Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

Molecular Cancer Therapeutics

Coexistence of *PIK3CA* and Other Oncogene Mutations in Lung Adenocarcinoma–Rationale for Comprehensive Mutation Profiling

Jamie E. Chaft[1], Maria E. Arcila[2], Paul K. Paik[1], Christopher Lau[2], Gregory J. Riely[1], M. Catherine Pietanza[1], Maureen F. Zakowski[3], Valerie Rusch[4], Camelia S. Sima[5], Marc Ladanyi[2], and Mark G. Kris[1]

Abstract

Phosphoinositide-3-kinase catalytic alpha polypeptide (*PIK3CA*) encodes the p110α subunit of the mitogenic signaling protein phosphoinositide 3-kinase (PI3K). *PIK3CA* mutations in the helical binding domain and the catalytic subunit of the protein have been associated with tumorigenesis and treatment resistance in various malignancies. Characteristics of patients with *PIK3CA*-mutant lung adenocarcinomas have not been reported. We examined epidermal growth factor receptor (*EGFR*), Kirsten rate sarcoma viral oncogene homolog (*KRAS*), v-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*), human epidermal growth factor receptor 2 (*HER2*), *PIK3CA*, v-akt murine thymoma vial oncogene homolog 1 (*AKT1*), v-ras neuroblastoma viral oncogene homolog (*NRAS*), dual specificity mitogen-activated protein kinase kinase 1 (*MEK1*), and anaplastic lymphoma kinase (*ALK*) in patients with adenocarcinoma of the lung to identify driver mutations. Clinical data were obtained from the medical records of individuals with mutations in *PIK3CA*. Twenty-three of 1,125 (2%, 95% CI: 1–3) patients had a mutation in *PIK3CA*, 12 in exon 9 (10 E545K and 2 E542K), and 11 in exon 20 (3 H1047L and 8 H1047R). The patients (57% women) had a median age of 66 at diagnosis (range: 34–78). Eight patients (35%) were never smokers. Sixteen of 23 (70%, 95% CI: 49–86) had coexisting mutations in other oncogenes—10 *KRAS*, 1 *MEK1*, 1 *BRAF*, 1 *ALK* rearrangement, and 3 *EGFR* exon 19 deletions. We conclude that *PIK3CA* mutations occur in lung adenocarcinomas, usually concurrently with *EGFR*, *KRAS*, and *ALK*. The impact of *PIK3CA* mutations on the efficacy of targeted therapies such as erlotinib and crizotinib is unknown. Given the high frequency of overlapping mutations, comprehensive genotyping should be carried out on tumor specimens from patients enrolling in clinical trials of PI3K and other targeted therapies. *Mol Cancer Ther; 11(2); 485–91. ©2011 AACR.*

Introduction

The identification and targeting of specific oncogenic driver mutations have revolutionized the treatment of lung adenocarcinoma. Although mutations in Kirsten rate sarcoma viral oncogene homolog (*KRAS*) were identified decades ago and remain a target of investigation, the first therapeutic advance in lung cancer was the discovery of mutations in the epidermal growth factor receptor (*EGFR*) gene in patients who had experienced dramatic benefit from treatment with EGFR tyrosine kinase inhibitors (1–3). Since then, a number of other oncogenic driver mutations (missense mutations, insertions, and deletions) have been identified in v-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*), phosphoinositide-3-kinase catalytic alpha polypeptide (*PIK3CA*), and human epidermal growth factor receptor 2 (*HER2*). In addition, the discovery of the anaplastic lymphoma kinase (*ALK*) fusion protein in lung cancer (4, 5) led to the rapid identification of the ALK inhibitor crizotinib revealing a 61% overall response rate in patients with *ALK* rearrangements (6, 7) and leading to expedited approval by the U.S. Food and Drug Administration. This early experience has bolstered the growing enthusiasm for our ability to target therapy for an individual based on the presence of a specific driver mutation in their tumor specimen.

Authors' Affiliations: [1]Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College, [2]Molecular Diagnostics Service, Department of Pathology, [3]Department of Pathology, [4]Thoracic Service, Department of Surgery, and [5]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York

Note: The mutation data were obtained from the Sanger Institute Catalogue of Somatic Mutations in Cancer web site, http://www.sanger.ac.uk/cosmic (Bamford and colleagues, 2004) and the Catalogue of Somatic Mutations in Cancer (COSMIC) database and website (Br J Cancer, 91, 355–358).

