Original Study

Effectiveness of Treatments for Advanced Non–Small-Cell Lung Cancer With Exon 20 Insertion Epidermal Growth Factor Receptor Mutations

Jenn-Yu Wu,[1] Chong-Jen Yu,[2] Jin-Yuan Shih[2]

Abstract

There are no approved effective treatments in advanced non–small-cell lung cancer (NSCLC) patients with exon 20 insertion epidermal growth factor receptor (*EGFR*) mutations. In the present survey of 3805 NSCLC patients screened for *EGFR* mutations, 84 patients had an exon 20 insertion. First-line pemetrexed-containing therapies were related to better outcomes. The mutation Ala763_Tyr764insPheGlnGluAla (A763_Y764 insFQEA) was related to a favorable response to tyrosine kinase inhibitors.

Background: Two main categories of epidermal growth factor receptor (*EGFR*) mutations in non–small-cell lung cancer (NSCLC) patients are deletions in exon 19 and L858R in exon 21. Treatments of advanced NSCLC patients with these mutations are well documented, and clinical responses to tyrosine kinase inhibitors (TKIs) are favorable. However, effective treatments for patients with exon 20 insertion mutations are not well verified. We investigated the clinical response to various treatments (chemotherapy and TKIs) of advanced NSCLC patients with *EGFR* exon 20 insertions. **Patients and Methods:** In this study, specimens from 3805 NSCLC patients were examined for *EGFR* mutations. Different first-line treatments and their effectiveness in NSCLC patients with exon 20 insertion *EGFR* mutations were investigated. **Results:** In the cohort of the 3805 patients, 2112 (55.5%) had *EGFR* mutations, including 84 patients (4.0%) with exon 20 insertion mutations. Efficacy of different first-line chemotherapy or TKIs were evaluated. Pemetrexed-containing therapies were related to better progression-free survival (6.2 vs. 2.7 months; $P < .001$) and overall survival (28.0 vs. 15.4 months; $P = .009$) than regimens without pemetrexed. Patients with exon 20 insertions had a poor response to TKIs. However, the mutation Ala763_Tyr764insPheGlnGluAla (A763_Y764 insFQEA) was related to a favorable response to TKIs. **Conclusion:** Exon 20 insertion *EGFR* mutations comprised a considerable group of the entire population of patients with *EGFR* mutations. Different first-line treatments and variants of the exon 20 insertions might be related to different outcomes in advanced NSCLC patients.

Clinical Lung Cancer, Vol. 20, No. 6, e620-30
Keywords: Epidermal growth factor receptor, Exon 20, Lung cancer, Mutations, Pemetrexed

Introduction

Treatment for patients with non–small-cell lung cancer (NSCLC) has been affected by mutations of the epidermal growth factor receptor (EGFR).[1] Clinical factors associated with the occurrence of *EGFR* mutations in NSCLC are never-smoking, adenocarcinoma, female sex, and Asian ethnicity.[2-5] EGFR tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, afatinib, dacomitinib, and osimertinib are highly effective as antitumor treatments for NSCLC patients with *EGFR* mutations.[6-9]

These mutations are located mainly in exons 18 to 21 of the EGFR tyrosine kinase domain. L858R in exon 21 and deletions in

Presented in part at the 23rd Congress of the Asian Pacific Society of Respirology 2018.

[1]Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan
[2]Department of Internal Medicine, National Taiwan University Hospital, and College of Medicine, National Taiwan University, Taipei, Taiwan

Submitted: Mar 1, 2019; Revised: Apr 26, 2019; Accepted: Jun 15, 2019; Epub: Jun 26, 2019

Address for correspondence: Jin-Yuan Shih, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, No 7 Chung-Shan South Road, Taipei, Taiwan
Fax: 886-2-23582867; e-mail contact: jyshih@ntu.edu.tw

1525-7304/$ - see frontmatter
https://doi.org/10.1016/j.cllc.2019.06.018

Table 1 Demographics of Patients Screened for *EGFR* Mutations

	All Patients Screened for *EGFR* Mutations	Patients With *EGFR* Mutations	Patients With Wild Type *EGFR*	*P*[a]	Patients With Exon 20 Insertion or Duplication
Patient n	3805	2112	1693		84
Median Age (Range), y	66 (25-102)	65 (27-101)	67 (25-102)	.864	64 (32-93)
Sex				<.001	
Male	1891	850	1041		30
Female	1914	1262	652		54
Smoking				<.001	
Smoker	899	258	641		13
Never smoker	2906	1854	1052		71
Histology				<.001	
Adenocarcinoma	3251	2025	1226		80
Nonadenocarcinoma	554	87	467		4
***EGFR* Mutation**					
Deletion in exon 19		897			
L858R		919			
Others		296			

Abbreviation: EGFR = epidermal growth factor receptor.
[a]Comparison of patients with an *EGFR* mutation and patients with wild type *EGFR*.

exon 19 are 2 major *EGFR* mutations, and comprise more than 80% of total *EGFR* mutations.[2,3,10-12] Another large proportion of *EGFR* mutations is insertions (or in-frame duplications) in exon 20, which account for 4% to 10% of all *EGFR* mutations in NSCLC patients.[13-16] The mutations are located within codon 762 to codon 774, and have the characteristics of oncogenic driver mutations, such as exon 19 deletions and L858R.[14,17] However, patients with exon 20 insertions who undergo TKI treatments including gefitinib, erlotinib and afatinib do not respond as well as patients with exon 19 deletions and L858R. Apart from TKI treatments, the effectiveness of chemotherapy in NSCLC patients with exon 20 insertions is not well documented. To increase our understanding of treatments for patients with NSCLC with exon 20 insertions, we surveyed a large group of NSCLC patients who were screened for *EGFR* mutations, and studied the clinical characteristics and treatments of those with exon 20 insertion *EGFR* mutations.

Patients and Methods

Characteristics of Patients

We reviewed the records of all patients with NSCLC who were diagnosed at National Taiwan University Hospital. The patients' clinical data were surveyed and recorded. Those who had smoked <100 cigarettes in their lifetime were defined as never-smokers. All patients in the hospital received complete cancer staging, including whole-body bone scintigraphy and computed tomography imaging of the head, chest, and abdomen. Lung cancer histology was stratified on the basis of the World Health Organization pathology classification (version 8).[18] Complete data on treatments received and responsiveness to treatments were recorded. Specimens of tumors were acquired using either needle biopsy/aspiration or surgical procedures, and were genotyped for mutational analysis. The institutional review board of the hospital approved the study. At the time of procurement of tumor specimens, written informed consent for use of tissue in molecular analysis was acquired from the patients.

