations in NSCLC, were not identified in the African American cohort. A heterozygous missense mutation in exon 20 of *EGFR* that involved a G-to-A substitution in nucleotide 2303G was identified, and it resulted in the amino acid change S768N, (ie, serine to asparagine). This sample also had increased *EGFR* gene copy number by FISH (FISH score of five) and wild-type *KRAS*. The somatic nature of this mutation was confirmed by analyzing DNA from a paired, uninvolved lymph node sample in which only wild-type *EGFR* sequences were found (Fig 1). Although this S768N mutation is not identical to the S768I (ie, serine to isoleucine) substitution, which in one review of NSCLC was previously reported to make up 2% of all detectable *EGFR* mutations,[29] we considered this a positive finding, as it affected the same residue. No other mutations were identified. Therefore, a 2% frequency of *EGFR* mutation in the African American cohort was observed, compared with a 17% frequency in the white cohort. In a multivariate analysis that was controlled for age, sex, histology, stage, and smoking status, this difference was statistically significant ($P = .022$). *EGFR* gene copy number by FISH was increased in 51% of African Americans compared with 32% of white patients, and this was significant also ($P = .018$). The frequency of *KRAS* mutation did not significantly differ between cohorts (Table 2). As anticipated, *KRAS* mutation was associated with nonsquamous histology ($P = .004$), but it was not associated with positive *EGFR* FISH ($P = 0.465$). Covariate associations with *EGFR* mutation in the African American cohort cannot be meaningfully reported, given the near absence of mutations, but it is noteworthy that the single mutation identified was in a former smoker.

Seven African American patients received treatment with a TKI. Table 3 summarizes their clinical courses. None harbored an *EGFR* mutation. Interestingly, one female patient with true *EGFR* amplification by FISH (wild-type *KRAS*) experienced stable disease for longer than 28 months with salvage gefitinib after chemotherapy.

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Ethnicity Group: African American (n = 53) No.	%	White (n = 102) No.	%	*P*
Age					.067
Median	64		60		
Range	46-81		25-84		
Sex					
Male	25		67		.026
Female	28		35		
Histology					
Squamous	18	34	26	26	.267
Adenocarcinoma	28	53	54	54	
Other	7	13	22	22	
Stage					
I	32		0		< .0001
II	6		0		
III	7		14		
IV	8		88		
Smoking history					
Ever smoker	46	87	87	84	.8
Never smoker	7	13	15	16	

DISCUSSION

African American patients with NSCLC were found to be significantly less likely than white counterparts to harbor activating mutations of *EGFR*. To confirm that this is indeed related to inherent differences between the two cohorts, we compiled available data from a number of large, well-studied cohorts (Table 4).[15-18,21,30-35] These results demonstrate that the unexpectedly low 2% frequency of *EGFR* mutations in our study group clearly stands out from other published series. It is highly unlikely that the low frequency of mutations detected in our study is attributable to technical difficulties, as only specimens with greater than 70% tumor were selected, and an expected rate of *KRAS* mutation was observed, which confirmed the quality of tissue and analysis. The only other published series to include a fair number of African American patients similarly found a low frequency of *EGFR* mutations.[25] The extremely low rate of oncogenic *EGFR* mutations we