Corresponding Author: Mark G. Kris, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, H1016, New York, NY 10065. Phone: 212-639-2939; Fax: 212-794-4357; E-mail: krism@mskcc.org

doi: 10.1158/1535-7163.MCT-11-0692

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

The characteristics of *EGFR*-mutant lung cancer and *ALK*-rearranged lung cancer have been well described (4, 8). Recent reports have summarized the characteristics of *BRAF*- (9) and *HER2*-mutant populations (10). However, the patients with tumors harboring mutations in *PIK3CA* have not been characterized.

PIK3CA encodes the p110α subunit of phosphoinositide 3-kinase (PI3K), an integral signaling molecule in the pathways driven by growth factor receptors such as HER/ERBB2. Activated PI3K phosphorylates AKT and leads to downstream activation of mTOR that is essential for cell survival and proliferation. Because activating mutations and overexpression of PI3K are known to be oncogenic, the PI3K pathway has been intensively studied (11).

Multiple mutations have been identified in *PIK3CA*, the oncogene that encodes the p110α subunit of PI3K (12). The mutations that occur with regularity and in highly conserved regions of the gene lead to amino acid substitutions in the helical binding domain encoded by exon 9 (E542K and E545K) and in the catalytic subunit of p110α encoded by exon 20 (H1047R or L). Mutations in the helical binding domain interfere with p85 binding and allow activation of PI3K. The mutations in the catalytic subunit are thought to increase kinase activity (13). These specific mutations have been shown to be sufficient for tumorigenesis both *in vivo* and *in vitro* (14, 15).

The rate of *PIK3CA* mutations reported in non-small cell lung cancer (NSCLC) is estimated from 1% to 4% (16–19). In the United States alone, this represents 9,000 patients per year who may benefit from a therapy targeting the PI3K pathway. Interestingly, unlike other oncogenic driver mutations in lung adenocarcinoma that are rarely found in squamous cell carcinoma, *PIK3CA* has been reported to be amplified and mutated in squamous cell carcinoma as well as adenocarcinoma of the lung (20). Preliminary data in the genotyping of squamous cell carcinoma carried out at our institution confirms the occurrence of *PIK3CA* mutations in 2% of squamous lung cancer (21).

As part of the ongoing Memorial Sloan-Kettering Lung Cancer Mutation Analysis Project, we have routinely tested for *PIK3CA* mutations in patients with adenocarcinoma of the lung since 2009 (22). Given the prevalence of *PIK3CA* mutations in other diseases, multiple drugs targeting PI3K and AKT/m-TOR are in development, including trials targeting *PIK3CA* mutations in lung cancers, we evaluated their clinical and molecular characteristics to learn more about this patient population.

Materials and Methods

Between January 2009 and June 2010, all patients evaluated by the thoracic medical oncology and surgery services were offered participation in an institutional tissue analysis program entitled the Lung Cancer Molecular Analysis Project. In patients with sufficient tissue, assessment for driver mutations was carried out in 9 genes: *EGFR*, *KRAS*, *BRAF*, *HER2*, *PIK3CA*, v-akt murine thymoma vial oncogene homolog 1 (*AKT1*), v-ras neuroblastoma viral oncogene homolog (*NRAS*), dual specificity mitogen-activated protein kinase kinase 1 (*MEK1*), and *ALK*. *EGFR* exon 19 deletions were identified through a PCR-based assay (23). *EGFR* exon 20 and 21 mutations, as well as activating mutations in *KRAS, BRAF, HER2, PIK3CA, AKT1, NRAS,* and *MEK1*, were assessed by a mass spectrometry–based nucleic acid assay on the Sequenom platform. The platform was designed to include mutations in these genes that have been reported as activating. All detected mutations were confirmed by direct sequencing. These methods have been previously described (24). Rearrangements involving *ALK* were determined by the *ALK* breakpoint FISH assay (Vysis LSI ALK Dual Color).