Effectiveness of Chemotherapy and EGFR TKI Treatment

Chemotherapy and TKI (gefitinib, erlotinib, or afatinib) treatments for advanced stage (stage IIIB or stage IV) NSCLC were identified and analyzed. Decisions on the timing and order of different chemotherapy regimens were made on the basis of the physicians' discretion. TKIs, including gefitinib, erlotinib, and afatinib, were prescribed at suggested dosages. Available chemotherapy drugs were platinum-based (including cisplatin and carboplatin), gemcitabine, taxanes (including docetaxel and paclitaxel), pemetrexed, and vinorelbine. Chest radiography and chest computed tomography scan (including liver and adrenal glands) were performed routinely (chest radiography every 2-4 weeks and computed tomography scan every 2-3 months) and as needed to confirm the response and progression of the disease. Treatment responses were defined as progressive disease, stable disease (SD), partial response (PR), and complete response (CR), on the basis of the criteria of the Response Evaluation Criteria in Solid Tumors version 1.1 group.[19] Response rate was defined as the patient number with CR and PR divided by the total patient number. Disease control status included CR, PR, and SD.[20] Overall survival (OS) was calculated from the date of initiation of chemotherapy or TKI treatment to death from any cause, or was censored at the last follow-up date. Progression-free survival (PFS) was defined as the time from the date of initiation of chemotherapy or TKI treatment to the date of disease progression or death. NSCLC patients diagnosed at the National Taiwan University Hospital between January 2000 and December 2017 were included in the study. The cutoff date for survival data collection was October 10, 2018.

Table 2 Summary of Clinical Information of Patients Treated With Chemotherapy or Tyrosine Kinase Inhibitors

Patient	Sex	Age, y	Smoking	Stage	Cell	*EGFR* Mutation	First-Line Regimen	Maximal Response	PFS, mo	OS, mo	Outcome
1	F	46	N	IV	AD	p. A763_Y764 insFQEA	Pem/Cis	PR	27.9	77.3	Alive
2	F	62	N	IV	AD	p. A763_Y764 insFQEA	Pem/Cis	PR	3.9	6.3	Dead
3	M	83	N	IV	AD	p. A763_Y764 insFQEA	Pem/Cis	PR	14.0	27.7	Alive
4	M	78	Y	IV	AD	p. A763_Y764 insFQEA	Pem/Cis	PD	2.8	6.5	Alive
5	F	72	N	IV	AD	p. A763_Y764 insFQEA	Gem/Cis	SD	7.0	17.0	Dead
6	F	42	N	IV	AD	p. A763_Y764 insFQEA	Gefitinib	PR	4.9	22.0	Dead
7	M	45	Y	IV	AD	p. A763_Y764 insFQEA	Erlotinib	PD	3.0	40.0	Dead
8	M	32	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	PR	2.7	17.3	Alive
9	F	62	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	PR	6.2	29.1	Dead
10	M	58	Y	IV	AD	p. A767_V769 dupASV	Pem/Cis	PR	6.7	14.6	Dead
11	F	49	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	SD	6.1	9.8	Dead
12	F	67	N	IIIb	AD	p. A767_V769 dupASV	Pem/Cis	SD	32.9	42.1	Alive
13	F	63	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	SD	7.1	7.1	Alive
14	F	62	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	SD	6.4	18.1	Dead
15	F	83	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	SD	3.4	5.9	Dead
16	F	60	N	IV	AD	p. A767_V769 dupASV	Pem/Cis	PD	3.2	10.6	Dead
17	F	61	N	IV	AD	p. A767_V769 dupASV	Pem	SD	14.3	58.8	Alive
18	M	54	N	IV	AD	p. A767_V769 dupASV	Tax/Cis	PR	5.4	37.2	Dead
19	M	54	N	IV	AD	p. A767_V769 dupASV	Tax/Cis	PD	2.9	2.9	Dead
20	F	59	Y	IV	AD	p. A767_V769 dupASV	Tax/Cis	PD	2.2	8.1	Dead
21	F	57	N	IV	AD	p. A767_V769 dupASV	Gem/Cis	PR	4.2	6.3	Dead
22	M	72	N	IV	SQ	p. A767_V769 dupASV	Gem/Cis	PD	2.2	8.4	Dead
23	F	41	N	IV	AD	p. A767_V769 dupASV	Gem/Cis	PD	1.0	1.0	Dead
24	F	76	N	IV	AD	p. A767_V769 dupASV	Vino	SD	5.0	15.4	Dead
25	F	51	N	IV	AD	p. A767_V769 dupASV	Vino	PD	3.9	13.9	Dead
26	F	60	N	IV	AD	p. A767_V769 dupASV	Gefitinib	PD	1.8	16.9	Dead
27	F	71	N	IV	AD	p. A767_V769 dupASV	Gefitinib	PD	0.8	43.5	Dead
28	F	84	N	IV	AD	p. A767_V769 dupASV	Gefitinib	PD	0.8	2.3	Dead
29	F	75	N	IV	AD	p. A767_V769 dupASV	Gefitinib	PD	1.9	4.8	Dead
30	M	87	Y	IV	AD	p. A767_V769 dupASV	Erlotinib	PD	3.0	16.8	Dead
31	M	58	N	IV	AD	p. A767_V769 dupASV	Afatinib	PD	2.7	35.0	Dead
32	M	66	N	IV	AD	p. S768_D770 dupSVD	Pem/Cis	SD	5.6	16.1	Dead
33	F	74	N	IV	AD	p. S768_D770 dupSVD	Pem/Cis	SD	9.0	9.0	Alive
34	F	93	N	IV	AD	p. S768_D770 dupSVD	Pem/Cis	PD	4.1	7.7	Dead
35	F	45	N	IV	AD	p. S768_D770 dupSVD	Pem/Cis	PD	1.0	28.0	Dead
36	M	58	N	IV	AD	p. S768_D770 dupSVD	Tax/Cis	PD	6.3	19.3	Dead

