EGFR mutation: Significance as a stratification factor in the era of molecular-targeted therapy

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Abstract. Somatic mutations of epidermal growth factor receptor (EGFR) are the strongest predictive markers for the response to EGFR-tyrosine kinase inhibitors (TKIs). Patients with EGFR mutations generally receive EGFR-TKI treatment, and their survival has been significantly improved compared with that before the development of EGFR-TKIs. This study aimed to clarify the impact of EGFR mutational status on the survival of patients with non-small cell lung cancer (NSCLC) receiving cytotoxic agents, but not EGFR-TKIs, as their first-line chemotherapy. In addition, we analyzed patients with EGFR mutations to determine whether the timing of EGFR-TKI administration affects overall survival (OS). A total of 83 NSCLC patients with stage IIIB/IV who received chemotherapy alone and whose EGFR mutational status was known were investigated. Univariate and multivariate analysis for OS was performed using parameters such as age, gender, performance status (PS), histology, disease stage, smoking status, EGFR mutational status and administration of a first-line regimen. Among the 52 patients with EGFR mutations who received EGFR-TKIs, OS between those who received EGFR-TKIs as their first-line treatment and after chemotherapy were similar. Among the 83 patients who received cytotoxic agents as their first-line chemotherapy, the multivariate analysis showed OS to be significantly associated with PS ($p<0.001$), histology ($p=0.039$) and EGFR mutational status ($p=0.040$). OS was almost similar among the 52 patients with EGFR mutations who received EGFR-TKIs in a first- and second-line setting (25.6 vs. 26.8 months, $p=0.914$). The EGFR mutational status had a significant impact on the survival of NSCLC patients, although these patients did not receive EGFR-TKIs as their first-line chemotherapy. In future randomized trials, even when EGFR-TKIs are not included in experimental regimens, patients may need to be stratified by EGFR mutational status in order that study results be evaluated appropriately.

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Introduction

Lung cancer is the leading cause of cancer-related death in many industrialized countries. Platinum-based combination chemotherapy has been shown to improve survival and quality of life in patients with advanced non-small cell lung cancer (NSCLC). However, chemotherapy for advanced NSCLC has been of limited benefit and appears to have reached a plateau, with response rates of approximately 30% and a median survival period of 8 months (1-4). Various molecular-targeted agents were developed, a number of which are now standard treatment, with or without conventional cytotoxic agents (5-7). Among these agents, tyrosine kinase inhibitors (TKIs) of epidermal growth factor receptor (EGFR) have produced a marked change in the clinical practice of NSCLC.

At present, two different types of EGFR-TKIs are widely used: gefitinib and erlotinib. In predicting the efficacy of these agents, certain clinical factors, such as histology, gender, smoking status and ethnicity, are regarded as significant (8). Somatic mutations of the tyrosine kinase domain of EGFR were found and were shown to be the most reliable predictive marker for the response to EGFR-TKIs (8-10). Findings of a recent population-based study showed that EGFR mutations significantly predict both a survival benefit of gefitinib and a favorable prognosis in patients with advanced lung adenocarcinoma (11). In the recent version of the American Society of Clinical Oncology (ASCO) guideline, gefitinib was accepted as the first-line chemotherapy for patients with activating EGFR mutations (12). The survival benefit is substantial and patients who are known to have EGFR mutations usually receive EGFR-TKIs during the treatment period.

Consequently, the EGFR mutational status may need to be incorporated as a stratification factor in randomized clinical trials even when EGFR-TKIs are not included in the experimental regimens as they appear to strongly affect survival when used in a second-line setting or beyond. This study aimed to show the significance of the EGFR mutational status as a stratification factor for future randomized trials by

clarifying the impact of the EGFR mutational status on the survival of NSCLC patients receiving cytotoxic agents, but not EGFR-TKIs, as first-line chemotherapy. Additionally, patients with EGFR mutations were examined to determine whether the timing of EGFR-TKI administration plays a role in patient outcome.