Clinical characteristics were obtained from the medical record. Smoking definitions are as follows: never (<100 cigarettes lifetime), current (active smoker within the past year), former (>100 cigarettes lifetime and no tobacco use within the last year). Pathologic stage was determined at the time of surgery according to the American joint commission on cancer (AJCC), 7th edition tumor-node-metastasis staging system (25). Binary correlative variables were evaluated with the Fisher exact test; continuous variables were evaluated with the Wilcoxon signed-rank test. Overall survival was calculated among patients diagnosed with stage IIIB/IV lung adenocarcinoma using the Kaplan–Meier method. Disease free survival is calculated from the date of surgery. Patients were followed from the date of diagnosis of stage IIIB/IV disease until death or the last available follow-up. Group comparison was done with the log-rank test. All research was conducted under appropriate Institutional Review Board/Privacy Board protocols and waivers.

Results

Twenty-three of 1,125 (2%, 95% CI: 1–3) patients had a mutation in *PIK3CA* (10 E545K, 2 E542K, 3 H1047L, and 8 H1047R; Fig. 1). There were no mutations identified in R88, N345, C420, and M1043. The clinical characteristics of the *PIK3CA*-mutant patients are presented in Table 1. Sixteen of 23 (70%, 95% CI: 49–86) had coexisting mutations in other oncogenes—10 *KRAS*, 1 *MEK1*, 1 *BRAF*, 1 *ALK* rearrangement, and 3 *EGFR* exon 19 deletions (Fig. 2). This is 1% (95% CI: <1–4) of the 260 *EGFR*-mutant cases, 3% (95% CI: <1–16) of the 34 *ALK*-rearranged cases, and 3% (95% CI: 1–5) of the 355 *KRAS*-mutant cases.

Patients had a median follow-up of 13 months (range 3–60 months). Of the 9 patients with early-stage disease, 5 received neoadjuvant chemotherapy and all underwent a complete resection. Four of the 5 patients treated with neoadjuvant chemotherapy received a cisplatin or carboplatin-based doublet. The other patient had the comutation *BRAF V600E* and had a marked treatment response to neoadjuvant gefitinib on a clinical trial (26).

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

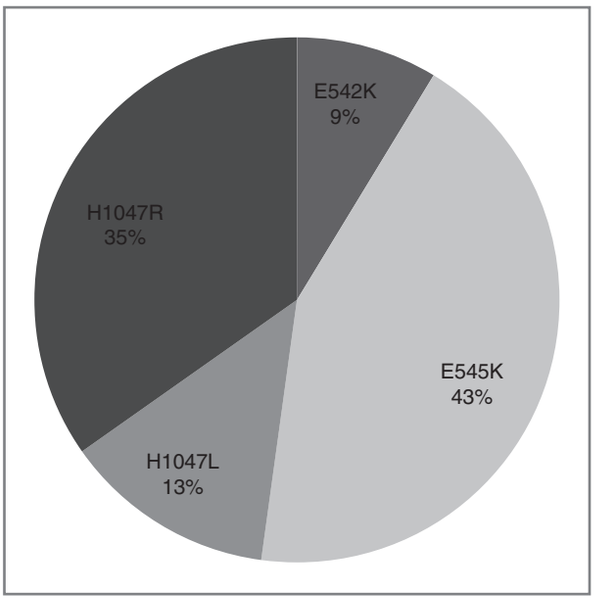

Figure 1. *PIK3CA* mutations in lung adenocarcinoma.

Table 1. Clinical characteristics

Characteristic ($N = 23$)	*PIK3CA* positive ($N = 23$)	*PIK3CA* negative ($N = 1,102$)	*P*-value
Age, median (range)	66 (34–78)	66 (24–96)	0.99
Sex, female (%)	13 (57%)	602 (55%)	0.83
Smoking history			
Never	8 (35%)	280 (25%)	0.34
Ever-smoker			
Former	10 (43%)	668 (61%)	
Current	5 (22%)	154 (14%)	
Stage			
Early (IA–IIIA)	9 (39%)	644 (58%)	0.2
Advanced (IIIB/IV)	14 (61%)	458 (42%)	

Five patients have had disease recurrence and 3 have died of disease. The mutation data, treatment course, pathologic stage, and survival of the patients are presented in Table 2.

