Mucinous Differentiation Correlates with Absence of *EGFR* Mutation and Presence of *KRAS* Mutation in Lung Adenocarcinomas with Bronchioloalveolar Features

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Somatic mutations in the epidermal growth factor receptor gene (*EGFR*) are detected in a subset of lung adenocarcinomas, particularly bronchioloalveolar carcinoma (BAC) and adenocarcinoma with bronchioloalveolar features (AWBF), and correlate with clinical response to tyrosine kinase inhibitors (TKIs). In contrast, lung adenocarcinomas refractory to TKIs often have activating mutations in *KRAS* but lack *EGFR* mutations. Some adenocarcinomas have mucinous histology, but the clinical and molecular significance of the mucinous pattern is less well studied. We analyzed 43 BAC and AWBF tumors submitted for *EGFR* mutation testing to identify histopathological features that predicted *EGFR* or *KRAS* mutations. *EGFR* mutations were detected in 14 of 30 (47%) non-mucinous tumors, whereas 0 of 13 mucinous tumors harbored an *EGFR* mutation ($P = 0.003$). Missense mutations in *KRAS* codon 12 were detected in six of seven (86%) mucinous adenocarcinomas but only 3 of 18 (17%) nonmucinous adenocarcinomas ($P = 0.003$). Thus, in BAC/AWBF mucinous differentiation was significantly correlated with the absence of *EGFR* mutation and presence of *KRAS* mutation, suggesting that mucinous BACs/AWBFs are unlikely to respond to TKIs. Therefore, our data suggest that *EGFR* sequence analysis could be avoided in BAC/AWBF when true mucinous morphology is identified, avoiding the associated testing costs. (*J Mol Diagn* 2007; 9:320–326; DOI: 10.2353/jmoldx.2007.060182)

The epidermal growth factor receptor (EGFR), a receptor protein tyrosine kinase (TK) of the ErbB family, is frequently expressed in solid tumors and promotes tumor growth and survival.¹ Non-small cell lung cancer (NSCLC), the most common category of lung cancer, frequently expresses EGFR.² Recently, somatic mutations in the TK domain of *EGFR* have been detected in a subset of NSCLC tumors^{3–5} that correlate with clinical response and survival after treatment with tyrosine kinase inhibitors (TKIs).^{3–8} Such mutations are more common in females, never-smokers, patients of East Asian origin, and adenocarcinomas versus other NSCLC histological categories.^{3,4,9–11} Some studies have suggested that *EGFR* mutations are more common in NSCLC displaying features of bronchioloalveolar carcinoma (BAC),^{3,5,10,12,13} a subtype of adenocarcinoma in which tumor cells spread out along the alveolar septa (lepidic growth pattern).¹⁴

In addition to *EGFR* mutations, *EGFR* gene amplification has been detected in NSCLC tumors.¹⁵ A composite endpoint of *EGFR* high polysomy plus *EGFR* gene locus amplification has been associated with improved survival after TKI treatment in some studies^{15–17} but not in others.¹⁸ Furthermore, in tumors with high-level *EGFR* amplification (more than or equal to six copies), the allele harboring an *EGFR* mutation is preferentially amplified,¹⁹ suggesting a strong dependency on EGFR signaling in these tumors.

Aberrant function of other components of the ErbB signaling pathway has also been described in lung cancer. These components include BRAF,²⁰ PIK3CA,²¹ and KRAS, a downstream GTPase.²² Approximately 30% of lung adenocarcinomas carry mutations in *KRAS*, which are most commonly activating missense mutations in codon 12²² and are strongly associated with a history of

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cigarette smoking.²³ Several studies suggest that in lung adenocarcinomas, *EGFR* and *KRAS* mutations are mutually exclusive,^{11,24–26} possibly reflecting different mechanisms of carcinogenesis. Indeed, NSCLC tumors that are refractory to TKIs often harbor mutations in *KRAS*,²⁴ suggesting that *KRAS* activation might confer TKI-resistance by activating signaling pathways downstream of *EGFR*. Thus, in NSCLC tumors lacking *EGFR* mutation, knowledge of *KRAS* mutation status may be of value in guiding appropriate pharmacotherapy.