Patients and methods

Patients. Between July 2003 and December 2009, 538 advanced (stage IIIB/IV) NSCLC patients were admitted to our department, and 327 patients received chemotherapy alone. Among them, 116 patients were examined for EGFR mutational status. Of the 116 patients, 83 received cytotoxic agents as their first-line treatment, and the remaining patients received EGFR-TKIs. Of the 116 patients, 52 had activating mutations of EGFR and also received EGFR-TKIs.

This study analyzed the correlation between clinical factors, including EGFR mutational status, evaluated prior to initial treatment, and overall survival (OS) in the 83 patients whose EGFR mutational status was known and who received cytotoxic agents as their first-line treatment (Cohort 1). Among the 52 patients who had EGFR mutations and received EGFR-TKIs (Cohort 2), OS was compared between the patients who received EGFR-TKIs as first-line treatment (first-line TKI group; n=24) and those who received EGFR-TKIs following chemotherapy (second-line TKI group; n=28).

Analysis of clinical factors. Analysis of factors such as age (<70/≥70 years), gender (female/male), Eastern Cooperative Oncology Group performance status (PS) (0-1/2-4), histology (adenocarcinoma/non-adenocarcinoma), disease stage (IIIB/IV), smoking status (+/-), EGFR mutational status (mutation/wild-type), and administration of a first-line regimen (platinum-based/single-agent) was carried out.

Mutational analysis of EGFR. Formalin-fixed paraffin-embedded tissue was cut into 6- to 8-mm sections and mounted on pretreated glass slides. Non-cancer cells and necrotic parts were manually removed from the slide under a microscope. The slides were deparaffinized, and DNA was extracted with phenol-chloroform and ethanol precipitation. The peptide nucleic acid/locked nucleic acid (PNALNA) polymerase chain reaction (PCR) clamp method, designed to detect 11 different EGFR mutations, was used for the determination of the EGFR gene mutation status in this study (13-15).

Tumor evaluation and statistical analysis. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) (16). OS was calculated from the commencement of first-line chemotherapy to either the time of death from any cause or the date when patients were last known to be alive. The survival curve was estimated using the Kaplan-Meier method and compared using the log-rank test. Individual clinical factors were compared using the χ^2 test. Multivariate analysis was conducted according to the Cox proportional hazards model. $P<0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS 11.0 statistical software (SPSS II for Windows, Standard version 11.0; SPSS Inc., Chicago, IL, USA).

Table I. Patient characteristics (Cohort 1).

Characteristics	n=83
Age (years)	
Median	65
Range	36-82
Gender	
Female	39
Male	44
Performance status	
0-1	70
2-4	13
Histology	
Adenocarcinoma	66
Squamous cell carcinoma	3
Large-cell carcinoma	14
Disease stage	
IIIB	16
IV	67
Smoking status	
Current smoker	20
Former smoker	25
Never smoker	38
EGFR	
Mutation	28
Wild-type	55
First-line chemotherapy	
Platinum-based	68
Single-agent	15
No. of regimens	
Median	3
Range	1-9
EGFR-TKI treatment	
+	52
-	31

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Results

Cohort 1

Patient characteristics. Table I shows the patient characteristics of Cohort 1. The median age of the patients was 65 years (range 36-82). Of the 83 patients, 39 (47%) were female, 66 (80%) had histologically confirmed adenocarcinoma and 38 (46%) were never smokers. Activated EGFR mutations were confirmed in 28 (34%) patients. A total of 68 (82%) patients received platinum-based regimens and 15 received a single-agent as their first-line chemotherapy. A total of 52 patients (63%) received EGFR-TKIs in a second-line setting or beyond. The EGFR mutant patients received EGFR-TKIs (100%), whereas 24 of 55 wild-type patients received EGFR-TKIs (44%).

Overall survival. According to the results of the univariate analysis, OS was significantly associated with gender

Table II. Univariate and multivariate analysis for overall survival (Cohort 1).