Table 2 Continued

Patient	Sex	Age, y	Smoking	Stage	Cell	*EGFR* Mutation	First-Line Regimen	Maximal Response	PFS, mo	OS, mo	Outcome
37	F	38	N	IV	AD	p. S768_D770 dupSVD	Gem/Cis	SD	7.0	28.8	Dead
38	F	53	N	IV	AD	p. S768_D770 dupSVD	Gem/Cis	PD	3.5	21.3	Dead
39	F	73	N	IV	AD	p. S768_D770 dupSVD	Gem	PD	0.9	3.1	Dead
40	F	63	N	IV	AD	p. S768_D770 dupSVD	Gefitinib	PD	1.9	2.9	Dead
41	F	40	N	IV	AD	p. S768_D770 dupSVD	Gefitinib	PD	3.9	34.6	Dead
42	F	48	N	IV	AD	p.V769_D770 insGSV	Gefitinib	PD	1.4	31.3	Dead
43	M	72	N	IV	AD	p. del 770D insGY	Gefitinib	PD	0.5	9.2	Dead
44	M	81	N	IV	AD	p. del 770D insGY	Erlotinib	PD	0.9	0.9	Dead
45	F	75	N	IV	AD	p. D770_N771 insG	Pem/Cis	PD	2.4	6.2	Dead
46	F	44	Y	IV	AD	p. D770_N771 insG	Gefitinib	PD	1.5	16.5	Dead
47	F	57	N	IIIb	AD	p. D770_N771 insGTT	Tax	PD	0.6	15.9	Dead
48	F	60	N	IV	AD	p. D770_N771 insY	Pem/Cis	PD	1.9	42.1	Dead
49	M	49	N	IV	AD	p. D770_P772 del 771N insTH	Tax/Cis	PD	4.1	21.0	Dead
50	M	84	N	IV	AD	p. del N771 insKH	Gem	PD	2.2	4.7	Dead
51	F	73	N	IV	AD	p. N771_P772 insS	Afatinib	PD	2.8	9.2	Dead
52	F	75	N	IV	AD	p. N771_H773 dupNPH	Pem/Cis	SD	13.8	17.9	Alive
53	F	62	N	IV	AD	p. N771_H773 dupNPH	Tax/Cis	PD	3.4	7.2	Dead
54	F	76	N	IV	AD	p. N771_H773 dupNPH	Gem	PD	1.4	1.8	Dead
55	M	67	N	IV	AD	p. P772_H773 insGHP	Pem/Cis	SD	24.5	41.0	Alive
56	M	67	Y	IV	AD	p. P772_H773 dupPH	Gem/Cis	PD	4.7	9.0	Dead
57	F	49	N	IIIb	AD	p. P772_H773 insTNP	Gefitinib	PD	0.8	75.3	Dead
58	F	64	N	IV	AD	p. H773_V774 insH	Pem/Cis	SD	7.1	22.2	Dead
59	M	57	N	IV	AD	p. H773_V774 dupHV	Pem/Cis	PR	8.1	31.5	Dead

Abbreviations: AD = adenocarcinoma; Cis = cisplatin; F = female; Gem = gemcitabine; M = male; N = no; OS = overall survival after treatment; Pem = pemetrexed; PFS = progression-free survival; SQ = squamous-cell carcinoma; Tax = taxanes; Vino = vinorelbine; Y = yes; A763_Y764 insFQEA = Ala763_Tyr764 insPheGlnGluAla; A767_V769 dupASV = Ala767_Val769 dupAlaSerVal; S768_D770 dupSVD = Ser768_Asp770 dupSerValAsp; V769_D770 insGSV = Val769_Asp770 insGlySerVal; del 770D insGY = deletion 770Asp insGlyTyr; D770_N771 insG = Asp770_Asn771 insGly; D770_N771 insGTT = Asp770_Asn771 insGlyThrThr; D770_N771 insY = Asp770_Asn771 insTyr; D770_P772 del 771N insTH = Asp770_Pro772 deletion 771Asn insThrHis; del N771 insKH = deletion 771Asn insLysHis; N771_P772 insS = Asn771_Pro772 insSer; N771_H773 dupNPH = Asn771_His773 dupAsnProHis; P772_H773 insGHP = Pro772_His773 insGlyHisPro; P772_H773 dupPH = Pro772_His773 dupProHis; P772_H773 insTNP = Pro772_His773 insThrAsnPro; H773_V774 insH = His773_Val774 insHis; H773_V774 dupHV = His773_Val774 dupHisVal.

Table 3 Summary of Clinical Information of Patients Who Did Not Receive Chemotherapy or EGFR TKIs

Patient	Sex	Age, y	Smoking	Stage	Cell	*EGFR* Mutation	Survival Time, mo[a]	Outcome
60	F	45	N	Ia	AD	p. A763_Y764 insFQEA	81.2	Alive
61	M	73	Y	Ia	AD	p. A763_Y764 insFQEA	54.4	Alive
62	F	77	N	Ia	AD	p. A767_V769 dupASV	87.9	Alive
63	F	65	N	Ib	AD	p. A767_V769 dupASV	148.3	Alive
64	F	68	N	Ib	AD	p. A767_V769 dupASV	86.8	Alive
65	M	68	Y	IV	AD	p. A767_V769 dupASV	1.1	Dead
66	M	53	Y	IV	AD	p. A767_V769 dupASV	2.0	Alive[b]
67	F	69	N	IV	AD	p. A767_V769 dupASV	0.8	Alive[b]
68	F	89	N	IV	AD	p. S768_D770 dupSVD	1.7	Dead
69	M	77	N	IV	SQ	p. S768_D770 dupSVD	0.7	Dead
70	F	87	N	IV	AD	p. S768_D770 dupSVD	34.9	Dead
71	M	83	Y	IV	AD	p. S768_D770 dupSVD	0.6	Dead
72	M	83	Y	IV	AD	p. S768_D770 dupSVD	1.8	Dead
73	M	83	Y	IV	SQ	p. S768_D770 dupSVD	1.7	Alive[b]
74	F	61	N	IIb	AD	p. S768_D770 dupSVD	0.7	Alive
75	F	49	N	Ia	AD	p. del D770 insGY	61.6	Alive
76	F	62	N	Ib	AD	p. D770_N771 insG	8.8	Alive
77	M	60	Y	IIIa	SQ	p. D770_N771 insG	5.5	Dead
78	M	74	N	IV	AD	p. N771_P772 dupN	1.9	Alive[b]
79	M	61	N	IV	AD	p. N771_P772 dupN	0.6	Alive[b]
80	F	48	N	IV	AD	p. N771_H773 dupNPH	5.9	Dead
81	F	50	N	IV	AD	p. N771_H773 dupNPH	0.8	Alive[b]
82	F	78	N	Ib	AD	p. N771_H773 dupNPH + p. H773_V774 insG	65.8	Alive
83	F	72	N	Ib	AD	p. P772_C775 dupPHVC	37.5	Alive
84	M	74	N	IV	AD	p. H773_V774 insGTNPH	1.3	Alive[b]

Abbreviations: AD = adenocarcinoma; EGFR = epidermal growth factor receptor; F = female; M = male; N = no; SQ = squamous cell carcinoma; TKI = tyrosine kinase inhibitor; Y = yes; A763_Y764 insFQEA = Ala763_Tyr764 insPheGlnGluAla; A767_V769 dupASV = Ala767_Val769 dupAlaSerVal; S768_D770 dupSVD = Ser768_Asp770 dupSerValAsp; del D770 insGY = deletion Asp770 insGlyTyr; D770_N771 insG = Asp770_Asn771 insGly; N771_P772 dupN = Asn771_Pro772 dupAsn; N771_H773 dupNPH = Asn771_His773 dupAsnProHis; H773_V774 insG = His773_Val774 insGly; P772_C775 dupPHVC = Pro772_Cys775 dupProHisValCys; H773_V774 insGTNPH = His773_V774 insGlyThrAsnProHis.
[a]From disease diagnosis to date of death date or last follow-up.
[b]Lost to follow-up.