The median survival of the 14 patients with stage IIIB and IV disease was 21 months. In this small group of patients, there was a shorter median survival in patients with a coexisting mutation (*EGFR*, *KRAS*, *BRAF*, and *ALK*) versus those with mutations in *PIK3CA* alone, median 13 versus 27 months ($P = 0.03$). Three patients had *EGFR* exon 19 deletions. One patient treated with first-line erlotinib had a prolonged radiographic partial response and then developed T790M-mediated resistance after 15 months on erlotinib. The other patient treated in the first-line had a partial radiographic response of 5 months duration and did not undergo a repeat biopsy at the time of progression. The third *EGFR*-mutant patient did not respond to second-line erlotinib; he had no evidence of T790M. The rebiopsy samples were not tested for persistence or loss of the *PIK3CA* mutation. The patient with an *ALK* rearrangement was initially treated with erlotinib with upfront progression and subsequently progressed again on docetaxel before being treated with crizotinib with stable disease as the best response. The remainder of patients were treated with standard first-line treatments (Table 2).

There was no difference in the stage or the frequency of coexisting mutations between patients with mutations in the *PIK3CA* kinase versus helical domain. Mutations in the kinase domain of *PIK3CA* occurred with higher frequency in patients who were never smokers ($P = 0.009$; Table 3).

Discussion

Consistent with the published literature, we have confirmed that *PIK3CA* mutations occur in approximately

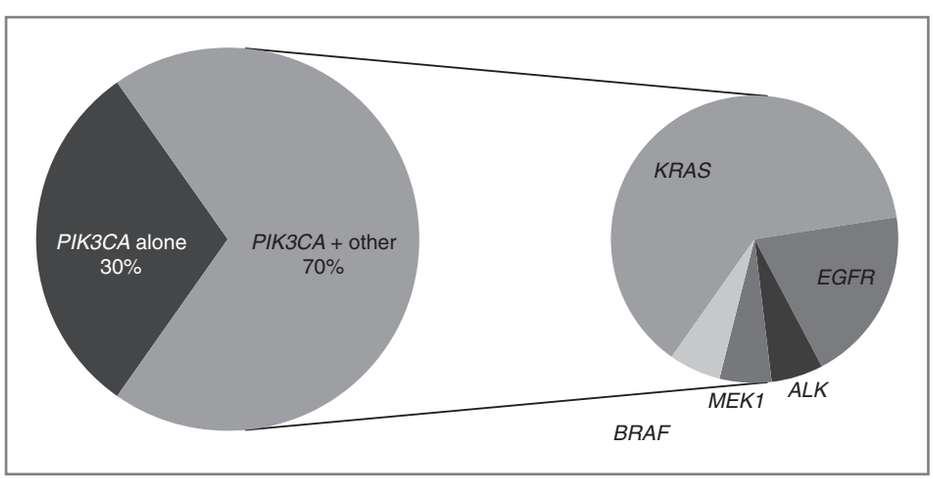

Figure 2. Coexisting mutations in patients with *PIK3CA*-mutant lung adenocarcinoma.

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

Table 2. Molecular and clinical characteristics

PIK3CA	Other	Age	Sex	Tobacco use	Pack years	Stage	Treatment neoadjuvant or first-line	Survival
E542K	*KRAS*	61	F	Current	30	IIB	Cisplatin & Doce	17+
E542K	*BRAF*	78	F	Former	30	IA	Gefitinib	15
E545K	*KRAS*	77	F	Former	20	IB	Carbo & Doce	3
E545K	—	70	F	Never	0	IIA	—	5+
E545K	*KRAS*	65	F	Current	45	IIIA	—	7
E545K	—	74	F	Former	40	IIIB	Carbo & Pacli & RT	27
E545K	—	50	M	Current	70	IV	Carbo & Pacli	21
E545K	*EGFR*	57	M	Current	50	IV	Carbo & Peme	11
E545K	*KRAS*	74	F	Former	50	IIIB	RT	10
E545K	*KRAS*	65	F	Current	40	IV	Carbo & Peme	6
E545K	*KRAS*	71	M	Former	55	IV	Cisplatin & Doce & B	16
E545K	*KRAS*	64	F	Former	15	IV	Unknown	13
H1047L	*KRAS*	66	M	Former	30	IA	—	3+
H1047L	—	34	F	Former	10	IV	Cisplatin & Peme	31
H1047L	*EGFR*	61	M	Never	0	IV	Erlotinib	21[a]
H1047R	—	71	M	Former	45	IA	Carbo & Pacli & RT	1+
H1047R	—	68	F	Never	0	IA	—	2+
H1047R	*KRAS*	38	M	Never	0	IIA	Cisplatin & Doce & B	4
H1047R	*EGFR*	57	F	Never	0	IV	Erlotinib	12[a]
H1047R	*MEK1*	69	M	Former	100	IV	Peme & Pacli & B	6[a]
H1047R	*KRAS*	76	M	Never	0	IV	Carbo & Peme	9
H1047R	—	64	F	Never	0	IV	Peme & Pacli & B	23[a]
H1047R	*ALK*	73	F	Never	0	IV	Erlotinib	23

