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REVIEW ARTICLE

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Biological and clinical implications of EGFR mutations in lung cancer

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Abstract
Background. Patients with non-small-cell lung cancer sometimes show a dramatic clinical response to gefitinib or erlotinib, small-molecule tyrosine kinase inhibitors (TKI) specific for the epidermal growth factor receptor (EGFR). However, until April 2004, it was unclear how to identify patients who would benefit from these drugs. Then, two groups from Boston reported that *EGFR* gene mutations in the kinase domain are strongly associated with gefitinib sensitivity. *EGFR* mutations are more frequent in Asians, females, nonsmokers, and adenocarcinomas than in their counterparts. These populations precisely coincide with those populations with higher response rates to TKIs. We and others subsequently confirmed and extended these findings.
Methods. We reviewed recent literatures on EGFR mutations and EGFR-TKIs. We discuss topics including the molecular epidemiology and biology of *EGFR* mutations in relation to EGFR-TKIs, the controversy about whether *EGFR* mutations account for all the clinical activity of EGFR-TKIs, and the mechanisms of acquired resistance to gefitinib or erlotinib.
Results. The discovery of EGFR mutations has great biologic and clinical implications in lung cancer. However, all but one phase III trials have so far failed to show a survival advantage of the treatment arm involving EGFR-TKIs.
Conclusion. It would be possible to individualize EGFR-TKI treatment of lung cancer by selecting patients according to EGFR mutational status and other biomarkers.

Key words Molecular targeted therapy · Tyrosine kinase inhibitor · Gefitinib · Individualized therapy · Predictive factor

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Introduction

Lung cancer is the leading cause of cancer-related death in Japan, as in Western countries, claiming nearly 60000 lives annually. Although various chemotherapeutic agents were developed in the 1990s, platinum doublet therapy reached a therapeutic plateau with an objective response rate of 30%–40% and a median survival time (MST) of 8–10 months for patients with stage IIIB or IV disease.[1]

To circumvent this situation, a new class of drugs that specifically targets certain molecular pathways leading to cancer phenotypes is being actively developed. Epidermal growth factor receptor (EGFR) is one such target for the treatment of non-small-cell lung cancer (NSCLC), because EGFR is frequently overexpressed and aberrantly activated in NSCLC. When EGFR binds to several specific ligands, multiple signaling pathways are activated including the RAS/RAF/ERK/MAPK pathway, resulting in cell proliferation, and the PI3K/AKT pathway, STAT (signal transducers and activators of transcription) 3 and 5 signal transduction pathways, resulting in the evasion of apoptosis.[2] Antibodies directed against the extracellular domain of EGFR (such as cetuximab, matuzumab, and panitumab) and small-molecule tyrosine kinase inhibitors (TKIs) that target the kinase domain (such as gefitinib and erlotinib) are in clinical use or in a late developmental stage.[3]

In the phase II trials of gefitinib, IDEAL 1 and 2, certain patient subgroups appeared to have a higher response rate: female and Japanese patients, and adenocarcinomas.[4,5] Miller et al. reported that smoking history and bronchioloalveolar pathological subtype predict sensitivity to gefitinib.[6] Overall, a partial radiographic response was observed in 21 (15%) of 139 patients with advanced NSCLC. Never-smokers had a significantly higher response rate than former/current smokers (36% vs 8%, respectively; $P < 0.001$) and multivariate analysis confirmed this association ($P = 0.006$).[6] Following the report of these findings, various groups confirmed that a response to gefitinib or erlotinib is consistently seen in a certain patient subgroup. An analysis

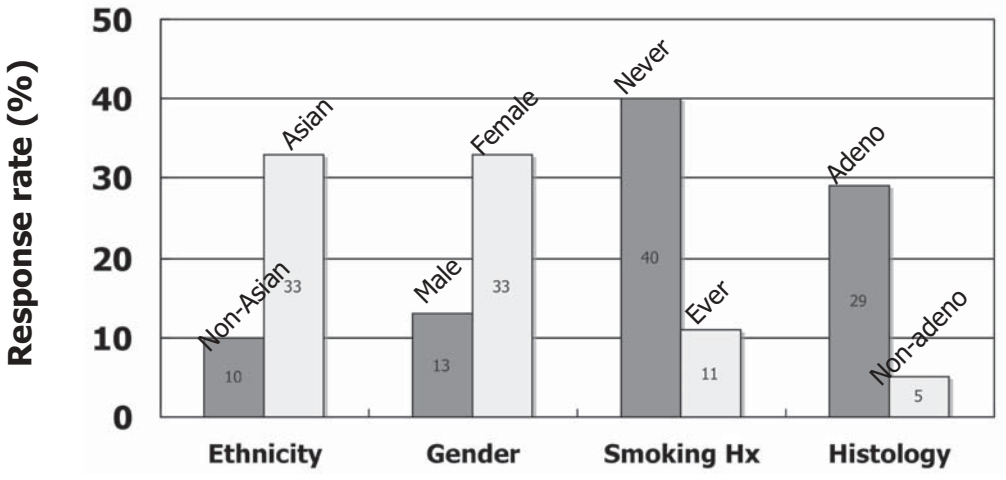

Fig. 1. Relationship between response rate and various clinical backgrounds. Data on 1974 patients compiled from the literature[4–6,23–25,28,31,44,46,52–59]

