Published in final edited form as:
Mol Cancer Ther. 2012 February ; 11(2): 485–491. doi:10.1158/1535-7163.MCT-11-0692.

Coexistence of *PIK3CA* and other oncogene mutations in lung adenocarcinoma – rationale for comprehensive mutation profiling

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Abstract

PIK3CA encodes the p110α subunit of the mitogenic signaling protein phosphatidylinositol 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 *EGFR*, *KRAS*, *BRAF*, *HER2*, *PIK3CA, AKT1, NRAS, MEK1*, and *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 1125 (2%, 95% confidence interval (CI) 1–3%) patients had a mutation in *PIK3CA*, 12 in Exon 9 (10 E545K, 2 E542K) and 11 in Exon 20 (3 H1047L, 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 performed on tumor specimens from patients enrolling on clinical trials of PI3K and other targeted therapies.

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Disclosures: None

Keywords

lung adenocarcinoma; oncogene; PIK3CA

Introduction

The identification and targeting of specific oncogenic driver-mutations has revolutionized the treatment of lung adenocarcinoma. While mutations in *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 *BRAF, PIK3CA*, and *HER2*. In addition, the discovery of the *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 US 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.

The characteristics of *EGFR*-mutant 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 phosphatidylinositol 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 (mammalian target of rapamycin) which is essential for cell survival and proliferation. Since activating mutations and over-expression 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, 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 NSCLC is estimated from 1–4%(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 which 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 performed 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.

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 performed in 9 genes: *EGFR*, *KRAS*, *BRAF*, *HER2*, *PIK3CA, AKT1, NRAS, 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 using a Mass spectrometry-based nucleic acid assay using 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 fluorescence *in situ* hybridization 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). Pathological stage was determined at the time of surgery according to the AJCC, 7th edition TNM 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 (OS) 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 performed with the log-rank test. All research was performed under appropriate institutional review board/privacy board protocols and waivers.

Results

Twenty-three of 1125 (2%, 95% CI 1–3%) patients had a mutation in *PIK3CA* (10 E545K, 2 E542K, 3 H1047L, 8 H1047R) (Figure 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 (Figure 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 co-mutation *BRAF V600E* and had a marked treatment response to neoadjuvant gefitinib on a clinical trial(26). Five patients have had disease recurrence and 3 have died of disease. The mutation data, treatment course, pathological 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*, *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 1st-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 1st-line had a partial radiographic response of 5 months duration and did not undergo a repeat biopsy at the time of progression. The 3rd *EGFR*-mutant patient did not respond to 2nd line erlotinib; he had no evidence of T790M. The re-biopsy 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 ~2% of patients with lung adenocarcinoma. While single cases of adenocarcinoma harboring *PIK3CA* mutations and co-mutations have been previously reported(27–30), we found that the majority of tumors with *PIK3CA* mutations had another driver mutation as well (Figure 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 co-exiting mutations, lack of two evident primary tumors microscopically and the data regarding *PIK3CA* mutation in other diseases(31–33) make the explanation of tumor heterogeneity unlikely. While 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 performed on all specimens. While 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 >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. While the frequency of these mutations has been defined (Figure 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* mutation status on the efficacy of cetuximab in metastatic colorectal cancer. While Prennen, *et al* 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 co-existence of *PIK3CA*-mutations with mutations in *KRAS*, *NRAS* and *BRAF* has been demonstrated 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.

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, where 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*-mutant and *KRAS*-mutant adenocarcinoma of the lung, where 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) (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 co-existing 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. While 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 demonstrated 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 utility and appropriateness of testing PI3K pathway targeted agents in patients whose tumors lack evidence of PI3K dependency and accentuates the importance of 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.

Acknowledgments

We thank L. Borsu for assistance with Sequenom assays. We thank D. Ang for assistance with Sequenom data review and E. Brzostowski and M. Pilloff for Sequenom data management. The MSKCC Sequenom facility was supported by the Anbinder Fund. The mutation data was obtained from the Sanger Institute Catalogue Of Somatic Mutations In Cancer web site, http://www.sanger.ac.uk/cosmic Bamford et al (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. Br J Cancer, 91,355–358.

Financial Support:

Kris - P01 CA129243, RC2 CA148394

Chaft - Conquer Cancer Foundation of ASCO Young Investigator Award

Abbreviations list

PIK3CA	phosphoinositide-3-kinase catalytic alpha polypeptide
PI3K	phosphatidylinositol 3-kinase
EGFR	epidermal growth factor receptor
KRAS	Kirsten rate sarcoma viral oncogene homolog
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
AKT1	v-akt murine thymoma vial oncogene homolog 1
NRAS	v-ras neuroblastoma viral oncogene homolog
MEK1	dual specificity mitogen-activated protein kinase kinase 1
ALK	anaplastic lymphoma kinase
CI	confidence interval
NSCLC	non-small cell lung cancer
mTOR	mammalian target of rapamycin
AJCC	American joint commission on cancer
PCR	polymerase chain reaction