Detection of EGFR *Mutation*

Mutational analysis was performed with tumor specimens, including fine-needle biopsy specimens, frozen tissues, or paraffin blocks of surgical specimens, and pleural effusions. Procurement of tumor specimens for *EGFR* mutational testing was performed before EGFR TKI treatment. Some data have been presented in our previous studies with reference to *EGFR* mutations.[5,13,21-24]

Tumor DNA from embedded paraffin blocks of tumors, and mRNA extracted from cell pellets from pleural effusion or frozen cancer tissue were sequenced. We used primer pairs to cover exons 18, 19, 20, and 21 of *EGFR*. The reverse transcription polymerase chain reaction (PCR) conditions and the primers used were reported in our previous study.[21] The cDNA amplicons were purified and sequenced. All sequencing reactions were done in forward and reverse directions using tracings from at least 2 PCRs. Direct sequencing can detect all of the variants of exon 20 insertion.

Statistical Analyses

To examine the relationship between categorical variables, Fisher exact test or χ^2 tests were performed where appropriate. Kaplan–Meier analysis was used to determine the PFS and OS, and differences between treatments (different chemotherapy or TKI treatments) were compared using the log rank test. All P values were 2-sided and a P value $< .05$ was considered significant. Statistical analyses were done with SPSS software version 21.0 (IBM Corp).

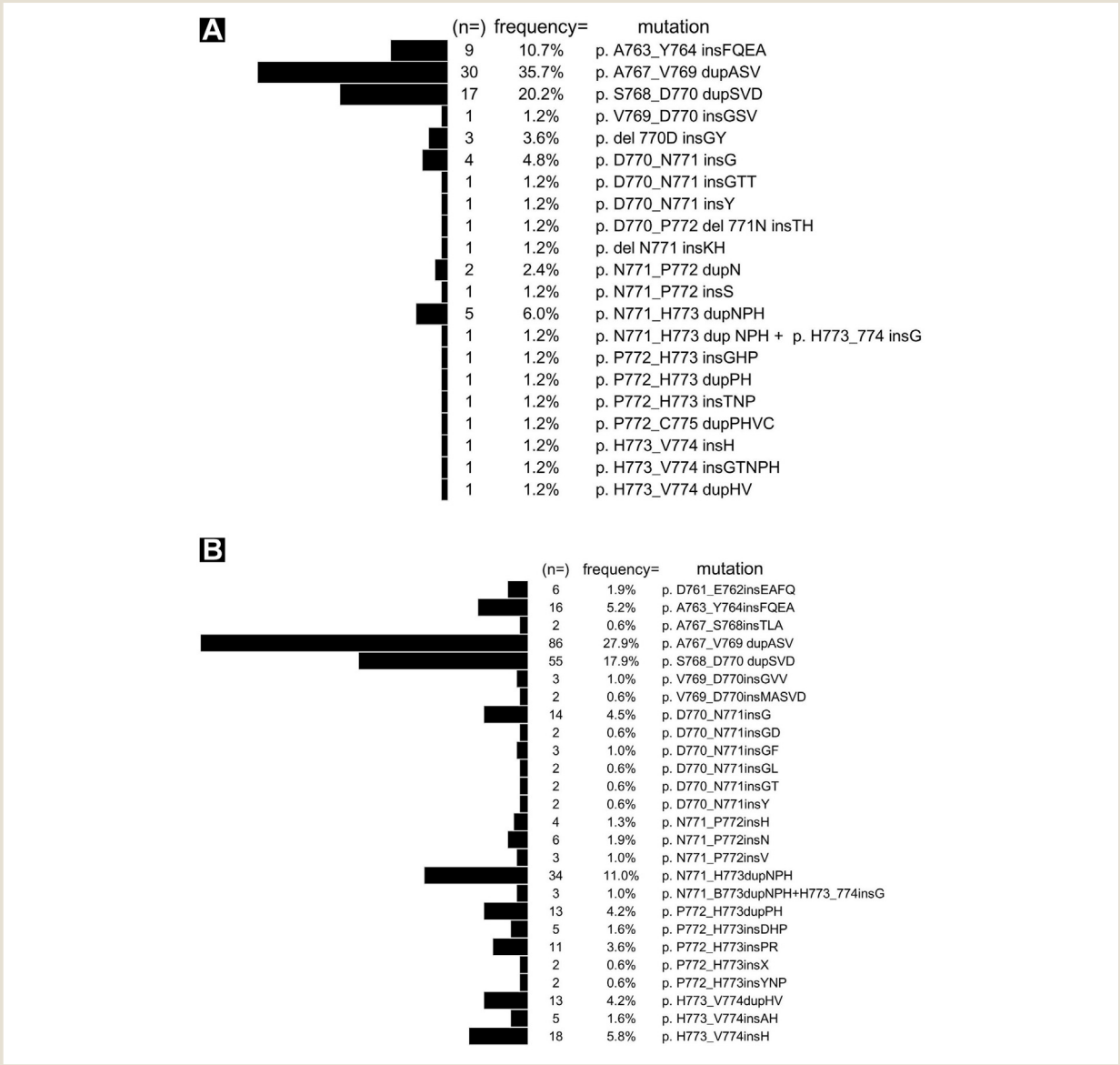
Results

Characteristics of Patients

Tumor specimens, which were obtained from surgical specimens, fine-needle biopsies, and body effusions (ie, pleural effusion, cerebrospinal fluid, ascites) from a total of 3805 NSCLC patients, were sequenced for *EGFR* mutations. Of the 3805 patients, 2112 (55.5%) had *EGFR* mutations. The *EGFR* mutations were more frequent in women, never-smokers, and in those with adenocarcinoma (Table 1).

Two major *EGFR* mutations were single deletions in exon 19 (897 patients [42.5%]) and L858R in exon 21 (919 patients [43.5%]) of the 2112 patients who had *EGFR* mutation, and 296 patients (14.0%) had other types of *EGFR* mutations. Eighty-four patients (84/2112 [4.0%]) had exon 20 insertion *EGFR*

Figure 1 Frequency of Specific *EGFR* Exon 20 Insertions (A) in the Study, and the Proportions of Different Exon 20 Insertion *EGFR* Mutations in Our Study and Those in the Literature (B)[25]



mutations. Some of these patients with exon 20 insertions were previously reported in our published studies.[3,13,24] The characteristics of the 3805 patients (*EGFR* mutation-positive and wild type *EGFR*), and the patients with exon 20 insertion mutations are presented in Table 1.