Although clinical screening for *EGFR* mutations in NSCLC is now available, consensus guidelines regarding the application of molecular profiling in NSCLC are lacking. To determine whether any specific histopathological features of NSCLC might be useful in guiding rational molecular profiling, we reviewed a large series of tumor specimens submitted to our hospital for *EGFR* mutation screening. In particular, we examined tumors with bronchioloalveolar features for predictors of *EGFR* mutation status, *KRAS* mutation status, and *EGFR* gene copy number, molecular features that correlate with either clinical responsiveness or refractoriness to TKI treatment.

Materials and Methods

Pathological and Clinical Review

Clinical *EGFR* mutation screening became available at Massachusetts General Hospital (MGH) in August 2004. Surgical pathology reports were reviewed from all tumor specimens submitted to MGH for *EGFR* screening from August 2004 to October 2005. Specimens included tumor biopsies, resections, and fine needle aspirations obtained from procedures performed at MGH as well as consult specimens submitted from outside institutions. Tumors were classified according to the World Health Organization criteria²⁷ into acinar, papillary, BAC, or mixed subtypes. For the purpose of analysis, all mixed adenocarcinomas with bronchioloalveolar features were grouped as adenocarcinoma with bronchioloalveolar features (AWBF). This defined the BAC/AWBF group of tumors, which was the cohort for the clinical analysis and for the central surgical pathology slide review. Histological examination of tissue from all available BAC/AWBF cases was performed by two of the authors (E.J.M., A.J.I.), and all tumors within the BAC/AWBF group were assessed for the presence of acinar and papillary features, according to World Health Organization criteria. Within the BAC/AWBF cohort, tumors were further assessed for the presence of mucinous features, a morphology in which the individual tumor cells are tall and well differentiated, have basally located nuclei, and produce mucin.^{14,28}

Patient demographics, cancer history, and smoking history were documented for all MGH patients using structured physician chart review. Staging was performed using American Joint Committee on Cancer staging criteria.²⁹ Patients were categorized using standard criteria including former smokers defined as patients who had quit smoking at least 1 year before their diagnosis of

lung cancer and never-smokers defined as patients who had smoked less than 100 cigarettes in their lifetime.³⁰ Pack-years of smoking were calculated by multiplying the number of packs smoked per day by the number of years of smoking.

EGFR Mutation Analysis

For each formalin-fixed, paraffin-embedded specimen, a 5- μ m tissue section was stained with hematoxylin and eosin and examined by light microscopy to select representative areas of tumor involvement appropriate for microdissection. Some tissues were sectioned and frozen, and representative areas of tumor were isolated in a similar manner. DNA isolation and mutation analysis of *EGFR* exons 18 to 24 were performed as previously described.³¹

KRAS Mutation Analysis

For BAC/AWBF cases, available tumor tissue was manually microdissected from serial, 5- μ m unstained sections of formalin-fixed, paraffin-embedded tissue (three slides; ~ 1 cm² tissue per slide). The tissue was deparaffinized in xylene and rehydrated in 100% ethanol. DNA was extracted using the Puregene DNA purification kit (Gentra Systems, Minneapolis, MN). Polymerase chain reaction (PCR) was conducted in 20- μ l volumes using 1 $\times$ Platinum *Taq* PCR buffer, 200 μ mol/L dNTPs, 3.0 mmol/L MgCl₂, 0.4 μ mol/L primers, and 1.0 U of Platinum *Taq* polymerase (Invitrogen, Carlsbad, CA) with 40 ng of tumor DNA as template. The following primers were used for PCR amplification of *KRAS* exon 2: forward, 5'-GGTGGAGTATTTGATAGTGATTAACC-3'; reverse, 5'-AGAATGGTCCTGCACCAGTAA-3'. Thermal cycling was performed in a Mastercycler ep (Eppendorf, Hamburg, Germany) with an initial denaturing step at 94°C for 2 minutes; followed by 38 cycles of denaturing at 94°C for 30 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 30 seconds; and a final elongation step at 72°C for 10 minutes. PCR products were purified using DNA Clean and Concentrator (Zymo Research, Orange, CA) and subjected to bidirectional dye-terminator sequencing using the same primers used for amplification. Sequencing fragments were detected by capillary electrophoresis using the ABI Prism 3130XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequence chromatograms were analyzed by ABI Seqscape software.