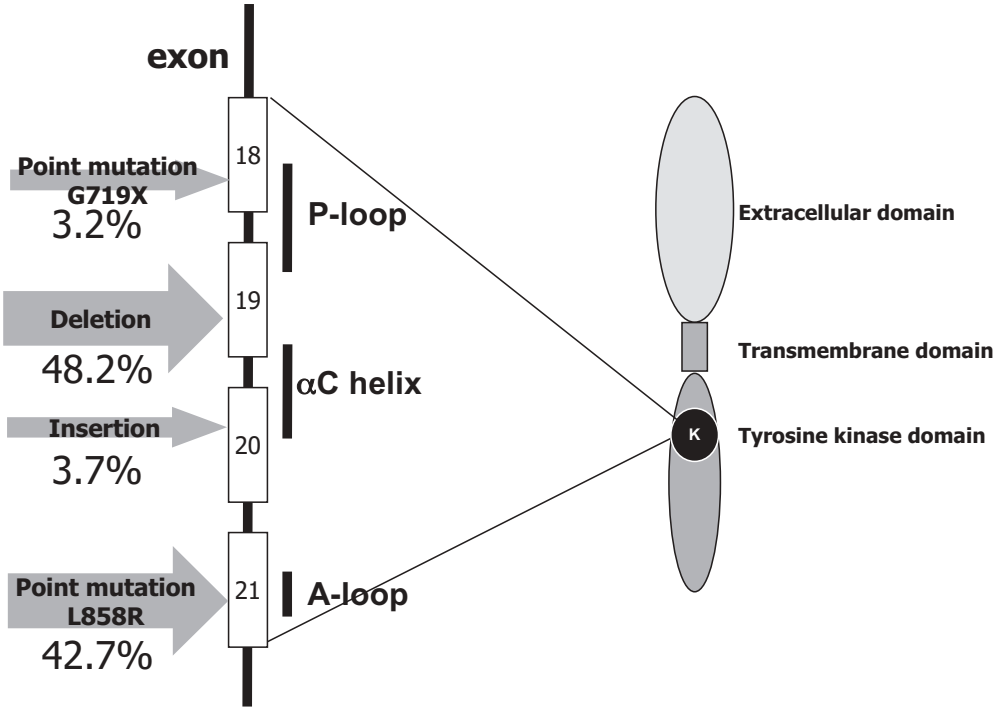

Fig. 2. Distribution of *EGFR* mutations ($n = 569$)[9,10,15,20,21,27,54,59–65]

of 1974 patients taken from previously published analyses (Fig. 1) indicated that the TKI response is significantly dependent on ethnicity, sex, smoking history, and histological type. However, it was not possible to predict gefitinib sensitivity by levels of EGFR overexpression, determined by immunohistochemistry or immunoblotting.[7,8] The factors that determine gefitinib sensitivity have long been an enigma.

In April 2004, two groups of researchers in Boston reported that activating mutations of the *EGFR* gene are present in a subset of NSCLCs and that tumors with *EGFR* mutations are highly sensitive to EGFR-TKI.[9,10] The populations with a higher response to gefitinib described above correspond to those with a higher incidence of *EGFR* mutations. Interestingly, *EGFR* mutations are the first molecular aberration identified as more frequent in nonsmoking patients. In this review, we discuss the current status of EGFR research in relation to kinase inhibitor therapy for lung cancer.

EGFR mutations

Figure 2 shows the distribution of the *EGFR* mutations reported so far (n = 569 from 14 studies). Mutations are present in the tyrosine kinase domain of *EGFR*. There are four main types of mutations: point mutations at codon 719 (G719X), deletions in exon 19, insertion mutations in exon 20, and a point mutation at codon 858 in exon 21. In exon

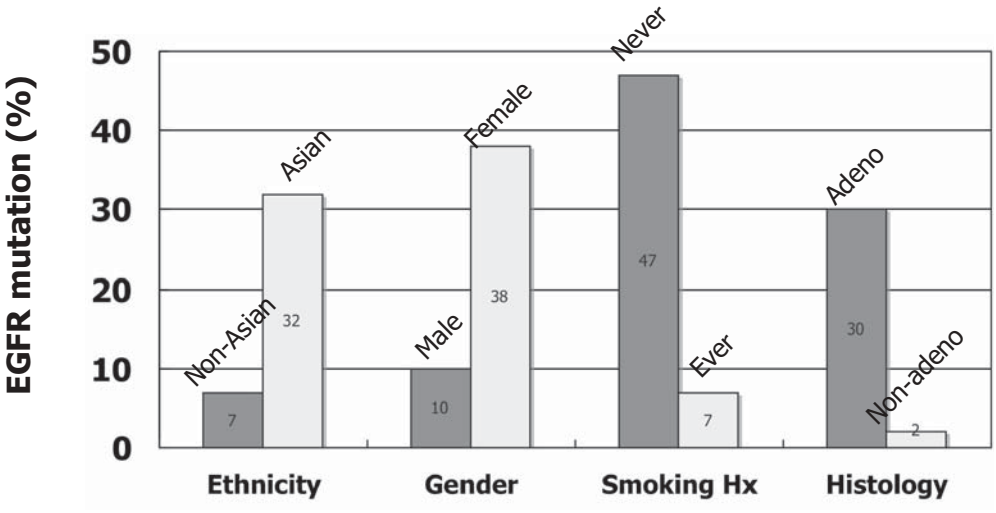

Fig. 3. Incidence of *EGFR* mutations according to particular clinical characteristics (compilation of the data from the literature used for Fig. 2; $n = 2880$). Overall incidence of *EGFR* mutations was 569/2880 = 19.8%

18, mutations are frequent at codon 719 (3.2%) and the patterns of amino acid substitutions are not uniform at this codon, resulting in changes from glycine to cysteine, serine, or alanine. Mutations resulting in the deletion of typically five amino acids at codons 746–750 (ELREA) in exon 19 and a leucine-to-arginine mutation at codon 858 (L858R) are two major types of mutations, which account for 90% of all mutations. These two types of *EGFR* mutations cause increased and sustained phosphorylation of EGFR itself and the phosphorylation of downstream molecules involved in antiapoptotic pathways (PI3K/AKT and STAT).[11] However, *EGFR* mutations have less effect on proliferation through the RAS/RAF/ERK/MAPK pathway.[11] There are several variant types of deletion mutations in exon 19, e.g., a larger deletion, deletion plus point mutation, deletion plus insertion, etc. There are also rarer point mutations and some patients have double mutations, but these usually accompany L858R. Interestingly, it is very rare for double mutations to occur among the four predominant types of mutations.