Variants of Exon 20 Insertions

The group of exon 20 insertions was composed of many variants. The most common variant of exon 20 insertion was Ala767_Val769 dupAlaSerVal (A767_V769 dupASV), which constituted 35.7% (30/84) of this cohort (Tables 2 and 3). The second and third most common variants were Ser768_Asp770 dupSerValAsp (S768_D770 dupSVD) (17/84 [20.2%]) and Ala763_Tyr764insPheGlnGluAla (A763_Y764 insFQEA) (9/84; 10.7%). The frequency of each individual exon 20 insertion *EGFR* mutation in this study are presented in Figure 1A. We also searched the Catalogue of Somatic Mutations in Cancer Web site database.[25] We used the database, filtered primary lung sites, found mutational type exon 20 insertion, and removed unclear cases. Exon 20 insertion mutations that were reported in the literature are presented in Figure 1B.

First-Line Treatments for Patients With Exon 20 Insertions

The treatment courses of all 84 patients with exon 20 insertion *EGFR* mutations were thoroughly surveyed. Fifty-nine patients had received chemotherapy or an EGFR TKI as first-line treatment

Table 4 Comparison of Different Categories of Chemotherapy or TKI

	Patient Number	Sex M/F	ECOG 0 to 2/3 or 4	Age Median (Range)	Median PFS	Median OS
Pemetrexed-Containing						
Yes	24	7/17	24/0	63 (32-93)	6.2	28.0
No	35	12/23	35/0	59 (38-87)	2.7	15.4
P	—	—	—	.420	<.001	.009
TKI-Containing						
Yes	16	5/11	16/0	62 (40-87)	1.8	16.8
No	43	14/29	43/0	62 (32-93)	4.2	16.1
P	—	—	—	.869	<.001	.941
Taxane-Containing						
Yes	7	4/3	7/0	57 (49-62)	3.4	15.9
No	52	15/37	52/0	63 (32-93)	3.4	16.9
P	—	—	—	.195	.361	.287
Gemcitabine-Containing						
Yes	10	3/7	10/0	70 (38-84)	3.4	6.3
No	49	16/33	49/0	62 (32-93)	2.2	18.1
P	—	—	—	.840	.297	.003

Abbreviations: ECOG = Eastern Cooperative Oncology Group performance status; F = female; M = male; OS = overall survival after treatment; PFS = progression-free survival; TKI = tyrosine kinase inhibitor.

(Table 2). Of the 59 patients who received first-line systemic therapies, 24 received pemetrexed-containing regimens, 7 received taxane-containing regimens, 10 received gemcitabine-containing regimens, and 16 patients received an EGFR TKI as first-line treatment.

First-line pemetrexed-containing regimens were associated with better PFS than regimens without pemetrexed (6.2 vs. 2.7 month; $P < .001$; Table 4 and Figure 2A). In patients treated with first-line pemetrexed-containing regimens, the response rate was 29% (7/24) and the disease control rate was 75% (18/24). OS after the start of first-line treatment was also longer in patients treated with pemetrexed-containing regimens (28.0 vs. 15.4 months; $P = .009$; Figure 2B).

Tyrosine Kinase Inhibitor Treatments in Patients With Exon 20 Insertions

The first-line TKI treatment response rate was 6.3% (1 patient had a PR, 15 had progressive disease). PFS after the start of first-line TKI treatment was not as favorable as that with chemotherapy treatment (1.8 vs. 4.2 months; $P < .001$; Figure 3A). There was no difference in OS between patients treated with first-line TKI and those who underwent first-line chemotherapy (16.8 vs. 16.1 months; $P = .941$; Figure 3B), possibly because most patients who received first-line TKIs also received second-line chemotherapy. Thirteen of 16 patients who received first-line TKIs received second-line chemotherapy (5 pemetrexed-containing regimens, 4 gemcitabine-containing regimens, 3 taxane-containing regimens, and 1 vinorelbine-containing regimen), with a median PFS of 4.8 months.

We collected data on the effectiveness of different lines of TKI treatment in patients with exon 20 insertions. A total of 39 patients received TKI treatment (including 16 patients as first-line, 8 as second-line, and 15 as third- or later line treatment). The objective response rate was 5.1% (2/39) and the disease control rate was 10.3% (4/39).

It is notable that of the 4 exon 20 insertion patients with disease control with TKI treatment, 3 patients had an A763_Y764 insFQEA mutation (2 PR, 1 SD). A total of 5 patients with A763_Y764 insFQEA received TKI treatment (2 patients as first-line, 1 as second-line, and 2 as third- or later line treatment), and the response rate was 40% (2/5) and the disease control rate was 60% (3/5). In all other exon 20 insertion patients, the response rate was 0% (0/34) and the disease control rate was 2.9% (1/34).

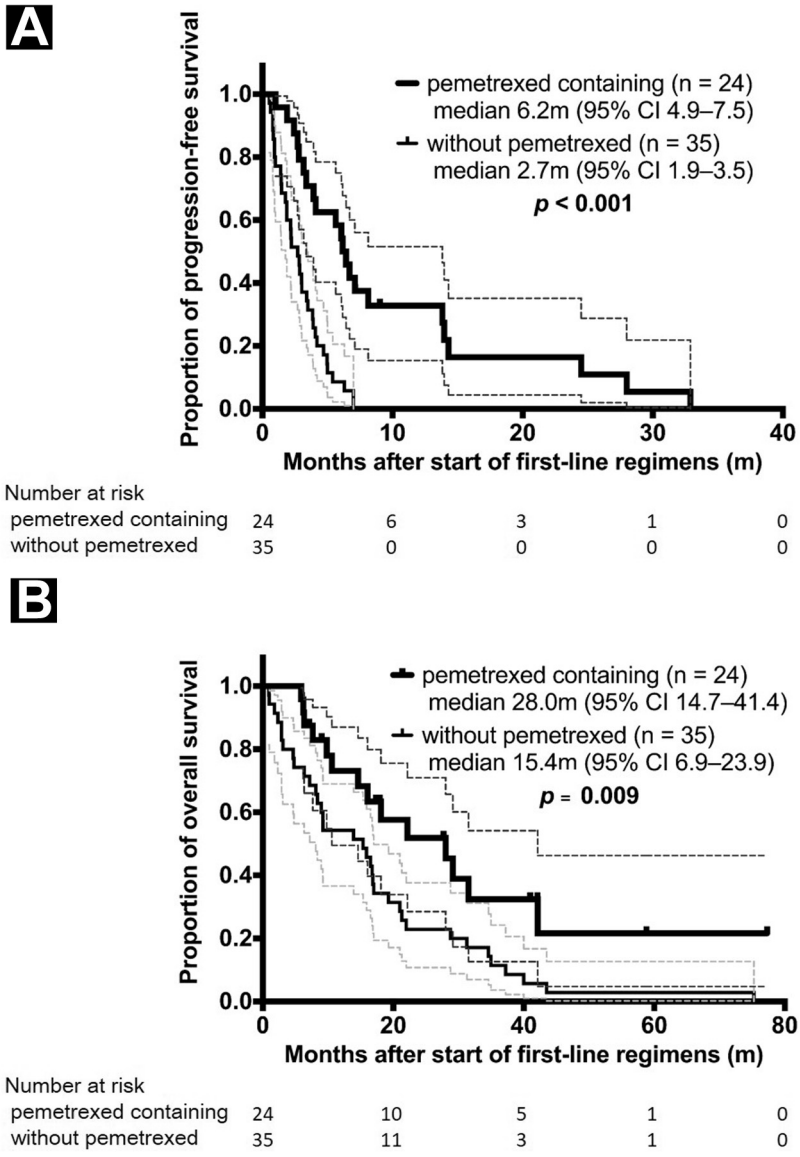
Overall Survival of Patients With Different EGFR *Mutations*