NOTE: Survival, disease free in early-stage patients (I–IIIA) and overall in advanced stage patients (IIIB/IV).

Abbreviations: Pacli, paclitaxel; Carbo, carboplatin; B, bevacizumab; Peme, pemetrexed; Doce, docetaxel; RT, radiotherapy; + disease free.

[a]Alive with disease.

2% of patients with lung adenocarcinoma. Although single cases of adenocarcinoma harboring *PIK3CA* mutations and comutations have been previously reported (27–30), we found that the majority of tumors with *PIK3CA* mutations had another driver mutation as well (Fig. 2). This is in contrast to the mutual exclusivity of driver oncogene mutations seen in adenocarcinoma of the lung harboring *EGFR, KRAS,* and *ALK,* raising the possibility of tumor heterogeneity, though the high frequency of coexiting mutations, lack of 2 evident primary tumors microscopically, and the data regarding *PIK3CA* mutation in other diseases (31–33) make the explanation of tumor heterogeneity unlikely. Although 2 cases of *PIK3CA* mutation acquisition have been reported after the development of acquired resistance to erlotinib in *EGFR*-mutant lung cancer (34), in this series the mutations were present before treatment with targeted therapies. Additional data are required to characterize the effect of concurrent *PIK3CA* mutations on responses to erlotinib and crizotinib in patients harboring *EGFR* mutations and *ALK* rearrangements.

Mutation detection in this study makes use of a mass spectrometry–based system (Sequenom). This assay was designed to detect known "hotspot" mutations in specific oncogenes based on the published literature and available databases. Some mutations are confirmed by direct Sanger sequencing but full sequencing is not carried out on all specimens. Although this directed approach may miss unknown point mutations that would be detected by direct sequencing, it is optimal for detecting recurrent oncogene mutations that are known to be activating and minimizes the identification of new mutations of unclear significance. Our panel detects more than 90% of the *PI3KCA* mutations reported in all histologies of lung cancer in the COSMIC database.

Beyond lung cancer, *PIK3CA* mutations have been identified in breast, ovarian, endometrial, and colorectal carcinomas. Although the frequency of these mutations has been defined (Fig. 3), the influence of *PIK3CA* mutations on pathogenesis, prognosis, and response to therapy is not uniform across disease types or between studies. An example of this is the effect of *PIK3CA*

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

Table 3. Comparison of *PIK3CA* helical (exon 9) and kinase (exon 20) domain mutations

Characteristic	Helical N (%)	Kinase N (%)	*P*-value
Stage			
Early (IA–IIIA)	5 (22)	4 (18)	1.0
Advanced (IIIB/IV)	7 (30)	7 (30)	
Comutation			
Yes	9 (39)	7 (30)	0.67
No	3 (13)	4 (18)	
Smoking			
Never	1 (4)	7 (30)	0.009
Former/current	11 (48)	4 (18)	

mutation status on the efficacy of cetuximab in metastatic colorectal cancer. Although Prennen and colleagues found no effect of *PIK3CA* mutation on response to cetuximab with or without irinotecan (35), DeRoock reported an inferior response rate to cetuximab plus chemotherapy in patients harboring mutations in *PIK3CA* exon 20 but not the more commonly mutated exon 9 (32). In breast cancer, response to trastuzumab in *PIK3CA*-mutant $HER2^+$ breast cancer cell lines is inferior to *PIK3CA* wild-type $HER2^+$ cell lines only with coincident PTEN loss (36) but not with intact PTEN (37) The coexistence of *PIK3CA* mutations with mutations in *KRAS, NRAS,* and *BRAF* has been shown in colorectal adenocarcinoma (32) and with *KRAS* in pancreatic adenocarcinoma and ovarian carcinoma (31). Initial reports in endometrial carcinoma claimed that mutations in *PIK3CA* and *KRAS* were mutually exclusive (38), but more recent studies have found both *KRAS* mutations and *PTEN* loss in patients with *PIK3CA* mutant endometrial carcinoma (33). In the absence of comprehensive mutational profiling, this heterogeneity between diseases and mutational profiles, may explain the seemingly contradictory clinical outcomes described above.