EGFR mutations and clinical features

Originally, *EGFR* mutations were predominantly found in females, nonsmokers, adenocarcinomas, and Japanese patients.[9,10] Subsequently, many different research groups have confirmed and extended these findings and their results, based on the 2880 mutations reported so far, are summarized in Fig. 3. The strong similarity of the graphs in Figs. 1 and 3 indicates that *EGFR* mutations are frequent in patient subsets that have a high response rate to TKIs.

Previously described genetic changes in lung cancer are almost always more frequent in smokers than nonsmokers. For example, mutations of the *TP53* gene,[12] or *KRAS* genes,[13] or deletion of the short arm of chromosome 3[14] are known to be more frequent in smokers. Indeed, we first showed that the frequency of *EGFR* mutations is inversely associated with smoking dose.[15] When we divided smokers into three categories according to smoke exposure, there

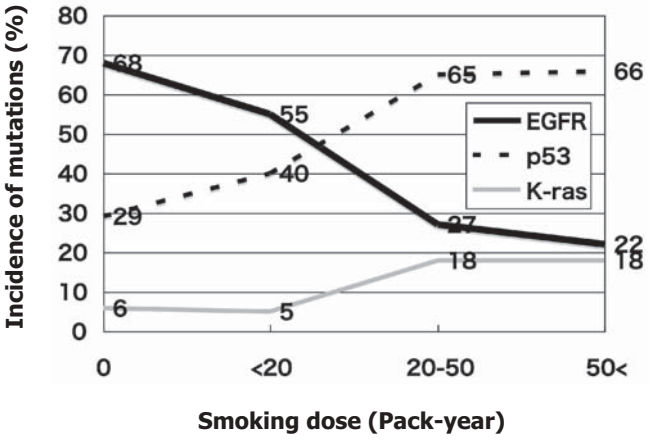

Fig. 4. Incidence of *EGFR*, *KRAS*, and *TP53* gene mutations in pulmonary adenocarcinomas according to smoking dose (data from Kosaka et al.[15])

was a trend such that the higher the exposure, the lower the incidence of *EGFR* mutations (Fig. 4). This is in distinct contrast to the *KRAS* and *TP53* mutations.

However, it cannot be construed that smoking has a preventive effect on *EGFR* mutations. Instead, it is reasonable to assume that *EGFR* mutations are caused by carcinogen(s) other than those contained in tobacco smoke, and that this apparent negative correlation with smoking dose results from the dilution of EGFR-positive tumors with the increased incidence of tumors with wild-type *EGFR* that occurs as smoking dose increases. This was the case in our recent case–control study of 152 patients with lung cancer and *EGFR* mutations, 283 patients with lung cancer and wild-type *EGFR*, and 2175 age- and sex-matched controls. For example, when the cumulative smoking exposure was divided into three groups, the odds ratio for lung cancer with wild-type *EGFR* increased from 1.00 to 2.72 (1–40 pack years) and further to 10.0 (>40 pack years; $P < 0.001$ for trend). In contrast, the odds ratios for patients with *EGFR* mutations were 1.00, 0.68, and 0.79 ($P = 0.303$ for trend) (K. Matsuo et al., unpublished).

It appears that the marked difference in the incidence of *EGFR* mutations with ethnicity might be at least partially attributable to differences in the incidence of nonsmoking patients among Japanese and American females. In our Japanese cohort, 83% of female patients and 10% of male patients were never-smokers.[15] In contrast, only 15% of 706 U.S. female patients and 6% of 1347 male patients with lung cancer were never-smokers.[16] However, smoking may not be the sole factor explaining these ethnic differences. It is known that the *EGFR* intron 1 polymorphic CA repeat (CA-SSR1) is longer in Asians than in Caucasians[17] and that a longer CA repeat leads to less gene transcription.[18] One can infer that lower transcription of *EGFR* may require mutational activation to obtain growth advantage in Asian patients.

EGFR mutations and pathology

In terms of morphological and pathological features, we found that *EGFR* mutations predominantly occur in adenocarcinomas of the terminal respiratory unit type.[19] We have proposed that these form a characteristic subset of adenocarcinomas putatively originating in the peripheral airway epithelium.[19] They have a papillolepidic growth pattern and frequently express thyroid transcription factor 1 or surfactant apoproteins. Interestingly, some atypical adenomatous hyperplasias, precursor lesions of this type of adenocarcinoma, occasionally harbor *EGFR* mutations, suggesting that *EGFR* mutations occur relatively early in pathogenesis.[19] This observation is closely associated with reports that gefitinib sensitivity is high in patients with adenocarcinomas with bronchioloalveolar cell carcinoma features.[6]

Relationship between *EGFR* mutations and mutations of other cancer genes

We and others have found that *EGFR* mutations never occur in tumors with *KRAS* mutations, thus exhibiting a mutually exclusive relationship.[15,20,21] Furthermore, mutations of *BRAF* and *ERBB2* are present in a very small fraction of adenocarcinomas of the lung (1% and 4%, respectively[22]) and these mutations also have a mutually exclusive relationship with *EGFR* and *KRAS* mutations. It is noteworthy that one of the major downstream pathways from EGFR is the RAS/RAF/ERK/MAPK pathway.

In contrast, *EGFR* mutations and *TP53* mutations appear to occur independently.[15] However, 23 *TP53* mutations relating to tobacco carcinogens (G-to-T transversions, mutations occurring at codons 157, 248, and 273) also have a mutually exclusive relationship with *EGFR* mutations, with two exceptions.[15] Again, this suggests that *EGFR* mutations occur independently of tobacco carcinogens.