Fluorescence in Situ Hybridization (FISH)

For BAC/AWBF tumors with mucinous morphology, available processed 5- μ m paraffin tumor sections were hybridized with bacterial artificial chromosomes CTD-2113A18 (chromosome 7p *EGFR* locus) and RP5-1129E22 (chromosome 7q reference probe). The bacterial artificial chromosomes were labeled by nick-translation with SpectrumOrange or SpectrumGreen dUTP (Vysis, Inc., Downers Grove, IL), respectively, and the

Figure 1. Study organization including summary of histological features of 119 primary lung cancers submitted for *EGFR* mutation status evaluation and summary of morphological and molecular features of the BAC/AWBF subset of tumors. N.O.S., not otherwise specified.

nuclei were counterstained with 4,6-diamidino-2-phenylindole. Fluorescence microscopy was performed with an Olympus BX-61 microscope (Olympus, Center Valley, PA). Signal quantitation of 100 cells was used to generate a 7p/7q ratio. A ratio of 7p:7q of >2.0 was considered *EGFR* locus amplification. The distribution of *EGFR* copy number in the cellular population was used to assess for polysomy, using criteria described previously.¹⁵

Statistical Analysis

Associations between *EGFR* and *KRAS* mutation status and histological features were evaluated with Fisher's exact test for dichotomous variables using SAS statistical software (version 8.02; SAS Institute, Cary, NC). A *P* value of less than 0.05 was considered significant. *KRAS* mutation analysis, *EGFR* gene copy number analysis, and medical record review were performed according to protocols approved by our institutional review boards. Some of the patients included in this analysis have been included in other clinical analyses.^{31,32}

Results

Clinical Specimens Submitted for *EGFR* Mutation Analysis

Among 119 primary lung cancers submitted to the MGH Department of Pathology for *EGFR* mutation status evaluation during the study period, 111 (93%) were adenocarcinomas, two were adenosquamous carcinomas, one was a squamous carcinoma, one was a large cell carcinoma, and four were NSCLCs not otherwise specified (Figure 1). Forty-four of the 111 adenocarcinomas were BAC/AWBF. Of these 44 cases, 39 patients were treated at MGH and thus were available for clinical chart review. Clinical characteristics of these 39 patients are shown in Table 1.

Morphological Correlates of *EGFR* Mutation Status in BAC/AWBF Tumors

EGFR mutations were detected in 15 (34%) tumors within the group of 44 BAC/AWBF (Supplemental Table at

<http://jmd.amjpathol.org/>) and in 13 (19%) of the 67 adenocarcinomas lacking BAC features (data not shown). This difference in prevalence of *EGFR* mutation was not statistically significant (*P* = 0.2). There was also no significant difference between the prevalence of *EGFR* mutations in pure BAC versus AWBF (*P* = 0.69). Among the BAC/AWBF cohort with *EGFR* mutations, the 2573T>G (L858R) mutation in exon 21 was detected in seven specimens (47%), whereas six tumors (40%) harbored in-frame deletions in exon 19 (see Supplemental Table at <http://jmd.amjpathol.org/>). These two types of mutations are the most common *EGFR* mutations in NSCLC, making up ~90% of described variants, and are both associated with TKI response.³³ A G719A mutation was detected in one AWBF and in one BAC, the latter of which also harbored a R776H mutation.

Histological review of the BAC/AWBFs (43 of 44 were available for review) revealed that the presence of mucinous histology (Figure 2A) was significantly associated with lack of *EGFR* mutation, whereas the absence of mucinous histology (Figure 2, B and C) was associated with the presence of *EGFR* mutation. Of the 13 BAC/AWBF tumors that were mucinous, none harbored an *EGFR* mutation, whereas 14 of the 30 (47%) nonmucinous tumors were *EGFR* mutation-positive (*P* = 0.003, Table 2).