The effectiveness of TKI treatments are favorable in patients with classical *EGFR* mutations (deletions in exon 19 and L858R) and in patents with other uncommon mutations (such as G719X or L861X), than in patients with exon 20 insertions. We are interested in the OS of advanced NSCLC patients with different *EGFR* mutations. We compared OS after first-line antitumor treatment of patients with different *EGFR* mutations who received at least 1 line of treatment. Patients with classical mutations and other uncommon mutations had better survival than those with exon 20 insertions (Figure 4).

Patients Without Chemotherapy or EGFR TKI Treatment

Of the 84 patients with exon 20 insertion mutations, 25 were not given chemotherapy or EGFR TKI treatment (Table 3). Nine patients (cases 60, 61, 62, 63, 64, 75, 76, 82, 83) had been diagnosed at an early stage, and underwent surgery without subsequent recurrence. Seven patients (cases 65, 68, 69, 70, 71, 72, 80) received supportive care after their diseases were diagnosed at an

Figure 2 Comparison of Progression-Free Survival (A) and Overall Survival (B) After the Start of First-Line Treatment With and Without Pemetrexed in Patients With Exon 20 Insertions

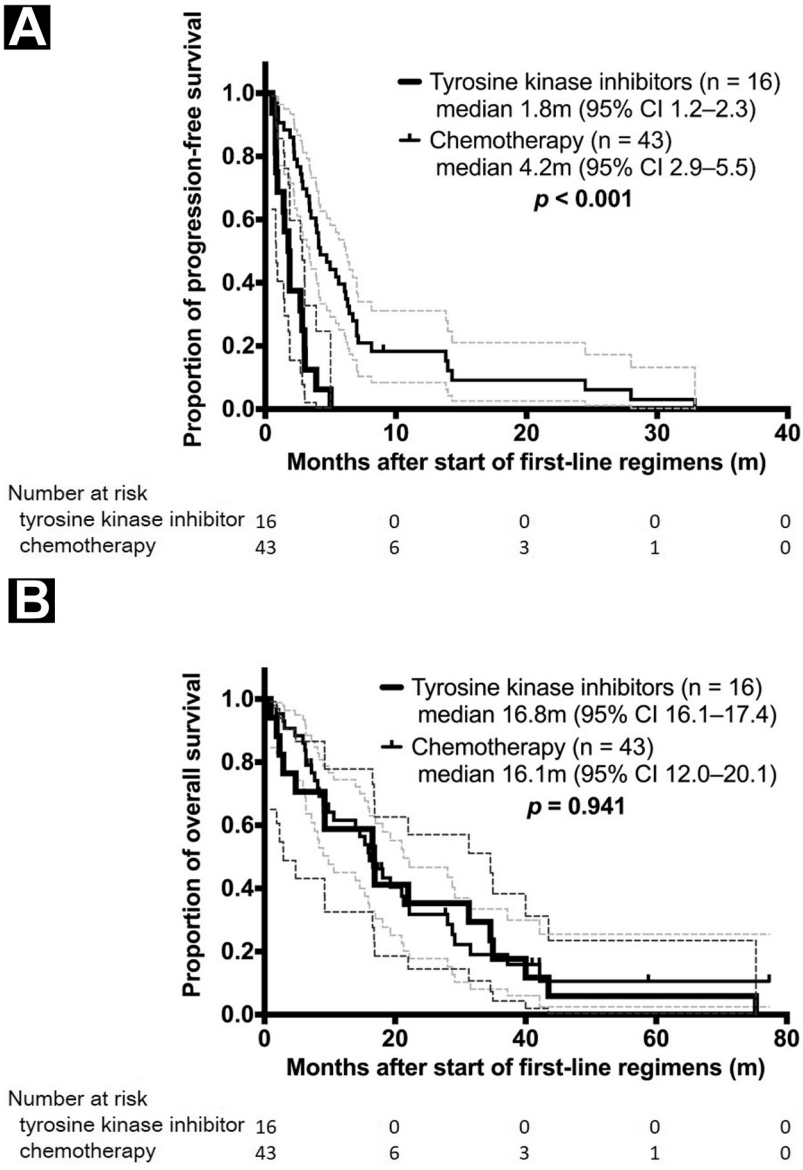


advanced stage. Seven patients (cases 66, 67, 73, 78, 79, 81, 84) were treated in other hospitals after a lung cancer diagnosis was made. We tried to survey the treatments of these 7 patients in other hospitals from the National Health Insurance Research Database of Taiwan, but could not obtain the data because all data are delinked and encrypted to protect patient privacy.[26] One patient underwent surgery for his stage IIIa tumor after neoadjuvant chemotherapy, but he died 1 month later because of unrelated complications without evidence of cancer recurrence (case 77). One patient underwent surgery at the initial IIb stage, and later was found to have a solitary brain metastasis (case 74). The patient received brain tumor excision and whole-brain radiotherapy 8 years before this writing, and was still alive with disease-free status at the data cutoff date.

Discussion

The *EGFR* exon 20 insertion mutation comprises a distinct proportion of all *EGFR* mutations in lung cancer. Our study was composed of a relatively large cohort of patients who received screening for *EGFR* mutations, and included 84 patients with exon 20 insertions. Exon 20 insertions accounted for 4.0% of all *EGFR* mutations in our study, and they were found in 2.2% (84/3805) of the NSCLC patient cohort. Among the first-line treatments for advanced NSCLC, pemetrexed-containing regimens were associated with better outcomes (PFS and OS) than regimens without pemetrexed. In general, responses to TKI were not favorable in patients with an exon 20 insertion, although patients with A763_Y764 insFQEA might have a good response to TKIs.