EGFR mutations and the TKI response

When *EGFR* mutations were first reported, the most interesting and exciting finding was that patients with this genetic alteration showed a striking response to EGFR-TKIs.[9,10] Figure 5 summarizes the relationship between

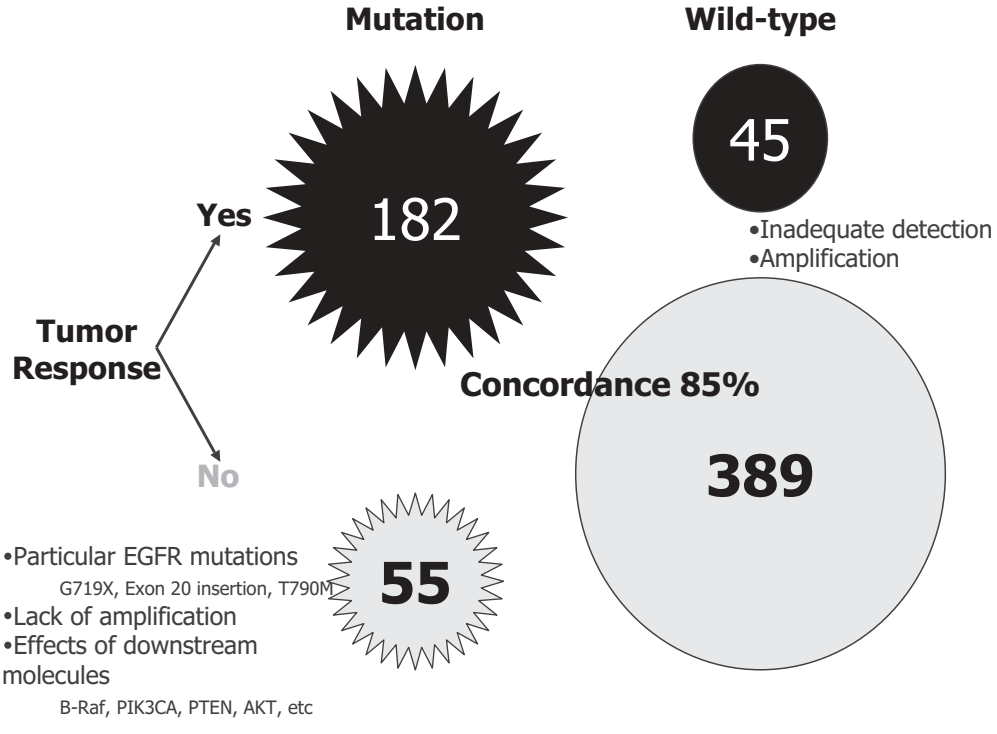

Fig. 5. Relationship between *EGFR* mutations and clinical responses to EGFR-TKI. Numbers of patients in each category are from a compilation of published data ($n = 671$).[9,10,23–28,31,54,58–60,64,66,67] The positive and negative predictive values of *EGFR* mutations (response rates in patients with or without *EGFR* mutations) were 77% and 10%, respectively. Possible reasons for the discrepancies are also listed

EGFR mutations and the response to EGFR-TKIs in 671 patients, compiled from the literature. In general, about 80% of NSCLCs with *EGFR* mutations respond to EGFR-TKIs, whereas 10% of tumors without *EGFR* mutations do so. Furthermore, several investigators have reported that patients with *EGFR* mutations have a significantly longer survival than those with wild-type *EGFR* when treated with gefitinib or erlotinib.[23–28] These results indicate that *EGFR* mutations are important in determining EGFR-TKI sensitivity. At the same time, they suggest that *EGFR* mutations are not the sole factor determining TKI sensitivity.

We first reported that gefitinib is more effective in patients with deletional *EGFR* mutations than in patients with other types of mutations, predominantly L858R.[23] Figure 6 shows the differences in response rates by classes of *EGFR* mutations from a compilation of 224 patients. The response rates of patients with an exon 19 deletion and L858R were 84% and 71%, respectively. In contrast, only about half the patients with G719X responded to gefitinib. Furthermore, patients with *EGFR* exon 19 deletions had significantly longer MSTs after treatment with erlotinib or gefitinib than those of patients with EGFR L858R (34 vs 8 months, respectively; log-rank $P = 0.01$).[29] Greulich et al. measured erlotinib sensitivity by the inhibition of cell-line transformation in vitro, using various *EGFR* mutant constructs and varying concentrations of erlotinib. They found that the order of sensitivity was: exon 19 deletion = L858R > G719X ≫ exon 20 insertion = wild type, which accords well with the clinical observations described above.[30]

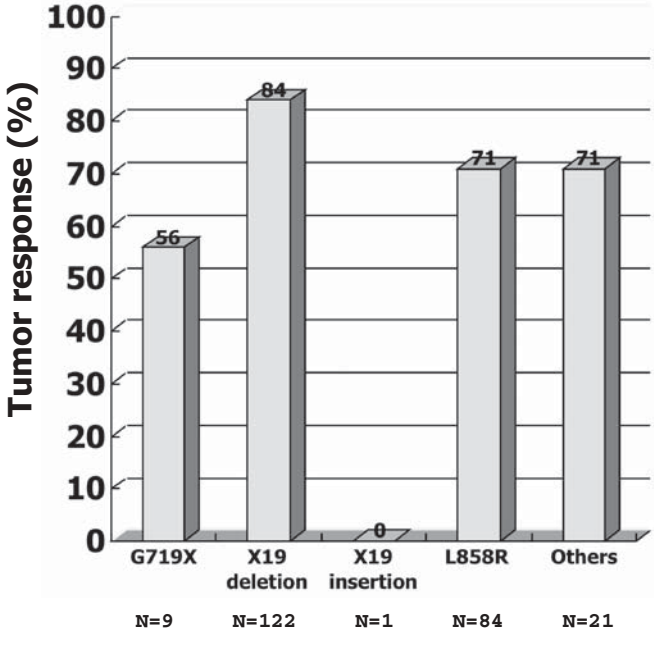

Fig. 6. Response rates to TKIs by classes of *EGFR* mutations (compilation of data from 16 papers used for Fig. 5; $n = 224$)