Table 1. Clinical Characteristics of the 39 Patients with BAC/AWBF

Median age 69 years (range, 45 to 85 years)	
Gender	
Female	26 (67%)
Male	13 (33%)
Race	
White	36 (92%)
Asian	2 (5%)
Unknown	1 (3%)
Disease stage*	
Stages I, II, or IIIA	27 (69%)
Stages IIIB or IV	12 (31%)
Smoking history	
Never smoked	9 (23%)
Former smoker	26 (67%)
Current smoker	4 (10%)

*American Joint Committee on Cancer staging system.²⁹

Figure 2. Morphological and molecular features of BAC/AWBF. **A:** Mucinous adenocarcinoma with prominent BAC component harboring *KRAS* mutation and lacking *EGFR* mutation. **Inset**, DNA sequence chromatogram displaying codons 12 and 13 of *KRAS* in the same tumor. A heterozygous G>A transition at nucleotide 35 of the *KRAS* coding sequencing is observed. **B:** Nonmucinous adenocarcinoma with prominent BAC component lacking *EGFR* mutation. **C:** Nonmucinous BAC with *EGFR* mutation. **Inset**, sequence tracing after codon 746 (GGA) consistent with L747_T751delinsP mutation (deletion confirmed by sequencing reverse strand; data not shown). **D:** *EGFR* gene copy number analysis by FISH demonstrating high polysomy in a mucinous BAC with *KRAS* mutation (35G>T) and lacking *EGFR* mutation. Red signal indicates probe hybridization to the chromosome 7p (*EGFR* locus), and green signal represents hybridization of the chromosome 7q reference probe. Original magnifications: $\times 200$ (A–C); $\times 1000$ (D).

Analysis of Mucinous BAC/AWBF Tumors

Given our finding that mucinous histology was not observed in any BAC/AWBF tumors that harbored *EGFR* mutations, we sought to determine whether the presence of mucinous histology in BAC/AWBF was associated with other molecular features previously associated with either clinical refractoriness or responsiveness to TKIs. Among the 25 BAC/AWBF with sufficient tissue available for analysis, missense mutations in *KRAS* exon 2 were detected in nine (36%) (Table 2).

Table 2. Mucinous and Nonmucinous Morphology in BAC/AWBF Tumors Harboring or Lacking *EGFR* TK Mutations and *KRAS* Exon 2 Mutations

	Mucinous	Nonmucinous
<i>EGFR</i> mutation detected	0% (0/13)	47% (14/30)
<i>EGFR</i> mutation not detected	100% (13/13)	53% (16/30)
<i>KRAS</i> mutation detected	86% (6/7)	17% (3/18)
<i>KRAS</i> mutation not detected	14% (1/7)	83% (15/18)

None of the tumors in which *KRAS* mutations were detected also harbored an *EGFR* mutation. *KRAS* mutations were significantly associated with mucinous morphology and were detected in six of seven (86%) cases with mucinous morphology (Figure 2A, inset), in contrast to 3 of 18 (17%) cases with nonmucinous morphology ($P = 0.003$, Table 2). In the mucinous cases, we found the normal DNA sequence at codon 12 (GGT; glycine) was mutated to TGT (cysteine) in one case, to GAT (aspartic acid) in two cases, and to GTT (valine) in three cases (Table 3). In addition, we performed FISH on the mucinous cases to assess *EGFR* gene copy number and detected high polysomy for the *EGFR* locus in four of the five cases analyzed, whereas *EGFR* gene amplification was not observed (Table 3; Figure 2D). Among the 39 patients with BAC/AWBF who were treated at MGH, only five have received TKI therapy and only 4 have died within our follow-up period; hence, there was insufficient power to perform response to treatment or survival analyses.

Table 3. *KRAS* Mutation Status and *EGFR* Gene Copy Number in Mucinous BAC/AWBF Lacking *EGFR* TK Mutations

Case	<i>KRAS</i> mutation status	Adenocarcinoma subtype	<i>EGFR</i> copy number
1	34G>T	Mixed (acinar and BAC, mucinous)	High polysomy
2	35G>A	Mixed (acinar and BAC, mucinous)	High polysomy
3	35G>A	Mixed (papillary and BAC, mucinous)	High polysomy
4	35G>T	BAC, mucinous	N.D.
5	35G>T	Mixed (acinar and BAC, mucinous)	High polysomy
6	35G>T	Mixed (papillary and BAC, mucinous)	Low polysomy
7	Wild type	BAC, mucinous	N.D.

N.D., not done.

