

Epidermal Growth Factor Receptor Mutations in Small Cell Lung Cancer

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Abstract Purpose: The vast majority of epidermal growth factor receptor (*EGFR*) mutations occur in lung adenocarcinoma, and even rare cases of other subtypes with this mutation, such as adenosquamous cell carcinoma, are associated with adenocarcinoma histology. According to this adenocarcinoma-specific nature of *EGFR* mutation, analysis of *EGFR* mutations with small cell lung cancers (SCLC) may provide a clue to its histogenesis.

Experimental Design: The mutational status of the *EGFR* gene was accessed in a cohort of 122 patients with SCLC; all patients were from a single institute. When the *EGFR* mutated, its gene copy number was also examined.

Results: *EGFR* mutations were detected in five SCLCs (4%). The patients were mainly in the light smoker and histologic combined subtype. All but one of the tumors harbored gene amplifications. Notably, in three tumors of the combined SCLC subtype, both components of adenocarcinoma and SCLC harbored an *EGFR* mutation, whereas gene amplification was detected only in the adenocarcinoma component. A partial response was achieved in a patient (with an *EGFR* mutation) who was treated with gefitinib.

Conclusions: Although *EGFR* mutations are rare in SCLC, a combined subtype of SCLC with adenocarcinoma in light smokers may have a chance of harboring *EGFR* mutations. For patients with an *EGFR* mutation, *EGFR* tyrosine kinase inhibitor can be a treatment option. In terms of molecular pathogenesis, it is suggested that some SCLCs may have developed from pre-existing adenocarcinomas with *EGFR* mutations, but the development may not be simply linear, taking into consideration the discordant distribution of *EGFR* amplification.

The vast majority of epidermal growth factor receptor (*EGFR*) gene mutations are detected in lung adenocarcinoma. A comprehensive analysis by Shigematsu and Gazdar reported that non-adenocarcinomatous lung cancers with *EGFR* gene mutations were restricted to <5% of lung cancers (1). Although it is rare in other histologic subtypes, adenosquamous cell carcinoma showed the highest frequency among lung cancers, followed by squamous cell carcinoma and large cell carcinoma. In contrast, small cell carcinoma was not listed among *EGFR*-mutated lung cancers following a comprehensive examination of 1,380 lung tumors, which suggests a different molecular

pathogenesis for this type of cancer. However, two patients (who had never smoked), recently reported having *EGFR* mutations with small cell lung cancers (SCLC; refs. 2, 3). In the first case, published in *The New England Journal of Medicine*, the patient with adenocarcinoma was initially treated with erlotinib. The recurrent tumor in the brain consisted of small cell carcinoma, which also harbored an *EGFR* mutation. Because the mutational status of the *EGFR* gene in the initial adenocarcinoma was not addressed, the clonal relationship between the two tumors was not clear. Another case was also a never-smoker who developed widespread SCLC. Mutational analysis revealed a typical *EGFR* gene deletion at exon 19. The tumor responded well to gefitinib treatment, and both primary and metastatic tumors regressed dramatically (3).

The incidence of *EGFR* mutation is quite high among the Japanese (~30-40% of non-small cell lung cancers on average) in contrast to ~10% of patients in the United States and in European countries (1, 4, 5). The clinicopathologic characteristics of patients with *EGFR* mutations include female sex, not smoking, and less frequent p53 mutation (4-6), which are very different from those of SCLC. It is therefore expected that *EGFR* mutations are very rare or absent in SCLC. A comprehensive analysis of *EGFR* mutations in SCLCs has not been reported in the literature; however, we believe it is important to determine its incidence, especially in mutation-endemic countries. In this study, we comprehensively examined a total of 122 SCLCs to address mutation incidence in SCLC.

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Translational Relevance

It is well known that epidermal growth factor receptor (*EGFR*) mutations are prevalent in female nonsmokers. However, *EGFR* mutations have recently been reported in some patients with small cell lung cancers (SCLC). In this study, we first examined a large series of SCLCs to address mutation incidence. Because the incidence of *EGFR* mutations differs between the United States and Japan, these data are important in determining the significance of ethnicity and frequency of *EGFR* mutations. As a result, a combined subtype of SCLC with adenocarcinoma in light smokers may have a chance of harboring *EGFR* mutations, although *EGFR* mutations are generally rare in SCLC. Notably, one such patient with an *EGFR* mutation achieved a partial response to gefitinib treatment. Although clinical relevance needs to be examined in more patients, *EGFR* tyrosine kinase inhibitor can be a treatment option for patients with SCLCs harboring an *EGFR* mutation.