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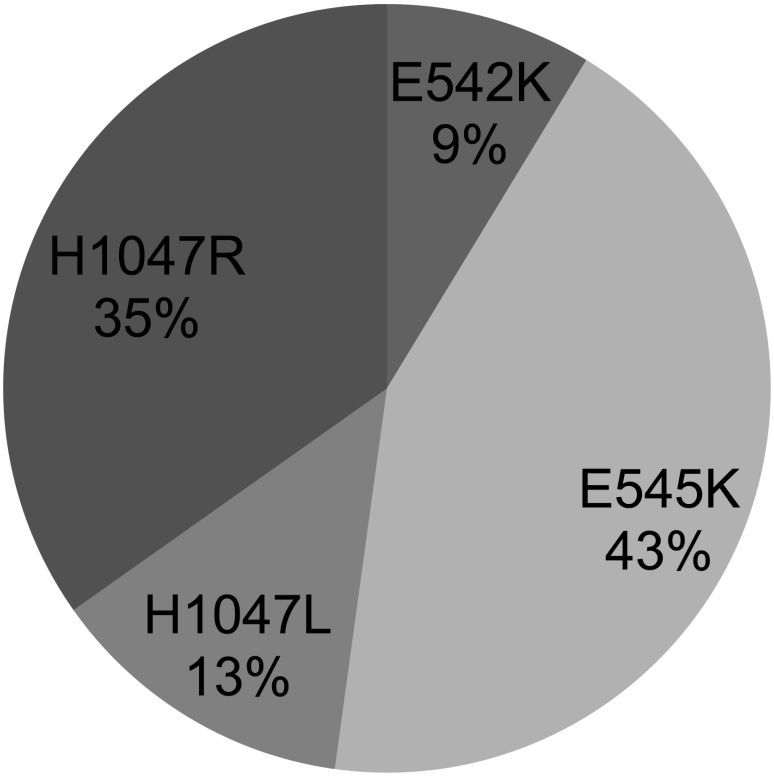

Figure 1.
PIK3CA mutations in lung adenocarcinoma

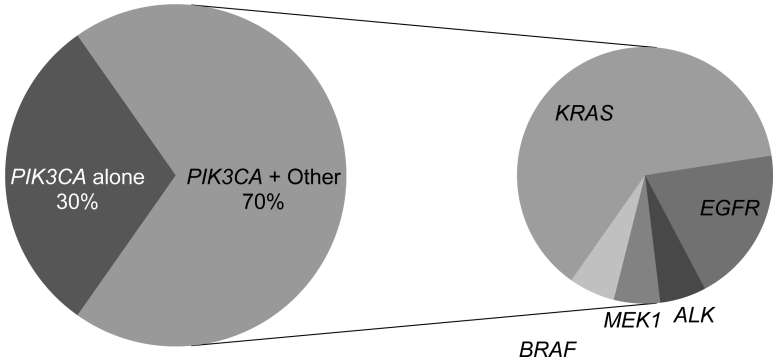

Figure 2.
Coexisting mutations in patients with *PIK3CA*-mutant lung adenocarcinoma

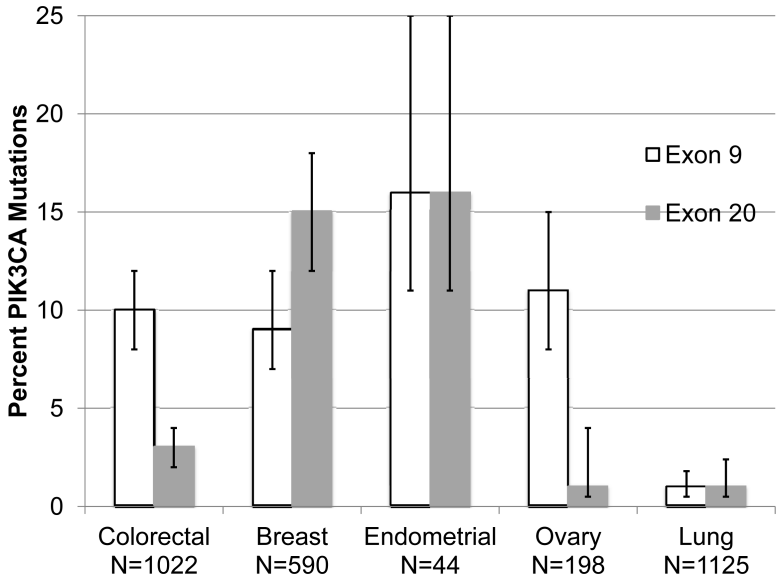

Figure 3.

PIK3CA Mutation Incidence with 95% Confidence intervals in Various Malignancies Colorectal(32), Breast(39), Endometrial(38), Ovary(45), and Lung Adenocarcinoma

Table 1

Clinical Characteristics

Characteristic (N=23)	***PIK3CA*-positive N=23**	***PIK3CA*-negative N=1102**	**p-value**
Age – Median (Range)	66 (34–78)	66 (24–96)	0.99
Sex – Female (%)	13 (57%)	602	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%)	

Table 2

Molecular and Clinical Characteristics

PIK3CA	Other	Age	Sex	Tobacco Use	Pack Yrs	Stage	Treatment Neoadjuvant or 1st line	Surv
E542K	*KRAS*	61	F	Current	30	IIB	Cisplatin + Docetaxel	17+
E542K	*BRAF*	78	F	Former	30	IA	Gefitinib	15
E545K	*KRAS*	77	F	Former	20	IB	Carbo + Docetaxel	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*
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*
H1047R	*MEK1*	69	M	Former	100	IV	Peme + Pacli + B	6*
H1047R	*KRAS*	76	M	Never	0	IV	Carbo + Peme	9
H1047R	-	64	F	Never	0	IV	Peme + Pacli + B	23*
H1047R	*ALK*	73	F	Never	0	IV	Erlotinib	23

Abbreviations: Surv (survival – disease free in early stage patients (I–IIIA) and overall in advanced stage patients (IIIB/IV)), Pacli (paclitaxel), Carbo (carboplatin), B (bevacizumab), Peme (pemetrexed), Doce (docetaxel) RT (radiotherapy), + (disease free)

* alive with disease

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)	
Co-mutation			
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)	