Discussion

We have correlated pathological features of NSCLC with the presence or absence of *EGFR* mutations in a series of tumors undergoing clinical *EGFR* mutation screening. *EGFR* mutations were detected in all subtypes of adenocarcinoma, including acinar, papillary, BAC, and mixed subtypes. In the clinical setting, classifying a tumor as pure BAC may be difficult if only limited tissue is available for histological examination. Accordingly, for the purpose of our analysis, we grouped BAC and mixed subtype adenocarcinomas with BAC features into one group, BAC/AWBF. In our study, we evaluated different types of surgical pathology specimens. For some the amount of evaluable material was limited, and a sampling bias may occur, resulting in, for example, the failure to detect a peripheral BAC component in a tumor or a diagnosis of pure BAC in a tumor that is actually AWBF.

Varying definitions of BAC used by different groups, as well as different patient demographics, may contribute to the marked differences reported in the frequency of BAC among cases with *EGFR* mutations, as well as the frequency of *EGFR* mutations among BACs. For example, Lynch and colleagues³ classified adenocarcinoma with any element of bronchioloalveolar carcinoma as BAC; defined this way, BAC was present in three of eight (37.5%) NSCLC tumors that harbored *EGFR* mutations in the initial *EGFR* mutation publication from MGH. Using a similar definition among the initial patient cohort tested for *EGFR* mutations at Memorial Sloan-Kettering Cancer Center, Pao and colleagues⁵ found that of 12 tumors with *EGFR* mutations, 11 (92%) had adenocarcinoma histology with features of BAC (one pure BAC, one BAC with focal invasion, nine AWBF). In a large series of 860 Italian patients, *EGFR* mutations were present in 22 (26%) of BAC cases defined using the nomenclature of Barkley

and Green³⁴ compared with 17 (6%) of conventional adenocarcinomas, $P < 0.0001$.¹² In a series of 35 Taiwanese patients, adenocarcinoma histology with any element of BAC was significantly associated with *EGFR* mutation (14 of 21 patients, 66%) compared with pure adenocarcinoma (3 of 13 patients, 23%, $P = 0.009$); however, a significant association between *EGFR* mutation and pure BAC as defined strictly by World Health Organization criteria²⁷ was not observed.¹⁰ Likewise in a series of 60 Japanese patients, any element of BAC was associated with an odds ratio of harboring an *EGFR* mutation of 5.0 (95% confidence interval, 2.0 to 12.6), yet strict World Health Organization classification of pure BAC was not correlated with mutation status.¹³ In contrast, among a series of 97 adenocarcinomas from NSCLC patients from the United States, classified according to World Health Organization criteria and also scored for the percentage of lepidic growth, no significant differences were found between *EGFR* mutations and the presence or percentage of BAC features.¹¹ Of note in this cohort, none of the seven adenocarcinomas that were pure BAC tumors harbored *EGFR* mutations.¹¹

We demonstrated that none of the BAC/AWBF tumors in our cohort that harbored *EGFR* mutations showed mucinous morphology. Similar findings have been observed in NSCLC patients from different demographic populations. A summary of the clinical and molecular features of mucinous BAC/AWBF reported in several studies is shown in Table 4. In a study of NSCLC cases in ethnically Chinese patients in Hong Kong, in which BAC tumors were defined by World Health Organization criteria, *EGFR* mutations were detected in none of the five cases of mucinous-type BAC but in 15 of 19 cases of nonmucinous BAC.²⁶ In the previously mentioned Italian series, in which NSCLC was subtyped according to World Health

Table 4. Comparison of Prevalence of *EGFR* Mutation, *KRAS* Mutation, Increased *EGFR* Copy Number, and Response to TKI Therapy Reported for Mucinous BAC/AWBF in Several Studies

Study	Proportion of cases with <i>EGFR</i> mutation	Proportion of cases with <i>KRAS</i> mutation	Proportion of cases with increased <i>EGFR</i> copy number*	Proportion of cases responding to TKI
Tam et al ²⁶	0/5 (0%)	N.D.	N.D.	N.D.
Marchetti et al ^{12†}	0/17 (0%)	13/17 (76%)	N.D.	N.D.
Hirsch et al ¹⁶	N.D.	N.D.	1/14 (7%)	0/11 (0%)
This study	0/13 (0%)	6/7 (86%)	4/5 (80%)	N.D.
Total	0/35 (0%)	19/24 (79%)	5/19 (26%)	0/11 (0%)

N.D., not determined/not reported.