Materials and Methods

Patients. Among 150 patients that were diagnosed with SCLC in the last 7 years at the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center in Nagoya, Japan, specimens from 122 patients were available for molecular genetic analysis, and these were the subject for the current study. This series included 102 specimens obtained by biopsy, and 20 from surgically resected tumors. Histologic diagnosis of SCLC was based on the standard criteria defined by WHO classification (7). The study was a part of a comprehensive lung cancer research program, which had been approved by the institutional review board.

EGFR mutation analysis. All the specimens were fixed with formalin, and the *EGFR* mutation was analyzed with the method described previously, using an unstained paraffin section (8). This technique allows the detection of tumor cells constituting as little as 5% of a mixture of tumor cells with normal tissue using a single paraffin section. When frozen tissues were available, the mutational status of *EGFR* was accessed with standard reverse transcription-PCR coupled direct sequencing, as described previously (4), in addition to DNA-based analysis. In this assay, the mutational status of the L858R point mutation and the deletion of exon 19 were obtained when we examined paraffin sections, whereas direct sequencing using RNA revealed the mutational status of the whole tyrosine kinase domain.

Copy number analysis of EGFR. Gene amplification was analyzed by fluorescence *in situ* hybridization, using the LSI *EGFR* SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis; Abbott Laboratories) according to the manufacturer's protocol. Fluorescence *in situ* hybridization was done on serial paraffin sections in the same tissue areas as the gene dosage analysis. A more than 4-fold increase of *EGFR* gene signals relative to CEP7 signals was considered a gene amplification. The results were confirmed by TaqMan-based gene dosage analysis as described previously (9).

Statistical analysis. Fisher's exact test for independence and unpaired *t* tests were used to show the correlation of clinicopathologic variables with *EGFR* mutation. $P < 0.05$ was considered statistically significant.

Results

SCLCs with EGFR mutation. Among 122 SCLCs examined (Table 1), we found *EGFR* mutations in five cases (4%). The mutations included L858R point mutations (three patients), a G719A point mutation (one patient), and a 15-bp deletion in exon 19 (one patient). Both frozen and paraffin tissues of 10

tumors, 2 of which harbored the above *EGFR* mutation, were available for analysis. They were examined using both reverse transcription-PCR coupled sequencing and assays for paraffin sections. The results were identical to those of the other analysis.

Clinicopathologic features of SCLCs with EGFR mutations. *EGFR* mutations were restricted to a very minor proportion (5 of 122; 4%) of SCLCs, and the clinicopathologic features of the patients with the mutation showed a trend similar to those of patients without the mutation. There were no significant differences in age, sex, and clinical stage at presentation. In contrast, accumulated smoking dose (pack-years) in patients with the mutation was much lower, and the difference was statistically significant (unpaired *t* test, $P = 0.02$). Indeed, three of the five patients with *EGFR* mutations were smokers with less than 40 pack-years. It is of note that one of the five patients was treated with gefitinib, and partial response was observed (case 2).

Morphologic features of SCLC with EGFR mutations. There are two subtypes of SCLC in the current WHO classification; thus, we examined whether the morphologic subtypes were associated with *EGFR* mutations. The combined subtype constituted a minor proportion (15 of 122, 12%) in this series, and three of them were positive for *EGFR* mutations (Table 1). Preferential mutation in the combined type were statistically significant (Fisher's exact test, $P < 0.01$). In two cases of the combined subtype (cases 1 and 3), SCLC components consisted of only a part of the nodule, and adenocarcinoma components constituted the predominant part. The representative morphologic features are displayed in Fig. 1. The other combined subtype (case 5) showed a mixture of SCLC and adenocarcinoma components throughout the tumor.

EGFR amplification in SCLCs. We have recently reported that *EGFR* amplification occurs in association with *EGFR* mutation (9). We therefore examined the *EGFR* gene copy number in the five SCLCs with *EGFR* mutations. Four of them showed gene amplification (Table 2), and the signals of the *EGFR* gene were loosely clustered (Fig. 2), suggesting a high degree of amplification, as is the case in homogeneously staining region patterns. Notably, three cases of combined SCLC subtypes harbored *EGFR* amplifications only in the adenocarcinoma component, but not in the SCLC component (Fig. 2).