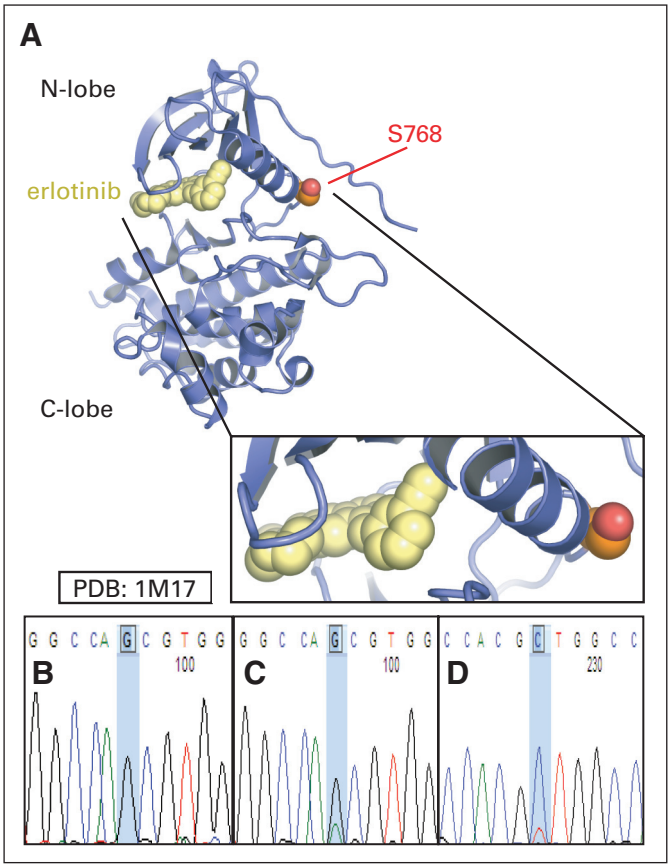

Fig 1. (A) Serine 768 (S768) in proximity to epidermal growth factor receptor adenosine triphosphate binding pocket. Panel made with pymol (http://www.pymol.org). (B) Wild-type sequence in negative lymph node. (C) Tumor S768N substitution, heterozygous peak AGC to AAC. (D) Tumor antisense S768N substitution, heterozygous peak GCT to GTT.

observed in African American patients with NSCLC predicts a low likelihood of major responses to EGFR TKIs in this setting. Response rates to gefitinib and erlotinib range widely, depending on the population studied. To our knowledge, there have been no studies reported with a substantial number of African American patients to allow a fair estimation of response and outcomes. An abstract reported from Cook County Hospital on erlotinib treatment of 33 patients with NSCLC, 18 of whom were African American, suggested significantly shorter duration of response for the African Americans.[36] Our personal experience mirrors this observation; however, given the retrospective nature of this analysis and the small number of patients exposed to an EGFR TKI, conclusions cannot be drawn regarding treatment responsiveness. Riely et al[37] conducted a retrospective chart review of 219 patients with NSCLC who had *EGFR* mutational analysis at Memorial Sloan-Kettering Cancer Center and identified 70 patients with classical *EGFR* mutations (ie, L858R and del19); these patients included six (43%) of 14 African American patients. However, eight of these 14 were never smokers, which suggests referral patterns of highly selected patients, which may account for the high mutation rate observed. Because our series drew from a community-based cohort, in which all patients resided in the Cleveland metropolitan area, we believe our series to more closely reflect the overall frequency of *EGFR* mutations in an unselected population.

It is highly interesting that none of the most common mutations, such as exon 19 deletions or L858R, were noted in this study, whereas these mutations can be found with an average frequency of 10% to 15% in the general population and of up to 40% to 50% in East-Asian patients. The etiology of these mutations remains poorly defined, but this study, among others, suggests a major genetic predilection, or lack thereof, to the acquisition of these mutations and provides impetus for studies that focus on genetic susceptibility to *EGFR*-mutant NSCLC. To our knowledge, the S768N mutation identified has not been previously reported. Interestingly, this sample also had high *EGFR* polysomy (FISH score of 5) but the functional role of this mutation is unclear. Exon 20 missense mutations that result in the amino acid change S768I have been previously reported in 2% of NSCLC with *EGFR* mutations among East-Asian patients,[38,39] but in vitro evaluation of the S768I mutation in transfected cell lines actually resulted in gefitinib resistance.[40] Asahina et al[41] reported a case of widely metastatic lung adenocarcinoma in a Japanese man who was a smoker, who harbored S768I and V769L mutations, and who was unresponsive to front-line gefitinib. However, he subsequently responded to couplet chemotherapy, which suggested primary resistance. Although, it has been clearly documented that paraffin embedding can lead to PCR artifacts,[42] we believe that the S768N mutation we detected is indeed real, as it was detected bidirectionally as a clear-cut heterozygous peak