			Univariate analysis	Multivariate analysis		
Characteristics	No. of patients	MST (months)	p-value	Risk ratio	95% CI	p-value
Age						
<70	53	23.4	0.082			
≥70	30	10.7				
Gender						
Female	39	25.9	0.019	1.734	0.878-3.427	0.113
Male	44	15.1				
Performance status						
0-1	70	22.5	<0.001	3.674	1.780-7.581	<0.001
2-4	13	5.2				
Histology						
Adenocarcinoma	66	23.4	<0.001	2.113	1.040-4.294	0.039
Non-adenocarcinoma	17	8.1				
Disease stage						
IIIB	16	19.3	0.722			
IV	67	15.1				
Smoking status						
Current/former	45	13.4	0.007	0.829	0.403-1.706	0.610
Never	38	26.5				
EGFR						
Mutation	28	26.8	0.003	2.053	1.033-4.080	0.040
Wild-type	55	10.6				
First-line regimen						
Platinum-based	68	20.1	0.082			
Single-agent	15	10.3				

MST, median survival time; EGFR, epidermal growth factor receptor.

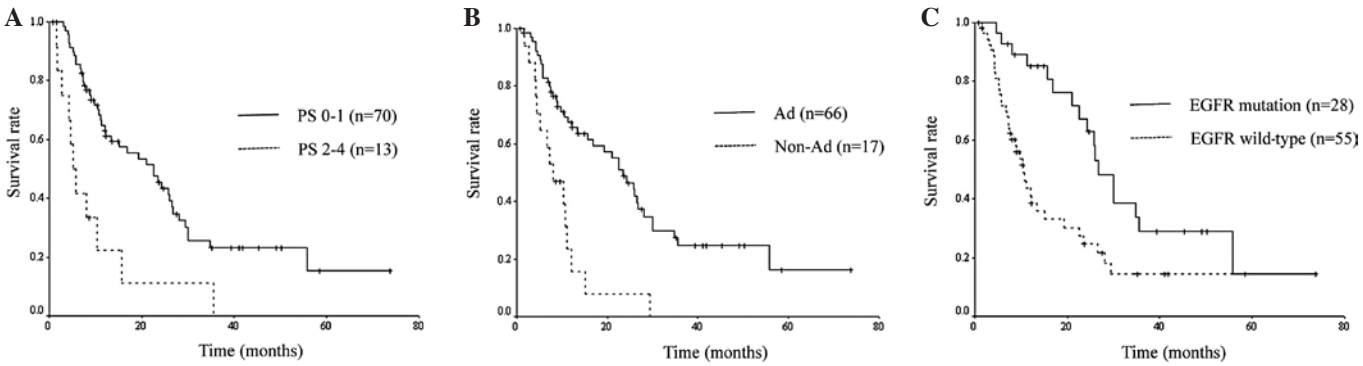

Figure 1. Overall survival curves according to (A) performance status (PS), (B) histology and (C) EGFR mutational status. Ad, adenocarcinoma.

(p=0.019), PS (p≤0.001), histology (p<0.001), smoking status (p=0.007) and EGFR mutational status (p=0.003). Multivariate analysis identified PS (p<0.001), histology (p=0.039) and EGFR mutational status (p=0.040) as independent prognostic factors for OS (Table II). Survival curves drawn according to PS, histology and EGFR mutational status are shown in Fig. 1.

Cohort 2

Patient characteristics. Table III shows the patient characteristics of Cohort 2. Compared to the second-line TKI group, the first-line TKI group comprised more elderly and more PS 2-4 patients, whereas the proportion of females was higher in the first-line TKI group. All patients had histologicaly confirmed adenocarcinoma.

Table III. Patient characteristics (Cohort 2).