EGFR gene copy number and TKI sensitivity

In May 2005, Cappuzzo et al. reported that *EGFR* gene amplification, as measured by fluorescence in situ hybridization (FISH), is more predictive of patient survival after gefitinib treatment than *EGFR* mutations.[31] However, this report does not necessarily refute the role of *EGFR* mutations as a predictive factor, because *EGFR* mutations only failed to significantly affect overall survival ($P = 0.09$); *EGFR* mutations were predictive of response rate and time to progression.[31] However, it should be noted that FISH positivity is defined as tumors in which more than 40% of tumor cells have more than four copies (high polysomy) in addition to those with *EGFR* gene amplification. It is biologically unclear whether high polysomy indicates the activation of the *EGFR* gene, resulting in effects similar to those caused by gene amplification. As shown in Table 1, whether mutation or copy number is more predictive of response and useful in patient selection remains controversial. Tsao et al. reported that *EGFR* gene amplification was most predictive of a stronger response and a longer survival in patients who received erlotinib in a phase III clinical trial (BR.21) that compared erlotinib with best supportive care.[32] They concluded that the detection of *EGFR* mutations is not necessary in selecting patients who will benefit from erlotinib therapy.[32] However, many investigators, particularly those in Japan, refute this point. In general, tumors with *EGFR* mutations tend also to have gene amplification.

Table 1. Effectiveness of EGFR-TKIs, and mutation or copy number of the *EGFR* gene in predicting the effectiveness of EGFR-TKIs, reported in selected recently published studies

First author[Ref.]	TKI	*n*	Mutation			Copy number		
			Response	TTP	OS	Response	TTP	OS
Han[24]	G	90	65% vs 14%	Yes	Yes	–	–	–
Mitsudomi[23]	G	59	83% vs 10%	–	Yes	–	–	–
Cappuzzo[31 a]	G	89	53% vs 5%	Yes	No	36% vs 3%	Yes	Yes
Bell[47 b]	G	79/90	46% vs 10%	Yes	No	29% vs 15%	Yes	No
Tsao[32 a]	E	177/125	16% vs 7%	–	No	20% vs 2.4%	–	Yes
Hirsch[48 a]	G	100	–	–	–	26% vs 11%	No	Yes
Takano[28 b]	G	66	82% vs 11%	Yes	Yes	72% vs 38%	Yes	No

G, gefitinib; E, erlotinib; TTP, time to progression; OS, overall survival
[a] Copy number was examined by fluorescence in situ hybridization
[b] Copy number was examined by quantitative polymerase chain reaction

Table 2. EGFR inhibitor activity against an $EGFR^{L858R/T790M}$-containing cell line (H1975)

Compound	Target	Clinical development	IC_{50} (nM)
CI-1033	EGFR	P-II	20
EKB-569	ERBB1,2	P-II	30
CL-387,785	EGFR	Research compound	51
SU-11464	EGFR	Research compound	500
ZD6474	VEGF2, EGFR	P-II	2000
GW572016	ERBB1,2,4	P-III	4000
Gefitinib	EGFR	Approved	7000
PKI-166	EGFR	P-II	8000
Erlotinib	EGFR	Approved	10000

Adapted from Carter et al.[42]

Shibata et al., using comparative genomic hybridizati on experiments, reported that the mutational status of the *EGFR* gene is significantly associated with the specific gain or loss of genetic material, including the amplification of the *EGFR* gene.[33] Mutation and amplification are probably both important in determining TKI sensitivity. To resolve this controversy, both *EGFR* mutations and amplification should be determined prospectively in future clinical trials.

Other parameters, such as the expression of phosphorylated AKT,[34] the amplification of *HER2*,[35] and the expression of EGFR protein,[31] are reported to affect sensitivity to EGFR-TKIs.

Resistance to gefitinib

Pao et al. first reported that lung cancers with *KRAS* mutations are resistant to EGFR-TKIs.[36] None of nine tumors with *KRAS* mutations responded to EGFR-TKIs.[36] As described previously, some tumors without *EGFR* mutations do respond to TKIs, but when these tumors harbor *KRAS* mutations, a tumor response to TKIs cannot be expected.

In contrast to the inherent resistance to gefitinib induced by *KRAS* mutations described above, it is common for patients to show progressive disease after presenting with an initial marked response to gefitinib. The mean duration of the response is about 3–7 months.[4,5] Most of these tumors have *EGFR* mutations that confer sensitivity to TKIs, such as exon 19 deletions and L858R, resulting in a good clinical response. However, the emergence of acquired resistance cannot be explained by the selection of tumor cells with wild-type *EGFR* genes, because their mutational status remains unchanged after they acquire resistance to TKIs. In February 2005, it was reported that a secondary mutation resulting in a threonine-to-methionine change at codon 790 is responsible for at least half the acquired resistance to gefitinib and erlotinib.[37,38] Crystal structure modeling has revealed that position T790 is located in the ATP-binding pocket of the catalytic region and appears to be critical for the binding of erlotinib and gefitinib. Substitution of the threonine at this codon with a bulkier residue, such as methionine, is thought to sterically hinder the binding of these two drugs.

In the case of chronic myeloid leukemia (CML), secondary mutations in the kinase domain of the *ABL1* gene are considered to be one of the mechanisms of acquired drug resistance to imatinib, a tyrosine kinase inhibitor specific for BCR–ABL1, KIT, and PDGFA.[39,40] The structural similarity between ABL1 and EGFR tyrosine kinases is fairly high, and the most common mutation related to acquired resistance is a threonine-to-isoleucine mutation at codon 315 (T315I) of ABL1, corresponding to T790M of EGFR. Reflecting this structural similarity, in 2003, before the discovery of the activating mutations of the *EGFR* gene in lung cancer, it was reported that artificially introduced T790M caused resistance to EGFR-specific 4-anilinoquinazoline inhibitors, including gefitinib and erlotinib[41]. In the case of CML, 20–30 other mutations of the *ABL1* gene, in addition to T315I, have been identified as mechanisms of acquired resistance to imatinib.[39] Although secondary *EGFR* mutations other than T790M are possible, only T790M has so far been detected in clinical samples.