Discussion

SCLC is a distinct neoplasm in terms of clinical aggressiveness, despite its high response to both chemotherapy and irradiation therapy. This aggressive cancer does not confer to

Table 1. Clinicopathologic features of SCLCs with and without *EGFR* mutations

	Mutated	Wild-type	P
No. of patients (total, N = 122)	5	117	
Age (median)	69	67	n.s.
Sex (female/male)	2/3	14/103	n.s.
Smoking history (median pack-years)	30	54	0.020
Disease stage (limited/extended disease)	4/1	81/33	n.s.
Histologic type (conventional/combined)	2/3	105/12	0.013

Abbreviation: n.s., not significant.

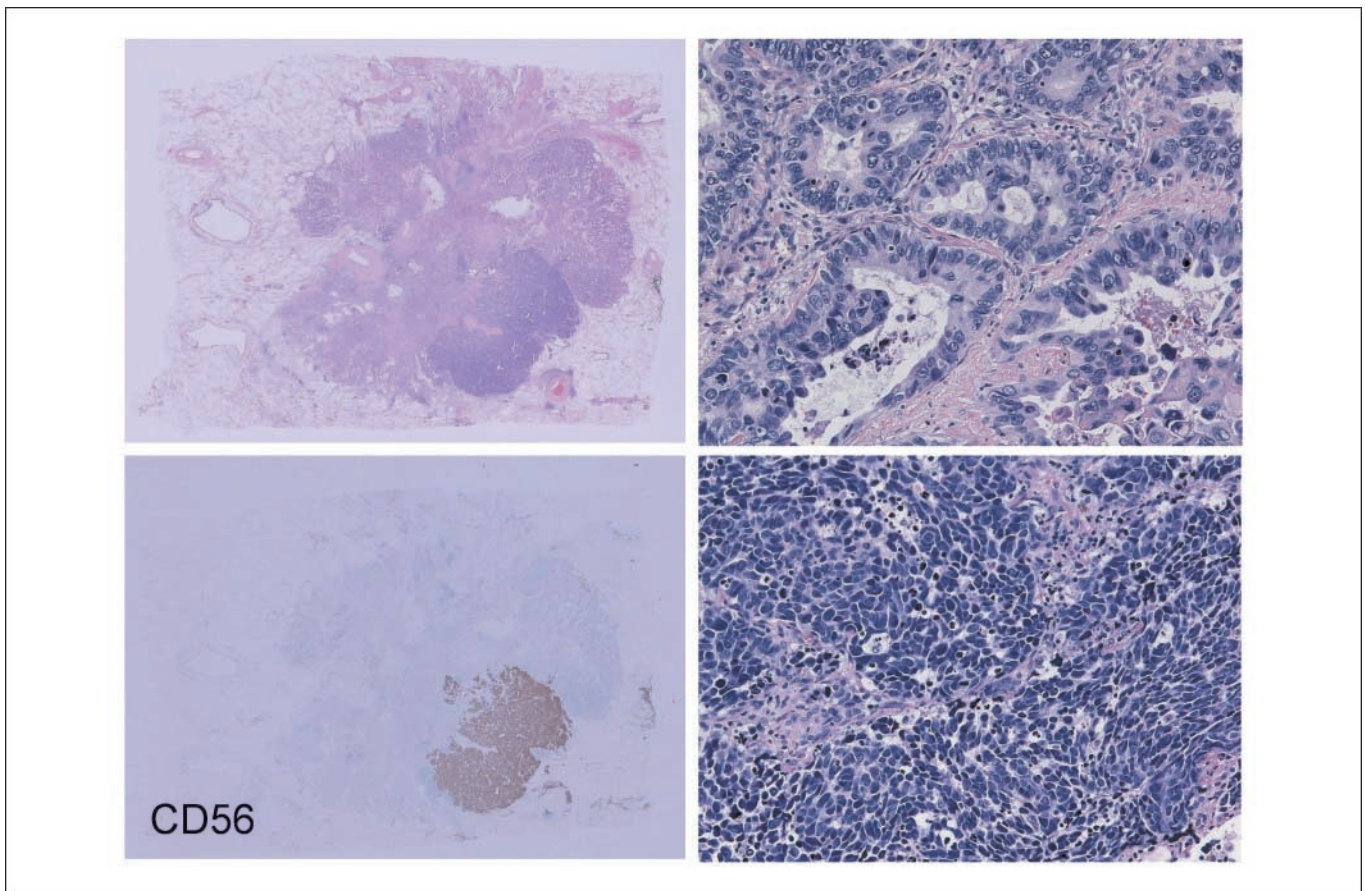


Fig. 1. Representative morphology of combined small cell carcinoma and adenocarcinoma (case 3). Approximately two-thirds of the area of the nodule (*top left*) consisted of an adenocarcinoma component, whereas the other area showed SCLC. Discrete expression of CD56 (neural cell adhesion molecule) corresponds to the component of SCLC (*bottom left*). High-power views of each component of adenocarcinoma (*top right*) and SCLC (*bottom left*). Both components harbor identical L858R *EGFR* mutations, although *EGFR* gene amplification was restricted to the adenocarcinoma component.

the lung, and it can develop in organs other than the lung, all of which share distinctive pathologic and immunohistochemical features, irrespective of their site of origin. These extrapulmonary carcinomas are characterized by frequent admixture with conventional carcinoma of the originating organ, such as adenocarcinoma in gastrointestinal tumors, and squamous cell carcinoma in head and neck cancers. This is true in SCLC. Nicholson et al. reported that 28 of 100 surgically resected SCLCs had a histologic component of non-small cell lung cancers (10). In our study, *EGFR* gene mutation was detected in 5 of 122 SCLCs. Because *EGFR* mutation was quite specific for adenocarcinoma, it is suggested that SCLCs with *EGFR* mutations are associated with adenocarcinoma. Indeed, three of the five combined SCLC had an adenocarcinoma component but not a squamous cell carcinoma component.

It has been suggested that the amine-precursor uptake and decarboxylase cells described by Pearse in 1969 (11) are the putative original cells of small carcinoma. These cells were described as comprising a neuroendocrine system in many organs, and as having ultrastructural features shared by small cell carcinomas. However, this hypothesis cannot explain the existence of combined SCLC, which is an admixture of small cell carcinoma and conventional adenocarcinoma or squamous cell carcinoma. Therefore, a multipotential cancer stem cell capable of divergent differentiation has been suggested as a