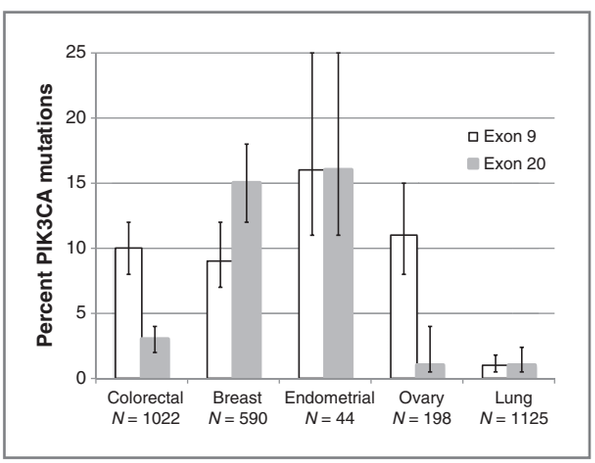

Figure 3. *PIK3CA* mutation incidence with 95% CIs in various malignancies colorectal (32), breast (39), endometrial (38), ovary (45), and lung adenocarcinoma.

Independent of treatment efficacy, mutations in distinct domains of *PIK3CA* may impart unique biologies. Studies in breast cancer have found clinical differences between patients with mutations in the helical and kinase domains with fewer lymph node metastases in individuals with mutations in the kinase domain (39) and inferior overall survival in those with mutations in the helical domain (40). These observations are further supported by findings in soft tissue sarcoma in which downstream activation of AKT is higher in tumors with helical domain mutations than those with kinase domain mutations (41). Our cohort of *PIK3CA*-mutant lung cancer patients has too many coexisting mutations to allow for comparison of outcomes between domain-specific mutation populations, although we can conclude that the never smokers were more likely to have mutations in the kinase domain. Interestingly, similar to the findings in *TP53*- and *KRAS*-mutant adenocarcinoma of the lung in which never smokers were more likely to harbor transition mutations (substitution purine for purine or pyrimidine for pyrimidine) and not transversion mutations (substitution pyrimidine for purine or purine for pyrimidine; refs. 42, 43), the *PIK3CA* kinase domain mutations, more commonly identified in never smokers, are transition and not transversion mutations.

Interestingly, in this small sample of patients with *PIK3CA*-mutant advanced disease, the presence of a coexisting oncogene mutation was correlated with an inferior outcome. Only one patient with a *PIK3CA* mutation received an experimental agent targeting the PI3K pathway, therefore, we cannot base the above average survival in this arm on effective targeted therapies. Although this sample size is too small to draw any conclusions, the survival findings are thought provoking.

Despite the uncertain effect of *PIK3CA* mutations on prognosis and response to standard therapies, it is an important target for drug development. We and others recommend the testing of agents specifically targeting PIK3CA only in patients with tumors that have evidence of dependency on PI3K pathway (*PIK3CA* mutation or *PTEN* loss). Cell lines with *PIK3CA* mutations are sensitive to downstream inhibitors such as everolimus, an inhibitor of mTOR, although this sensitivity can be abrogated by coincident mutation in *KRAS* (44). This is an expected yet important observation in light of the high frequency of coincident mutations found in this study. A recent report showed a 7-fold increase in response rate (35% vs. 5%) of PI3K pathway–targeted agents in patients with evidence of *PIK3CA* mutation in tumor specimens (31). These data call into question the use and appropriateness of testing PI3K pathway–targeted agents in patients whose tumors lack evidence of PI3K dependency and accentuates the importance of

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

comprehensive genotyping of tumor specimens in all patients under consideration for molecularly targeted therapies.