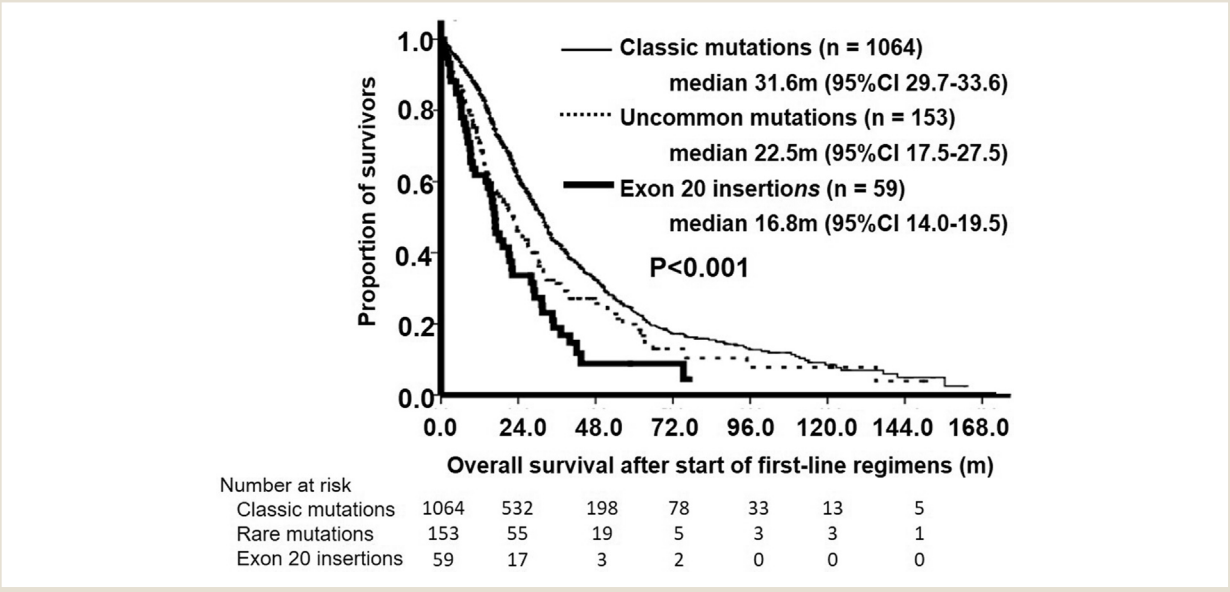
Figure 3 Comparison of Progression-Free Survival (A) and Overall Survival (B) After the Start of First-Line Tyrosine Kinase Inhibitors and First-Line Chemotherapy in Patients With Exon 20 Insertions



The effectiveness of chemotherapy in *EGFR*-mutant patients has been reported in many studies, and most of the mutations in these studies were L858R or exon 19 deletions. In their study, Sequist et al[8] compared a first-line TKI (afatinib, 230 patients) and chemotherapy (pemetrexed with cisplatin, 115 patients) in *EGFR*-mutant patients. The median PFS was 6.9 months for the chemotherapy group. L858R with exon 19 deletions accounted for 90.5% (104/115 patients) of the chemotherapy group, and only 3 patients had exon 20 insertion mutations. In their study, Wu et al[9] compared a first-line TKI (afatinib, 242 patients) with chemotherapy (gemcitabine with cisplatin, 122 patients) in patients with *EGFR* mutations, and the PFS was 5.6 months for the chemotherapy group. In the chemotherapy group, 108 patients had L858R or exon 19 deletion mutations. Only 1 patient had an exon 20 insertion; that patient had SD after chemotherapy and the PFS was 5.2 months. Maemondo et al[27] compared the use of a first-line TKI (gefitinib, 114 patients) with chemotherapy (paclitaxel with carboplatin, 114 patients) in *EGFR*-mutant patients; the median PFS was 5.5 months for the chemotherapy group. No patient in this study had an exon 20 insertion.[27]

As far as we can determine, ours is the largest study to investigate the efficacy of chemotherapy or TKI treatments and to focus on exon 20 insertion patients. Use of first-line pemetrexed-containing regimens is related to better PFS and OS than regimens without pemetrexed. Our study results might help clinical physicians choose

Figure 4 Overall Survival After the Start of First-Line Treatment in Patients With Different *EGFR* Mutations



the treatment for advanced NSCLC patients with exon 20 insertion *EGFR* mutations.

In the in vitro study by Yasuda et al,[28] cells with T790M and most of the cells with exon 20 insertion mutations had a high 50% inhibitory concentration of erlotinib. Only 1 cell line with exon 20 insertions (A763_Y764 insFQEA) was inhibited by a low erlotinib concentration, similar to cells with TKI-sensitive mutations (deletion Leu747_Pro753 insSer and L858R). The most common exon 20 insertion mutations are within the amino acids Y764 to V774, and are nonsensitizing to TKIs. A763_Y764 insFQEA is different from most other exon 20 insertions. It shifts the register of the C-helix toward its N-terminus and alters the length of the β3-αC loop. At a structural and enzyme kinetic level, A763_Y764 insFQEA might be more similar to L858R and exon 19 deletion mutations.

Because the current TKI treatments for patients with exon 20 insertion mutations are not promising, the development of new regimens is urgent and ongoing. Osimertinib, a new-generation of TKI, was approved by both the Food and Drug Administration and European Commission for the treatment of patients with T790M, which is resistant to gefitinib and erlotinib. Osimertinib showed effectiveness with cell lines harboring exon 20 insertions (Asp770_Asn771 dupSerValAsp and A767_V769 dupASV) in vitro and with *EGFR*-mutant xenograft mice in vivo.[29] Another new TKI, TAS6417, effected a regression of tumors with exon 20 insertion mutations that was more potent than that of wild type *EGFR*, in a cell model and an animal study.[30] The effectiveness of poziotinib, a pan-erythroblastic oncogene B (pan-ErbB) inhibitor, against *EGFR* exon 20 insertions was investigated in a phase II interventional study (NCT03066206), and showed a 64% (7/11) response rate.[31] Potent treatments of patients with exon 20 insertions are anticipated and can be expected. As we wait for these potent treatments to be realized, we should consider the use of more effective pemetrexed-containing regimens than we currently have.

In the literature, exon 20 *EGFR* mutations account for 4% to 10% of all *EGFR* mutations in NSCLC patients.[13-16] In one recent Korean study, the prevalence of exon 20 insertion was 1.6% (56 of 3539 patients with positive *EGFR* mutations).[32] In our study, exon 20 insertions constituted 4.0% of all *EGFR* mutations using direct sequencing. However, the methods we used in this study could sequence the whole exon 20 of *EGFR*. As we know, next-generation sequencing with higher sensitivity might detect low-frequency *EGFR* mutation variants that are not detected using direct sequencing. More exon 20 insertion mutations might be detected if next-generation sequencing methods are comprehensively used.

In this study, 9 patients with exon 20 insertion *EGFR* mutations were diagnosed in early stages and received tumor resection. They were followed-up regularly, and no recurrence of malignancy was noted. Suggestions for follow-up and management for early stage NSCLC patients with exon 20 insertions are not clarified, and standard management for NSCLC patients might be reasonable for these patients.