To overcome acquired resistance, a new class of EGFR-TKIs is being developed that can be used as second-generation drugs. Carter et al. found that the EGFR inhibitors EKB-569 and CI-1033, but not GW-572016 and ZD-6474, potently inhibit EGFR (L858R, T790M) kinase (Table 2).[42] EKB-569 and CI-1033 are already in clinical trials.

TKIs and clinical trials

In four randomized trials comparing TKI plus platinum doublet and platinum doublet (i.e., INTACT 1 and 2 using gefitinib, and TALENT and TRIBUTE using erlotinib), the addition of TKI did not yield a survival advantage over platinum doublet. However, subgroup analysis in the TRIBUTE trial showed that the addition of erlotinib to carboplatin plus paclitaxel conferred an advantage in overall survival in patients who were never-smokers (MST 22.5 months vs 10.1 months for others; $P = 0.01$).[43]

In a randomized placebo-controlled trial (BR.21) to determine whether erlotinib prolongs survival in patients with NSCLC after the failure of first- or second-line chemotherapy, erlotinib significantly prolonged survival, with an MST of 6.7 months vs 4.7 months (hazard ratio 0.70; $P < 0.001$).[44] In contrast, a similar placebo-controlled randomized trial using gefitinib instead of erlotinib (ISEL trial)

failed to show an overall survival advantage in the gefitinib treatment group (MST of 5.6 months vs 5.1 months; $P = 0.087$).[45] However, gefitinib prolonged survival in never-smokers (MST 8.9 months vs 6.1 months; $P = 0.012$), as well as in Asian patients (MST 9.5 months vs 5.5 months; $P = 0.010$) in preplanned subset analyses.[45] Following these results, the U.S. Food and Drug Administration limits the indication of gefitinib to cancer patients who are currently benefiting or have previously benefited from gefitinib treatment, or are enrolled in clinical trials as of June 2005.

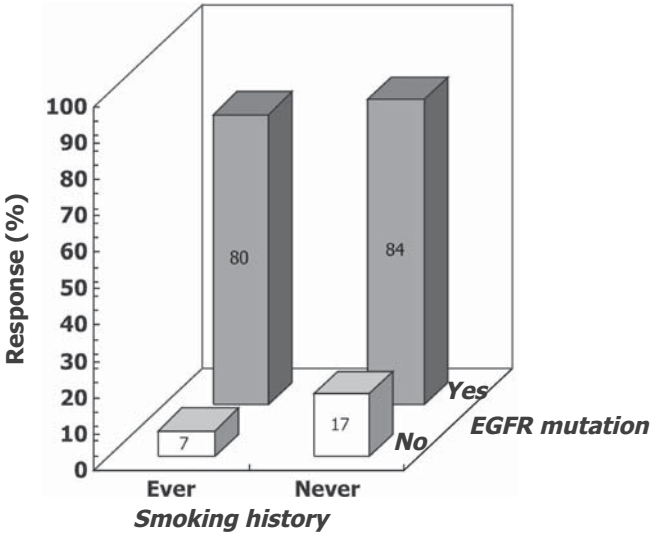

Fig. 7. Smoking history, *EGFR* mutation, and response to gefitinib (data from Mitsudomi et al.[23])

As has been described, EGFR-TKIs are not universally effective for lung cancer, but these drugs are effective in patients who have particular clinical or biological characteristics, e.g., Asian, nonsmoking female patients with adenocarcinomas with *EGFR* mutations. The different outcomes of the BR.21 and ISEL trials are at least partly attributable to differences in the degree of dilution in the two trials of patients with the abovementioned characteristics by those without such characteristics. Therefore, patients who would benefit from gefitinib therapy should be concentrated in future clinical trials. Smoking history and *EGFR* mutations are good predictors of response in patients treated with EGFR-TKIs. Which of these two markers should we use in future clinical trials? In our exploratory subset analysis, tumor response was observed in 16/19 patients with both *EGFR* mutations and no smoking history.[23] Whereas a response was seen in 1/6 never-smokers without *EGFR* mutations, a response was seen in 8/10 smokers with *EGFR* mutations.[23] Therefore, our limited experience indicates that *EGFR* mutations may be superior to smoking history in

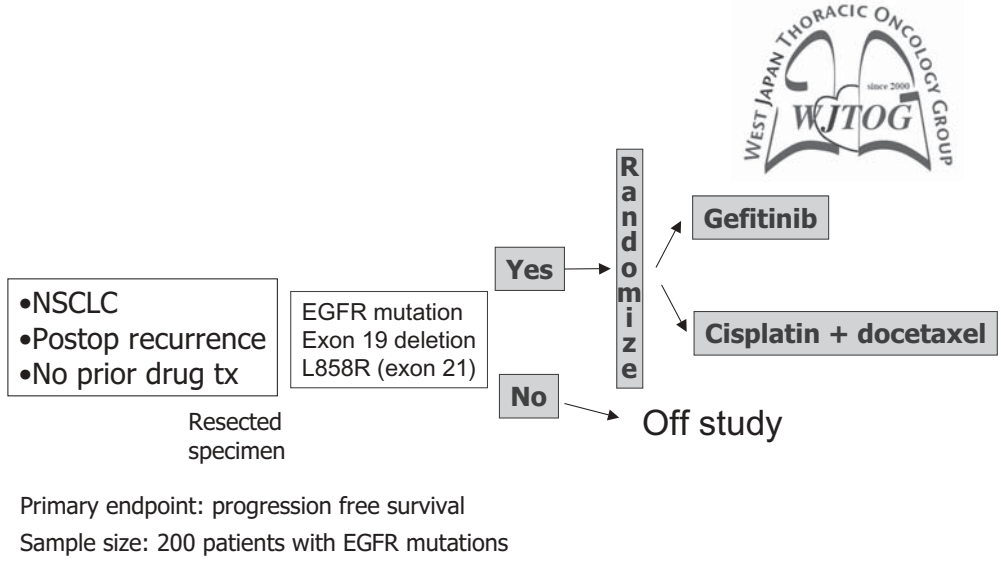

Fig. 8. Ongoing phase III trial comparing gefitinib monotherapy with cisplatin plus docetaxel in patients with recurrent disease after they had undergone pulmonary resection for non-small-cell lung cancer (*NSCLC*) (WJTOG3405)