Table 2. Biomarker Analysis Results

	Ethnicity Group					Analysis			
	African American (n = 53)		White (n = 102)			Univariate	Multivariate		
Assay	No.	%	No.	Total No.	%	*P*	*P*	OR	95% CI
EGFR mutation–positive	1	2	15	89	17	.005	.022	11.36	1.41 to 90.91
KRAS mutation–positive	12	23	16	76	21	.83	.409	1.48	0.59 to 3.74
EGFR FISH–positive*	27	51	33	102	32	.024	.018	2.51	1.17 to 5.37
Polysomy (5)	21	40	20	102	20	.007			
Amplification (6)	6	11	13	102	13	.798			

Abbreviations: OR, odds ratio; EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; FISH, fluorescent in situ hybridization.
*For the FISH score, two readers independently scored 50 tumor cells in at least three tumor areas per specimen. Scores of 5 and 6 were considered FISH-positive scores. A FISH score of 5 required four or more copies in ≥ 40% of cells; a FISH score of 6 required gene amplification, which was defined by the presence of EGFR gene clusters, by a gene/chromosome ratio per cell of two or greater, or by 15 or more copies of EGFR in ≥ 10% of analyzed cells. Discrepancies between readers were resolved by a third reader.

Table 3. Clinical Characteristics of African American Patients Treated With Oral Gefitinib or Erlotinib

Characteristic							
Clinical Course	Patient Age (years)	Patient Sex	Stage	Histology	Patient Smoking Status	*EGFR* FISH	*KRAS* Mutation
Chemotherapy, salvage gefitinib ≥ 28 months, then lost to follow-up	60	F	IIIa	Adeno	Yes	Amplified (6)	—
Gefitinib 11 months then stopped with GI bleeding	74	M	IIIb	Adeno	Yes	—	—
Gefitinib, rapid failure, then hospice	52	M	IV	Large-cell	Yes	Polysomy (5)	G12V
Erlotinib, rapid failure, then hospice	60	F	IIIa	Squamous	Yes	—	—
Erlotinib, rapid failure, then salvage chemotherapy	71	F	IV	Adeno	Yes	—	—
Erlotinib, rapid failure, then hospice	81	F	IV	Adeno	Never	—	G12V
Adjuvant erlotinib 4 months, then study protocol completed	78	F	Ib	Adeno/squamous	Never	Polysomy (5)	—

Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; adeno, adenocarcinoma.

(Fig 1). It is also unlikely to represent a germline polymorphism, because it was not detected in DNA obtained from uninvolved, paired nodal tissue. Feinmesser et al[43] engineered an S-to-A mutation at amino acid 768 of exon 20 EGFR by site-directed mutagenesis, which led to increased *EGFR* activity through decreased CaM kinase II mediated phosphorylation. An S-to-N substitution at this site may also have an effect similar to the S-to-A mutation (Fig 1).[44] Therefore, we have reported this as a positive mutation—in fact, the only mutation identified in 53 samples—given a recognized locus and theoretical mechanism.

A significantly higher frequency of *EGFR* FISH positivity was observed for African American patients with NSCLC compared with white counterparts. Given that FISH positivity in several studies correlated with benefit from EGFR TKIs and anti-EGFR monoclonal antibody therapy, the high rate of FISH positivity in African American patients is noteworthy. Interestingly, the difference in *EGFR* FISH positivity between the African American and white cohorts in this study derived solely from high polysomy (FISH = five), and there was an essentially equal frequency of true amplification (FISH = six). True *EGFR* amplification appears closely associated with the presence of *EGFR* mutations and preferential amplification of the mutant allele, but this correlation does not appear to exist for polysomy. Whether increased *EGFR* gene copy numbers because of polysomy have the same biologic relevance as true EGFR gene amplification remains unclear, but increased gene copy number per se is associated with poorer survival in NSCLC.[21,45] As listed in Table 4, the frequency of FISH positivity varies over a wide range (ie, 31% to 69%) among large published series, which may represent improvement with time in the sensitivity of the FISH assay. Given the imbalance in stage distribution between the two groups in this study, (ie, white cohort was restricted to stages III and IV), it might be argued that the higher frequency of positive *EGFR* FISH among the African American cohort