Characteristics	First-line TKI group (n=24)	Second-line TKI group (n=28)	p-value
Age (years)			
Median	74	61	0.001
Range	34-86	39-74	
Gender			
Female	20	15	0.023
Male	4	13	
Performance status			
0-1	16	24	0.104
2-4	8	4	
Histology			
Adenocarcinoma	24	28	-
Non-adenocarcinoma	0	0	
Disease stage			
IIIB	4	5	0.910
IV	20	23	

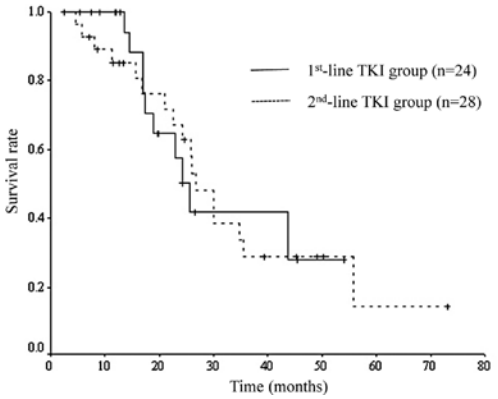

Figure 2. Overall survival curves according to the timing of EGFR-TKI administration in patients with EGFR mutations.

Overall survival. OS of the first-line TKI group was 25.6 months and that of the second-line TKI group was 26.8 months. No significant difference was noted between the two groups (p=0.914). Survival curves of the two groups are shown in Fig. 2.

Discussion

Recently, effective second-line chemotherapies have been developed using agents, such as docetaxel, pemetrexed, gefitinb and erlotinib, for the treatment of NSCLC (12). Therefore, OS is not necessarily the most favorable primary endpoint in randomized trials since the exact difference of effectiveness between the investigated regimens does not often translate into OS due to effective second-line chemotherapy or beyond. Consequently, progression-free survival has been selected as a primary endpoint in a number of recent randomized trials. However, progression-free survival is less reliable than OS due to its arbitrariness, and it remains debatable which is more adequate as a primary endpoint (17-19). Should a definite biomarker be found that predicts the response of second-line chemotherapy and patients are stratified based on such a biomarker, then OS is likely to be the most favorable endpoint.

The development of EGFR-TKIs and the finding of EGFR mutations is a significant event in chemotherapy for NSCLC, since individualized therapy is now feasible (20). Currently, the EGFR mutation is the most powerful and widespread biomarker in NSCLC. A number of biomarkers, such as excision repair cross-complementation group 1 (ERCC1)21 for cisplatin, ribonucleotide reductase subunit M1 (RRM1)22 for gemcitabine and thymidylate synthase (TS)23 for pemetrexed, have been identified. However, no other biomarker apart from the EGFR mutation has been of clinical use.

In the present study, the correlation between clinical factors, including the EGFR mutational status evaluated prior to the initial treatment, and OS was analyzed. We found that the EGFR mutational status was an independent prognostic factor for OS as well as PS and histology in NSCLC patients who received cytotoxic agents, but not EGFR-TKIs, as their first-line treatment. In addition, the efficacy of EGFR-TKIs was similar regardless of the timing of the administration when the patients had EGFR mutations, as previously reported (24). Patients who are known to have EGFR mutations are generally treated with EGFR-TKIs; in the present study, any patients who had EGFR mutations were treated with EGFR-TKIs.

The prevalence of EGFR mutations is much higher in Asia than in Western populations (8). Therefore, the importance of EGFR mutations as a stratification factor is prominent in Asian-oriented trials, although the same may not be the case of Western-oriented trials. Our results should be confirmed in non-Asian regions as well. More globalized clinical trials are currently underway and the role of EGFR mutations as a stratification factor appears to be of significance in such global trials.

In conclusion, the EGFR mutational status was an independent prognostic factor for survival in NSCLC patients who received cytotoxic agents, but not EGFR-TKIs, as their first-line chemotherapy. In future randomized trials, particularly in Asia, even when EGFR-TKIs are not included in the

experimental regimens, patients may need to be stratified by EGFR mutational status to for study results to be evaluated appropriately.

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