Table 3. Results of phase III clinical trials involving EGFR-TKIs

TKI	Trial[Ref.]	Design	Result
G	INTACT I[49]	GP + G vs GP	Negative
G	INTACT II[50]	TC + G vs TC	Negative
E	TRIBUTE[43]	TC + E vs TC	Negative
E	TALENT	GP + E vs GP	Negative
E	BR.21[44]	E vs BSC	Positive
G	ISEL[51]	G vs BSC	Negative
G	S0023	PE/TRT → D → G vs PE/TRT → D	Terminated

G, gefitinib; E, erlotinib; GP, gemcitabine+cisplatin; TC, paclitaxel+cisplatin; BSC, best supportive care; PE, cisplatin+etoposide; D, docetaxel; TRT, thoracic radiotherapy

the selection of patients who would benefit from TKI treatment. Obviously, the detection of *EGFR* mutations requires laborious laboratory work. Hence, smoking history can be used in contexts in which *EGFR* gene testing is not readily available. In this way, the survival benefit of EGFR-TKIs, especially gefitinib, should be demonstrated in future clinical trials in a defined subset of patients with lung cancer. We, the West Japan Thoracic Oncology Group (WJTOG), have just launched a phase III clinical trial comparing gefitinib monotherapy with cisplatin plus docetaxel in lung cancer patients with *EGFR* mutations who have had recurrent disease after pulmonary surgery. The primary endpoint is progression-free survival and the sample size is 200 patients with *EGFR* mutations. To assure tumor specimens of good quality to avoid possible false negative results for mutation analyses, we decided to limit patients who had postoperative recurrence. We also limit our mutation search to deletions in exon 19 and L858R, because it would be less laborious and these two are most reliable predictor for response or survival. The primary endpoint is progression-free survival, to avoid confounding by possible crossover between two arms.

Conclusions

The development of EGFR-TKIs and the discovery of *EGFR* gene mutations have provided a great opportunity to develop individualized therapies for lung cancer. In Japan, a considerable fraction of patients undergoing gefitinib treatment suffer from fatal interstitial lung disease (ILD) (approximately 6% by prospective analysis). Pre-existing pulmonary fibrosis and smoking history are regarded as risk factors for ILD.[46] In this regard, it is also necessary to select patients who are likely to benefit from gefitinib therapy.

References

1. Schiller JH, Harrington D, Belani CP, et al. (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346:92–98
2. Arteaga CL (2002) Overview of epidermal growth factor receptor biology and its role as a therapeutic target in human neoplasia. Semin Oncol 29:3–9
3. Ciardiello F, Tortora G (2001) A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res 7:2958–2970
4. Fukuoka M, Yano S, Giaccone G, et al. (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237–2246
5. Kris MG, Natale RB, Herbst RS, et al. (2003) Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 290:2149–2158
6. Miller VA, Kris MG, Shah N, et al. (2004) Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 22:1103–1109
7. Cappuzzo F, Gregorc V, Rossi E, et al. (2003) Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol 21:2658–2663
8. Suzuki T, Nakagawa T, Endo H, et al. (2003) The sensitivity of lung cancer cell lines to the EGFR-selective tyrosine kinase inhibitor ZD1839 ("Iressa") is not related to the expression of EGFR or HER-2 or to K-ras gene status. Lung Cancer 42:35–41
9. Paez JG, Janne PA, Lee JC, et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500
10. Lynch TJ, Bell DW, Sordella R, et al. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
11. Sordella R, Bell DW, Haber DA, et al. (2004) Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305:1163–1167
12. Suzuki H, Takahashi T, Kuroishi T, et al. (1992) p53 mutations in non-small cell lung cancer in Japan: Association between mutations and smoking. Cancer Res 52:734–736
13. Ahrendt SA, Decker PA, Alawi EA, et al. (2001) Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. Cancer 92:1525–1530
14. Mitsudomi T, Oyama T, Nishida K, et al. (1996) Loss of heterozygosity at 3p in non-small cell lung cancer and its prognostic implication. Clin Cancer Res 2:1185–1189
15. Kosaka T, Yatabe Y, Endoh H, et al. (2004) Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 64:8919–8923
16. Kobrinsky NL, Klug MG, Hokanson PJ, et al. (2003) Impact of smoking on cancer stage at diagnosis. J Clin Oncol 21:907–913
17. Liu W, Innocenti F, Chen P, et al. (2003) Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. Clin Cancer Res 9:1009–1012
18. Gebhardt F, Zanker KS, Brandt B (1999) Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem 274:13176–13180
19. Yatabe Y, Kosaka T, Takahashi T, et al. (2005) EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. Am J Surg Pathol 29:633–639
20. Marchetti A, Martella C, Felicioni L, et al. (2005) EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 23:857–865
21. Shigematsu H, Lin L, Takahashi T, et al. (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 97:339–346
22. Shigematsu H, Gazdar AF (2006) Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer 118:257–262
23. Mitsudomi T, Kosaka T, Endoh H, et al. (2005) Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 23:2513–2520
24. Han SW, Kim TY, Hwang PG, et al. (2005) Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 23:2493–2501
25. Kim KS, Jeong JY, Kim YC, et al. (2005) Predictors of the response to gefitinib in refractory non-small cell lung cancer. Clin Cancer Res 11:2244–2251
26. Cortes-Funes H, Gomez C, Rosell R, et al. (2005) Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. Ann Oncol 16:1081–1086
27. Taron M, Ichinose Y, Rosell R, et al. (2005) Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. Clin Cancer Res 11:5878–5885
28. Takano T, Ohe Y, Sakamoto H, et al. (2005) Epidermal growth factor receptor gene mutations and increased copy numbers pre-