Table 4. Prior Studies Examining *EGFR* Mutation, *EGFR* FISH, and *KRAS* Mutation in NSCLC

		Mutation Evaluation											
		EGFR Mutation–Positive			*EGFR* FISH–Positive			*KRAS* Mutation–Positive			Adenocarcinoma		
Study	No. of Patients	No.	Total No.	%	No.	Total No.	%	No.	Total No.	%	No.	Total No.	%
African American arm, this study	53	1	53	2	27	53	51	12	53	23	28	53	53
White arm, Cappuzzo 2005[15]	102	15	89	17	33	102	32	16	76	21	54	102	54
Hirsch et al, 2008[30]	143	16	119	13	76	143	53	29	135	21	106	143	74
Richardson et al, 2008 (RADIANT)[31]	278	32	270	12	192	278	69	56	270	21	149	278	54
Hirsch et al, 2008 (TRIBUTE)[32]	245	30	179	17	100	245	41	42	192	22	146	245	60
Kim et al, 2008 (INTEREST)[16]	1,466	44	297	15	174	374	47	49	275	18	797	1,466	54
Crino et al, 2008 (INVITE)[17]	196	7	65	11	54	158	34	NA	NA	NA	79	196	40
Zhu et al, 2008 (BR.21)[1]	328	37	204	17	61	159	38	30	206	15	365	731	50
Hirsch et al, 2008 (SWOG 0342)[18]	229	NA	NA	NA	45	76	59	NA	NA	NA	130	229	57
Sone et al, 2007[33]	59	17	59	29	26	54	48	NA	NA	NA	44	59	75
Hirsch et al, 2006 (ISEL)[34]	1,692	26	215	12	114	370	31	12	152	8	812	1,692	48
Han et al, 2006[35]	69	15	69	22	31	66	47	9	69	13	43	69	62

Abbreviations: NSCLC, non–small-cell lung cancer; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NA, not applicable; RADIANT, RAD001 in Advanced Neuroendocrine Tumor study; TRIBUTE, Tarceva Responses in Conjunction with Paclitaxel and Carboplatin study; INTEREST, Iressa NSCLC Trial Evaluating Response and Survival Against Taxotere; INVITE, IRESSA in NSCLC *v* Vinorelbine Investigation in the Elderly study; SWOG, Southwest Oncology Group; ISEL, Iressa Survival Evaluation in Lung Cancer trial.

is a reflection of this skew. Several reports, however, have noted a higher frequency of *EGFR* FISH-positivity with advancing tumor stage[13] which would predict a lower frequency of *EGFR* FISH positivity among the African American cohort—the opposite of what we have observed. We are currently pursuing a larger-scale, retrospective review of EGFR TKI responsiveness in African American patients with advanced NSCLC. Given our observation of a high frequency of *EGFR* gene copy number changes in African American patients, and the low frequency of *EGFR* mutation, it is conceivable that use of the anti-EGFR monoclonal antibody cetuximab along with chemotherapy and/or radiation may be an effective strategy in the management of these patients, whereas an EGFR TKI would not be expected to yield major responses.[46] Prospective studies that incorporate appropriate biomarker analyses are necessary to determine the optimal use of these compounds in this population. Our findings add to a growing body of evidence that highlights the genetic heterogeneity of the *EGFR* pathway in NSCLC among different populations and that underscores the need for incorporation of these differences in the design of clinical trials with agents targeted at inhibition of this pathway.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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