putative origin of small cell carcinoma. Alternatively, the SCLC component may arise as a consequence of undifferentiated transformation from conventional carcinoma. Case 2 in the present study supported the latter scenario, because SCLC is the only component that metastasized to the lymph nodes. Furthermore, the vast majority of lung cancers harboring *EGFR* mutations are adenocarcinomas, supporting the idea that the adenocarcinomas existed prior to the development of SCLC in at least three of the cases of SCLC with *EGFR* mutations.

However, the results of *EGFR* amplification analyses support the former possibility. In three cases of combined subtype of SCLC with an *EGFR* mutation, only the adenocarcinoma component, not the SCLC component, harbored the amplification. This is in contrast to the uniform detection of *EGFR* mutations in both components. Because *EGFR* mutations in SCLC are rather rare, it is unlikely that the two components are independent of their origin. Rather, it is believed that they originated from a common ancestor. Therefore, it is suggested that the mutation occurred before a point branching off to SCLC and adenocarcinoma components, whereas gene amplification was acquired after that point. Cases 3 and 5 may be considered to have followed this scenario. However, case 1 was inconsistent with it because SCLC emerged after the therapy.

In case 1, the initial adenocarcinoma harbored both *EGFR* mutation and amplification. Subsequently, SCLC, which lacked

gene amplification, developed after the chemotherapy and gefitinib therapy. It was unlikely that the amplification was removed from cancer cells due to therapy. We have recently reported heterogeneous distribution of *EGFR* amplification in lung adenocarcinoma (9), and thus we suggested that only a clone without amplification was selected, survived, and was subsequently transformed to SCLC. The reported SCLC with *EGFR* mutation followed this pattern of progression (2, 3, 12), and lack of *EGFR* expression in SCLC may be a clue to this phenomenon. Under heavy selection pressure by gefitinib therapy, only a clone which is independent of *EGFR*-driven growth signals has a chance to expand. Transformation to SCLC fulfills this condition because *EGFR* expression in the SCLC was at a very low or undetectable level (13–15). Indeed, the SCLC component lacked *EGFR* expression, in contrast to positive expression in the initial adenocarcinoma and adenocarcinoma components (data not shown). This may be another mechanism for tolerance to the *EGFR* tyrosine kinase inhibitor, in addition to secondary genetic alterations.