dict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 23:6829–6837
29. Riely GJ, Pao W, Pham D, et al. (2006) Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 12:839–844
30. Greulich H, Chen T-H, Feng W, et al. (2005) Oncogenic transformation by inhibitor-sensitive and resistant EGFR mutations. PLoS Med 2:e313
31. Cappuzzo F, Hirsch FR, Rossi E, et al. (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. J Natl Cancer Inst 97:643–655
32. Tsao MS, Sakurada A, Cutz JC, et al. (2005) Erlotinib in lung cancer – molecular and clinical predictors of outcome. N Engl J Med 353:133–144
33. Shibata T, Uryu S, Kokubu A, et al. (2005) Genetic classification of lung adenocarcinoma based on array-based comparative genomic hybridization analysis: its association with clinicopathologic features. Clin Cancer Res 11:6177–6185
34. Cappuzzo F, Magrini E, Ceresoli GL, et al. (2004) Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. J Natl Cancer Inst 96:1133–1141
35. Cappuzzo F, Varella-Garcia M, Shigematsu H, et al. (2005) Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 23:5007–5018
36. Pao W, Wang TY, Riely GJ, et al. (2005) KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2:e17
37. Kobayashi S, Boggon TJ, Dayaram T, et al. (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352:786–792
38. Pao W, Miller VA, Politi KA, et al. (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2:e73
39. Shah NP, Nicoll JM, Nagar B, et al. (2002) Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2:117–125
40. Gorre ME, Mohammed M, Ellwood K, et al. (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880
41. Blencke S, Ullrich A, Daub H (2003) Mutation of threonine 766 in the epidermal growth factor receptor reveals a hotspot for resistance formation against selective tyrosine kinase inhibitors. J Biol Chem 278:15435–15440
42. Carter TA, Wodicka LM, Shah NP, et al. (2005) Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. Proc Natl Acad Sci USA 102:11011–11016
43. Herbst RS, Prager D, Hermann R, et al. (2005) TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 23:5892–5899
44. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. (2005) Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353:123–132
45. Thatcher N, Chang A, Parikh P (2005) Results of a Phase III placebo-controlled study (ISEL) of gefitinib (IRESSA) plus best supportive care (BSC) in patients with advanced non-cmall-cell lung cancer (NSCLC) who had received 1 or 2 prior chemotherapy regimens. Proc Am Assoc Cancer Res 46 (suppl): Abstract# LB-6
46. Takano T, Ohe Y, Kusumoto M, et al. (2004) Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib. Lung Cancer 45:93–104
47. Bell DW, Lynch TJ, Haserlat SM, et al. (2005) Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 23:8081–8092
48. Hirsch FR, Varella-Garcia M, McCoy J, et al. (2005) Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 23:6838–6845
49. Giaccone G, Herbst RS, Manegold C, et al. (2004) Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial – INTACT 1. J Clin Oncol 22:777–784
50. Herbst RS, Giaccone G, Schiller JH, et al. (2004) Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial – INTACT 2. J Clin Oncol 22:785–794
51. Thatcher N, Chang A, Parikh P, et al. (2005) Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 366:1527–1537
52. Perez-Soler R (2004) Phase II clinical trial data with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib (OSI-774) in non-small-cell lung cancer. Clin Lung Cancer 6 Suppl 1:S20–S23
53. Kaneda H, Tamura K, Kurata T, et al. (2004) Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer. Lung Cancer 46:247–254
54. Huang SF, Liu HP, Li LH, et al. (2004) High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 10:8195–8203
55. Kris MG, Sandler A, Miller V, et al. (2004) Cigarette smoking history predicts sensitivity to erlotinib: results of a phase II trial in patients with bronchioloalveolar carcinoma (BAC). J Clin Oncol 22:Abstract# 7062
56. Lim ST, Wong EH, Chuah KL, et al. (2005) Gefitinib is more effective in never-smokers with non-small-cell lung cancer: experience among Asian patients. Br J Cancer 93:23–28
57. Lee DH, Han JY, Lee HG, et al. (2005) Gefitinib as a first-line therapy of advanced or metastatic adenocarcinoma of the lung in never-smokers. Clin Cancer Res 11:3032–3037
58. Zhang XT, Li LY, Mu XL, et al. (2005) The EGFR mutation and its correlation with response of gefitinib in previously treated Chinese patients with advanced non-small-cell lung cancer. Ann Oncol 16:1334–1342
59. Tokumo M, Toyooka S, Kiura K, et al. (2005) The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res 11:1167–1173
60. Pao W, Miller V, Zakowski M, et al. (2004) 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 USA 101:13306–13311
61. Yang SH, Mechanic LE, Yang P, et al. (2005) Mutations in the tyrosine kinase domain of the epidermal growth factor receptor in non-small cell lung cancer. Clin Cancer Res 11:2106–2110
62. Soung YH, Lee JW, Kim SY, et al. (2005) Mutational analysis of EGFR and K-RAS genes in lung adenocarcinomas. Virchows Arch 446:483–488
63. Qin BM, Chen X, Zhu JD, et al. (2005) Identification of EGFR kinase domain mutations among lung cancer patients in China: implication for targeted cancer therapy. Cell Res 15:212–217
64. Chou TY, Chiu CH, Li LH, et al. (2005) Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res 11:3750–3757
65. Sonobe M, Manabe T, Wada H, et al. (2005) Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma. Br J Cancer 93:355–363
66. Mu XL, Li LY, Zhang XT, et al. (2005) Gefitinib-sensitive mutations of the epidermal growth factor receptor tyrosine kinase domain in Chinese patients with non-small cell lung cancer. Clin Cancer Res 11:4289–4294
67. Tomizawa Y, Iijima H, Sunaga N, et al. (2005) Clinicopathologic significance of the mutations of the epidermal growth factor receptor gene in patients with non-small cell lung cancer. Clin Cancer Res 11:6816–6822