Our data indicate that the majority of patients with lung adenocarcinoma harboring mutations in *PIK3CA* have coexisting mutations in other oncogenes. A shortcoming of this study is that the high throughput system used did not allow for testing of *PTEN* or *TP53* loss; this is a step that we feel is essential moving forward and plan to incorporate into future studies to fully understand the effect of PI3K pathway alterations in lung adenocarcinoma. The timing of acquisition of *PIK3CA* mutation (and/or *PTEN* loss) in relation to that of other oncogenes and the contribution to tumor biology and response to therapy is unclear. As agents targeting various pathways including PI3K are in development, comprehensive (and perhaps sequential) mutation profiling should be carried out on tumor specimens from all patients to assess the impact of coincident mutations on the response to the targeted agents.

Disclosure of Potential Conflicts of Interest

G.J. Riely is a consultant or is on the advisory boards of Boehringer-Ingelheim, Chugai, Tragara, and Ariad.

Acknowledgments

The authors thank L. Borsu for assistance with Sequenom assays, D. Ang for assistance with Sequenom data review, and E. Brzostowski and M. Pilloff for Sequenom data management.

Grant Support

This work was also supported by grants P01 CA129243 and RC2 CA148394 to M.G. Kris and Conquer Cancer Foundation of ASCO Young Investigator Award to J.E. Chaft. The MSKCC Sequenom facility was supported by the Anbinder Fund.

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

Received September 6, 2011; revised October 18, 2011; accepted November 1, 2011; published OnlineFirst December 1, 2011.

References

1. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
2. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
3. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.
4. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247–53.
5. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561–6.
6. Camidge DR, Bang Y, Kwak EL, Shaw AT, Iafrate AJ, Maki RG, et al. Progression-free survival from a phase I study of crizotinib (PF-02341066) in patients with ALK-positive non-small cell lung cancer. J Clin Oncol 2011;29s:abstr 2501.
7. Crino L, Kim D, Riely GJ, Janne PA, Blackhall FH, Camidge DR, et al. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): PROFILE 1005. J Clin Oncol 2011;29s:abstr 7514.
8. Miller VA, Kris MG, Shah N, Patel J, Azzoli C, Gomez J, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 2004;22:1103–9.
9. Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, et al. Clinical characteristics of patients with lung adenocarcinomas harboring braf mutations. J Clin Oncol 2011;29:2046–51.
10. Arcila ME, Chaft JE, Nafa K, Kris MG, Zakowski MF, Ladanyi M. Molecular and clinicopathologic characteristics of HER2-mutant lung adenocarcinoma. J Clin Oncol 2011;29s:abstr 10596.
11. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62.
12. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
13. Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 2007;317:239–42.
14. Bader AG, Kang S, Vogt PK. Cancer-specific mutations in PIK3CA are oncogenic *in vivo*. Proc Natl Acad Sci U S A 2006;103:1475–9.
15. Samuels Y, Diaz LA Jr., Schmidt-Kittler O, Cummins JM, Delong L, Cheong I, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 2005;7:561–73.
16. Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. Cell Cycle 2004;3:1221–4.
17. Okudela K, Suzuki M, Kageyama S, Bunai T, Nagura K, Igarashi H, et al. PIK3CA mutation and amplification in human lung cancer. Pathol Int 2007;57:664–71.
18. Bianconi F, Pistola L, Chiari R, Minotti V, Colella R, Giuffrida D, et al. Phosphoinositide-3-kinase catalytic alpha and kras mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol 2011;6:707–15.
19. Lee SY, Kim MJ, Jin G, Yoo SS, Park JY, Choi JE, et al. Somatic mutations in epidermal growth factor receptor signaling pathway genes in non-small cell lung cancers. J Thorac Oncol 2010;5:1734–40.
20. Kawano O, Sasaki H, Okuda K, Yukiue H, Yokoyama T, Yano M, et al. PIK3CA gene amplification in Japanese non-small cell lung cancer. Lung Cancer 2007;58:159–60.
21. Rekhtman N, Paik PK, Tafe L, Riely GJ, Miller VA, Kris MG, et al. Screening for EGFR, KRAS, and PIK3CA mutations in well characterized, immunohistochemically confirmed squamous cell carcinoma of the lung. J Clin Oncol 2011;29s:abstr e21143.
22. Kris MG, Lau CY, Ang D, Brzostowski E, Riely GJ, Rusch VW, et al. Initial results of LC-MAP: an institutional program to routinely profile tumor specimens for the presence of mutations in targetable pathways in all patients with lung adenocarcinoma. J Clin Oncol 2010;28s:abstr 7009.
23. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. J Mol Diagn 2005;7:396–403.
24. Arcila M, Lau C, Nafa K, Ladanyi M. Detection of KRAS and BRAF mutations in colorectal carcinoma roles for high-sensitivity locked nucleic acid-PCR sequencing and broad-spectrum mass spectrometry genotyping. J Mol Diagn 2011;13:64–73.