Clinically, it is noteworthy that a partial response was achieved in one of the patients with an *EGFR* mutation who was treated with gefitinib. Because *EGFR* expression is at a very low or undetectable level in SCLC, it would be expected that *EGFR* tyrosine kinase inhibitors are not effective against SCLC even if the *EGFR* is mutated. However, a similar marked reduction of such cancers by *EGFR* tyrosine kinase inhibitor treatment has also been reported (2, 3). *EGFR* tyrosine kinase inhibitors may be a treatment option for SCLC with *EGFR* mutations, and a mutation test may be helpful to select such patients in addition to clinical characteristics, including the light smoker and histologic combined subtypes.

In summary, we examined 122 SCLCs and found 5 (4%) of them harboring *EGFR* mutations. The SCLCs with *EGFR* mutations were seen in the light smoker and histologic combined subtypes. Because of the specific involvement of *EGFR* mutations in adenocarcinoma, it is suggested that the SCLCs may have developed from pre-existing adenocarcinomas. However, we have concluded that this development may

Table 2. Clinicopathologic features of five SCLCs with *EGFR* mutations

Case	Sex/Age (y)	Pack-years smoking	<i>EGFR</i> mutation	<i>EGFR</i> amplification	Stage	Sample and histologic subtype	Clinical course
1	F/36	0	L858R	Amplified (>6)*	ED	Resected tumor; combined type (diagnosis of adenocarcinoma with a biopsy prior to surgery)	Stage IV adenocarcinoma was treated with CBDCA and PAC, followed by gefitinib, because of positive <i>EGFR</i> mutation with a biopsy specimen. Partial response was achieved but the tumor regrew. It was surgically resected, and histologically revealed to be combined small and adenocarcinoma
2	M/81	40	G719A	Amplified (>6)	ED	Biopsy specimen; conventional type	Stage IV SCLC was treated with gefitinib, because of the detection of G719A mutation using a lung biopsy specimen. A partial response was obtained
3	M/69	30	L858R	Amplified (>6)*	LD	Biopsy specimen, combined type	A lung cancer (cT ₁ N ₀ M ₀) was surgically removed, and subsequent pathologic examination revealed combined SCLC. Adjuvant chemotherapy (CDDP and CPT-11) were administered. The patient is alive without recurrence
4	F/89	2.5	L858R	Low polysomy	LD	Biopsy specimen; conventional type	A biopsy specimen for lung cancer (cT ₂ N ₀ M ₀) was diagnosed as SCLC. The patient refused any therapy, and was not a part of follow-up
5	M/65	67.5	Ex.19Del	Amplified (>6)*	LD	Resected tumor; combined type (cytological diagnosis of SCLC prior to surgery)	cT ₁ N ₁ M ₀ cancer was treated with CDDP and TXT, followed by surgical resection of the tumor. Combined SCLC was revealed, and the patient was treated with adjuvant chemotherapy and irradiation. Three years later, SCLC recurred

Abbreviations: F, female; M, male; LD, limited disease; ED, extended disease; CBDCA, carboplatin; PAC, paclitaxel; CDDP, cisplatin; CPT-11, irinotecan.

* Only in the adenocarcinoma component.