*Increased *EGFR* copy number defined as a composite endpoint of high polysomy and gene amplification by FISH as per Cappuzzo et al.¹⁵

†BAC cases defined using nomenclature of Barkley and Green.³⁴

Organization criteria and in which BAC was histologically subtyped according to the criteria of Barkley and Green,³⁴ *EGFR* mutations were detected in 0 of 17 cases of mucinous BAC but in 22 of 69 (32%) cases of nonmucinous BAC.¹²

In addition to the association between mucinous differentiation and the absence of *EGFR* mutation, we established that *KRAS* mutations are common in mucinous BAC/AWBF, occurring in 86% of this cohort. Our findings confirm the previous association of *KRAS* mutations with mucinous BAC.³⁵ Furthermore, multiple studies have suggested that *EGFR* mutations and *KRAS* mutations are almost always mutually exclusive in NSCLC.^{11,12,24–26,36} Our finding that mucinous differentiation correlates with the presence of *KRAS* mutations and the absence of *EGFR* mutations further strengthens this hypothesis.

The *KRAS* mutations we detected in exon 2 have all been previously reported in NSCLC cases refractory to TKI treatment. Pao and colleagues²⁴ examined 60 lung adenocarcinomas from patients treated with TKIs, and found that whereas 17 of 22 (77%) TKI-sensitive adenocarcinomas harbored mutations in *EGFR*, none of them harbored *KRAS* exon 2 mutations. Furthermore, of 38 tumors that were TKI refractory, nine (24%) harbored *KRAS* mutations. In addition, *KRAS* mutations were associated with poor outcome after TKI treatment in a randomized trial of primary chemotherapy (carboplatin and paclitaxel) with or without concurrent TKI treatment in advanced NSCLC patients.³⁷ Progression time and survival were decreased in the subgroup with mutant *KRAS* that was treated with TKI and chemotherapy compared with the subgroup with mutant *KRAS* treated with chemotherapy alone.³⁷

EGFR gene copy number is another molecular feature that has been shown to influence the outcome of NSCLC patients treated with TKIs. In a study of patients with BAC or adenocarcinoma with BAC features, increased *EGFR* copy number, defined as a composite endpoint of high polysomy and gene amplification by FISH as per Cappuzzo and colleagues,¹⁵ was associated with improved survival after TKI therapy. Interestingly, in the same study, both clinical response to treatment and *EGFR* FISH positivity were more common in patients with BAC with nonmucinous morphology. Thirty percent (6 of 20) of patients with nonmucinous BAC had a response to TKI therapy, whereas 0 of 11 patients with mucinous BAC responded, and *EGFR* FISH was positive in 11 of 35 cases (31%) of nonmucinous BAC, but only 1 of 14 (7%) cases of mucinous BAC. The *KRAS* mutation status of these mucinous cases was not examined.¹⁶ Defining high polysomy according to the criteria of Cappuzzo and colleagues,¹⁵ our *EGFR* copy number analysis detected high polysomy in four of five (80%) examined mucinous BAC/AWBF that harbored *KRAS* exon 2 mutations but did not detect gene amplification in any mucinous BAC/AWBF cases.

When considering the implications of our study, it should be noted that our series consisted specifically of tumors submitted for *EGFR* mutation screening and may not be entirely representative of unselected adenocarcinomas in the general population. Our cohort may include

an overrepresentation of patients from certain demographic groups (ie, females, Asians, and nonsmokers) and of certain tumor histologies (ie, BAC/AWBF). Because our patient population was heterogeneous and not part of a single prospective treatment protocol, we lack the ability to compare treatment response and the power to perform survival analyses.

In summary, our findings indicate that mucinous BAC/AWBFs lack sensitizing *EGFR* mutations and most often contain activating *KRAS* mutation and thus are unlikely to respond to *EGFR* TKIs. From a patient testing standpoint, our data suggest that *EGFR* sequence analysis could be avoided when true mucinous morphology is identified, avoiding the associated testing costs. Our finding that high polysomy for *EGFR* coexists with *KRAS* mutation in some mucinous BAC/AWBF raises the possibility that high polysomy may fail to effectively predict TKI responsiveness in this subset of tumors. Further clinical trials are needed to improve understanding of the relationship between adenocarcinoma histological subtype, *EGFR* mutation status, *EGFR* gene copy number status, *KRAS* mutation status, and the interrelationship between these factors with the response to TKI therapy.

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