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

25. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706–14.
26. Rizvi NA, Rusch V, Pao W, Chaft JE, Ladanyi M, Miller VA, et al. Molecular characteristics predict clinical outcomes: prospective trial correlating response to the egfr tyrosine kinase inhibitor gefitinib with the presence of sensitizing mutations in the tyrosine binding domain of the egfr gene. Clin Cancer Res 2011;17:3500–6.
27. Sequist LV, Heist RS, Shaw AT, Fidias P, Temel JS, Lennes IT, et al. SNaPshot genotyping of non-small cell lung cancers in clinical practice. J Clin Oncol 2011;29s:abstr 7518.
28. Endoh H, Yatabe Y, Kosaka T, Kuwano H, Mitsudomi T. PTEN and PIK3CA expression is associated with prolonged survival after gefitinib treatment in EGFR-mutated lung cancer patients. J Thorac Oncol 2006;1:629–34.
29. Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, et al. PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 2008;68:6913–21.
30. Ludovini V, Bianconi F, Pistola L, Chiari R, Minotti V, Colella R, et al. Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol 2011;6:707–15.
31. Janku F, Tsimberidou AM, Garrido-Laguna I, Wang X, Luthra R, Hong DS, et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitor. Mol Cancer Ther 2011;10:558–65.
32. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 2010;11:753–62.
33. Rudd ML, Price JC, Fogoros S, Godwin AK, Sgroi DC, Merino M, et al. A unique spectrum of somatic PIK3CA (p110a) mutations within primary endometrial carcinomas. Clin Cancer Res 2011;17:1331–40.
34. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
35. Prenen H, De Schutter J, Jacobs B, De Roock W, Biesmans B, Claes B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 2009;15:3184–8.
36. Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. Ann Oncol 2010;21:255–62.
37. Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, et al. PTEN, PIK3CA, p-AKT, and p-p70S6K status: association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. Am J Pathol 2010;177:1647–56.
38. Kang S, Seo SS, Chang HJ, Yoo CW, Park SY, Dong SM. Mutual exclusiveness between PIK3CA and KRAS mutations in endometrial carcinoma. Int J Gynecol Cancer 2008;18:1339–43.
39. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, et al. PIK3CA mutation associates with improved outcome in breast cancer. Clin Cancer Res 2009;15:5049–59.
40. Barbareschi M, Buttitta F, Felicioni L, Cotrupi S, Barassi F, Del Grammastro M, et al. Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. Clin Cancer Res 2007;13:6064–9.
41. Barretina J, Taylor BS, Banerji S, Ramos AH, Lagos-Quintana M, Decarolis PL, et al. Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. Nat Genet 2010;42:715–21.
42. Le Calvez F, Mukeria A, Hunt JD, Kelm O, Hung RJ, Taniere P, et al. TP53 and KRAS mutation load and types in lung cancers in relation to tobacco smoke: distinct patterns in never, former, and current smokers. Cancer Res 2005;65:5076–83.
43. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 2008;14:5731–4.
44. Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, Macarulla T, et al. Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest 2010;120:2858–66.
45. Levine DA, Bogomolniy F, Yee CJ, Lash A, Barakat RR, Borgen PI, et al. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. Clin Cancer Res 2005;11:2875–8.

Published OnlineFirst December 1, 2011; DOI: 10.1158/1535-7163.MCT-11-0692

Molecular Cancer Therapeutics

Coexistence of *PIK3CA* and Other Oncogene Mutations in Lung Adenocarcinoma–Rationale for Comprehensive Mutation Profiling

Jamie E. Chaft, Maria E. Arcila, Paul K. Paik, et al.

Mol Cancer Ther 2012;11:485-491. Published OnlineFirst December 1, 2011.