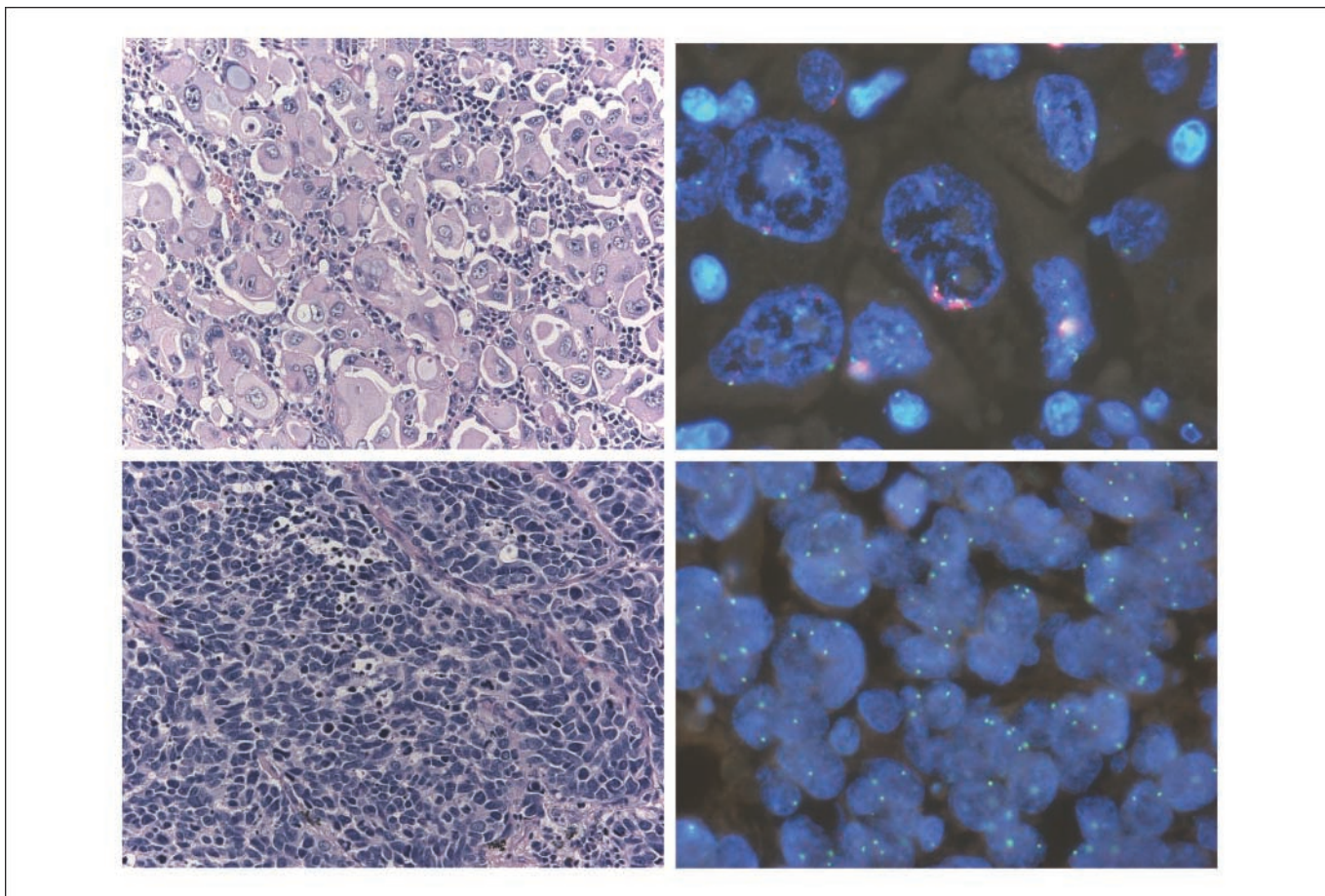


Fig. 2. *EGFR* amplification in SCLC with *EGFR* mutation (case 1). A female nonsmoker who had developed stage IV adenocarcinoma was treated with carboplatin and paclitaxel. The tumor recurred at the neck lymph node (*top left*), which was biopsied. Because molecular analysis using the tissue revealed a L858R mutation, she was subsequently treated with gefitinib. Although the tumor responded initially, rapid regrowth of the lung nodule was evident, and it was removed surgically. The SCLC component constituted most of the regrown nodule. *EGFR* mutation was detected in both adenocarcinomas in the lymph node and in the regrown SCLC, *EGFR* amplification was identified only in the adenocarcinoma but not in the regrown SCLC (*right*).

not be simply linear, considering the discordant distribution of *EGFR* amplification.

Disclosure of Potential Conflicts of Interest

T. Mitsudomi has a minor conflict with AstraZeneca, Chugai Pharm, Astellas, Daiichi-Sankyo, Sanofi-Aventis, Taiho Pharm, and Bristol Meyers.

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