Conclusion

In our study we investigated, to our knowledge, the largest group of lung cancer patients with exon 20 insertion *EGFR* mutations, and the effectiveness of various treatments for patients with advanced NSCLC with exon 20 insertions. These exon 20 insertion mutations constitute a considerable part of all *EGFR* mutations. Treatments with pemetrexed-containing regimens are associated with better clinical efficacy. Effectiveness of TKIs (afatinib, erlotinib, and gefitinib) was not favorable in patients with exon 20 insertions, except in those with A763_Y764 insFQEA. These study results

might assist physicians in choosing treatments for NSCLC patients with exon 20 insertion *EGFR* mutations.

Clinical Practice Points

- Most studies that reported the effectiveness of chemotherapy in *EGFR*-mutant patients included those with L858R in exon 21 or deletions in exon 19, and very few studies validated treatment effectiveness in patients with exon 20 insertions.
- This was a large series of patients with *EGFR* exon 20 insertions treated with different chemotherapy regimens or TKIs.
- Among the first-line treatments for advanced NSCLC patients with exon 20 insertions, pemetrexed-containing regimens were associated with better outcomes than regimens without pemetrexed.
- Effectiveness of first-line TKIs were not favorable in most patients with an exon 20 insertion, although patients with A763_Y764 insFQEA might have a good response to TKIs.

Acknowledgments

This study was supported by grants MOHW103-TDU-PB-211-144002 (Department of Health, Executive Yuan, Taiwan), and 104-S2675, 105-S3032, 106-S3488 (National Taiwan University Hospital, Taiwan).

Disclosure

Jin-Yuan Shih has served as an advisory board member and received speaking honoraria from AstraZeneca, Roche, Boehringer Ingelheim, Eli Lilly, Pfizer, Novartis, Merck Sharp & Dohme, Ono Pharmaceutical Chugai, AbbVie, and Bristol-Myers Squibb. The remaining authors have stated that they have no conflicts of interest.

References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361:947-57.
2. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009; 361:958-67.
3. Wu JY, Yu CJ, Yang CH, et al. First- or second-line therapy with gefitinib produces equal survival in non−small-cell lung cancer. *Am J Respir Crit Care Med* 2008; 178:847-53.
4. Mitsudomi T, Kosaka T, Endoh H, et al. 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* 2005; 23:2513-20.
5. Shih JY, Gow CH, Yu CJ, et al. Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced non−small-cell lung cancer. *Int J Cancer* 2006; 118: 963-9.
6. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005; 353:133-44.
7. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non−small-cell lung cancer. *J Clin Oncol* 2006; 24:5034-42.
8. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013; 31:3327-34.
9. Wu YL, Zhou C, Hu CP, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non−small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *J Clin Oncol* 2013; 15:213-22.
10. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497-500.
11. Gu D, Scaringe WA, Li K, et al. Database of somatic mutations in EGFR with analyses revealing indel hotspots but no smoking-associated signature. *Hum Mutat* 2007; 28:760-70.
12. Pallis AG, Voutsina A, Kalikaki A, et al. "Classical" but not "other" mutations of EGFR kinase domain are associated with clinical outcome in gefitinib-treated patients with non−small-cell lung cancer. *Br J Cancer* 2007; 97:1560-6.
13. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin Cancer Res* 2008; 14:4877-82.
14. Greulich H, Chen TH, Feng W, et al. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005; 2:e313.
15. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non−small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol* 2012; 13:e23-31.
16. Oxnard GR, Lo PC, Nishino M, et al. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. *J Thorac Oncol* 2013; 8: 179-84.
17. Costa DB. Kinase inhibitor-responsive genotypes in EGFR mutated lung adenocarcinomas: moving past common point mutations or indels into uncommon kinase domain duplications and rearrangements. *Transl Lung Cancer Res* 2016; 5: 332-7.
18. Travis W, Brambilla E, Burke AP, Marx A, Nicholson AG. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Fourth edition. Lyon: IARC Press; 2015.
19. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version1.1). *Eur J Cancer* 2009; 45: 228-47.
20. Lara PN Jr, Redman MW, Kelly K, et al. Disease control rate at 8 weeks predicts clinical benefit in advanced non−small-cell lung cancer: results from Southwest Oncology Group randomized trials. *J Clin Oncol* 2008; 26:463-7.
21. Wu SG, Gow CH, Yu CJ, et al. Frequent epidermal growth factor receptor gene mutations in malignant pleural effusion of lung adenocarcinoma. *Eur Respir J* 2008; 32:924-30.
22. Wu JY, Yu CJ, Shih JY, et al. Influence of first-line chemotherapy and EGFR mutations on second-line gefitinib in advanced non−small-cell lung cancer. *Lung Cancer* 2010; 67:348-54.
23. Wu JY, Wu SG, Yang CH, et al. Comparison of gefitinib and erlotinib in advanced NSCLC and the effect of EGFR mutations. *Lung Cancer* 2011; 72: 205-12.
24. Lin YT, Liu YN, Wu SG, et al. Epidermal growth factor receptor tyrosine kinase inhibitor-sensitive exon 19 insertion and exon 20 insertion in patients with advanced non−small-cell lung cancer. *Clin Lung Cancer* 2017; 18:324-32.
25. COSMIC. Catalogue of Somatic Mutations in Cancer. Mutations. Positive data, Available at: https://cancer.sanger.ac.uk/cosmic/gene/samples?all_data=&coords=AA%3AAA&dr=&end=1211&gd=&id=150&ln=EGFR&mut=insertion_inframe&mut=insertion_frameshift&seqlen=1211&sn=lung&src=gene&start=1. Accessed: April 24, 2019.
26. Ministry of Health and Welfare. Health and Welfare Data Science Center. Data evaluation standard procedure; 2017 [in Chinese], Available at: https://dep.mohw.gov.tw/DOS/lp-2506-113.html. Accessed: April 24, 2019.
27. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non−small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; 362:2380-8.
28. Yasuda H, Park E, Yun CH, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. *Sci Transl Med* 2013; 5:216ra177.
29. Floc'h N, Martin MJ, Riess JW, et al. Antitumor activity of osimertinib, an irreversible mutant-selective EGFR tyrosine kinase inhibitor, in NSCLC harboring EGFR exon 20 insertions. *Mol Cancer Ther* 2018; 17:885-96.
30. Hasako S, Terasaka M, Abe N, et al. TAS6417, a novel epidermal growth factor receptor inhibitor targeting exon 20 insertion mutations. *Mol Cancer Ther* 2018; 17:1648-58.
31. Robichaux JP, Elamin YY, Tan Z, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non−small-cell lung cancer. *Nat Med* 2018; 24:638-46.
32. Byeon S, Kim Y, Lim SW, et al. Clinical outcomes of EGFR exon 20 insertion mutations in advanced non−small-cell lung cancer in Korea. *Cancer Res Treat* 2019; 51:623-31.