Importance of driver gene mutation assessment and targeted therapy for patients with early-stage non-small cell lung cancer and non-R0 resection

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Abstract. Patients with non-small cell lung cancer (NSCLC) and incomplete resection have poor clinical outcomes. The present study aimed to identify risk factors for disease progression and mortality. A total of 65 patients with early-stage NSCLC that underwent operation but had a non-R0 resection between August 2011 and December 2020 were included in the present study, and the clinicopathological features and driver gene mutation status were analyzed. The median follow-up time was 36.2 months; 39 patients (60.0%) experienced disease progression and 3 patients (4.6%) died. In total, 22 patients (33.8%) harbored mutations in driver genes. Multivariate analysis demonstrated that the presence of driver gene mutations was associated with an increased risk of disease progression [adjusted odds ratio, 24.08; 95% confidence interval (CI), 2.77-209.01; P=0.004]. Tumors classed as Eastern Cooperative Oncology Group performance status 2 [adjusted hazard ratio (HR), 3.49; 95% CI, 1.10-11.03; P=0.033], stage II-IIIB tumors (adjusted HR, 2.55; 95% CI, 1.06-6.17; P=0.037) and the presence of a driver gene mutation (adjusted HR, 3.28; 95% CI, 1.55-6.94; P=0.002) were associated with a significantly reduced progression-free survival (PFS). Driver gene-targeted therapy was associated with an increased post-progression survival for patients that were reported to have disease progression (adjusted HR, 0.38; 95% CI, 0.16-0.91; P=0.030). There was no significant impact of driver gene mutation status on the overall survival (OS) of patients. Although the presence of a driver gene mutation was associated with an increased risk of disease progression and a reduced PFS, it was demonstrated that patients with disease progression may benefit from driver gene-targeted therapy, as patients with driver gene-targeted therapy had a similar OS compared with that of patients with a driver gene-negative or unknown status. Therefore, early comprehensive analysis of driver gene mutation status may be recommended for early-stage NSCLC cancer patients experiencing non-R0 resection.

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Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B; CI, confidence interval; EGFR, epidermal growth factor receptor; ECOG, Eastern Cooperative Oncology Group; PS, performance status; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; ROS1, ROS proto-oncogene 1; STAS, spread through air space

Key words: non-small cell lung cancer, R0 resection, incomplete resection, driver gene mutation, targeted therapy

Introduction

Lung cancer has a high incidence and is the leading cause of cancer associated mortality worldwide (1). Histologically, lung

cancer can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC) and NSCLC patients possess a higher opportunity of surgical resection (1). Furthermore, >50% of patients with lung cancer are diagnosed at locally advanced or metastatic stages (2,3). Although there have been advances in the understanding of the pathophysiology and the development of novel treatments (4), curative treatments for advanced lung cancer are limited and a diagnosis of advanced lung cancer is predictive of a poor clinical outcome. For patients with early-stage lung cancer, complete resection remains the most reliable treatment option and is currently the only option that is potentially curative. In terms of resection margin status, a complete resection is referred to as R0, while microscopic and macroscopic residual tumors are referred to as R1 and R2 resections, respectively (5).

However, complete resection of early-stage lung cancer does not guarantee a curative response. It is reported that 30-55% of patients with early-stage lung cancer and R0 resection may experience disease recurrence and succumb to lung cancer (2,6). Although neoadjuvant and adjuvant chemotherapy may improve the outcome of patients with lung cancer, the benefits in five-year survival rates are limited (7,8). Currently, numerous novel agents, including immunotherapies, such as nivolumab, durvalumab, and pembrolizumab as well as targeted therapies, such as osimertinib and alectinib, are approved by U.S. Food and Drug Administration and European Medicines Agency for the treatment of patients with early-stage lung cancer (9-11). However, previous studies only include early-stage non-small cell lung cancer (NSCLC) patients with R0 resection (9-11). To the best of the authors' knowledge, the predicted outcomes and appropriate management of early-stage NSCLC patients with non-R0 resection is still to be elucidated.

Between 3-7% of patients with early-stage lung cancer experience incomplete resection of lung tumors (12,13). There is a positive association between the risk of incomplete resection and the increasing tumor and/or node stage (12). Additionally, patients with R1 and R2 resection margin status have a notably reduced survival time compared with patients with R0 resections (12). According to current treatment guidelines (5,14), the treatment options for patients with incomplete resection include resection and radiotherapy, either alone or in combination with chemotherapy. For patients with advanced NSCLC, nine driver mutations have been recommended to be assessed and corresponding targeted therapies should be prescribed according to the results. Of them, *epidermal growth factor receptor* (*EGFR*) mutation is the most common genetic alteration (5). The role of driver gene mutation assessment and the corresponding targeted therapies, such as EGFR-tyrosine kinase inhibitor (TKI), for example, osimertinib, for non-R0 resected *EGFR*-mutant NSCLC patients, is currently unclear. Therefore, the present study investigated the characteristics, outcomes and prognostic factors of patients with early-stage NSCLC and non-R0 resection, and focused on driver gene mutation detection and driver gene-targeted therapy.

Patients and methods

Patient criteria. The present study was retrospective and included patients with lung cancer that were diagnosed and treated at Taichung Veterans General Hospital (Taichung, Taiwan) between August 2011 and December 2020. Included patients were required to have: i) Pathologically confirmed NSCLC; ii) a resectable disease prior to operation; iii) history of surgical resection of lung tumor and/or mediastinal lymph node dissection; iv) non-R0 resection status; v) a precise history of diagnosis; vi) received all the treatments; and vii) survival follow-up data. Patients were excluded if they had: i) Small cell lung carcinoma; ii) other active malignancies; or iii) incomplete data records.

The present study was approved by the Institutional Review Board (IRB) of Taichung Veterans General Hospital (IRB nos. CF12019 and CF20175; Taichung, Taiwan). Written informed consent for clinical data records and genetic testing was obtained from all patients.

Data records for analysis. Clinical data used for analyses included the age, sex, smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), histological type, driver gene mutation status, tumor stage, operation types, resection margin status, pathological features, history of radiotherapy and antineoplastic treatment, and the survival follow-up data of the patients. Lung cancer tumor, node and metastases staging was conducted according to the 8th edition of the American Joint Committee on Cancer staging system (15).

Driver gene mutation status. Patients with available tumor specimens were tested for mutations in six driver genes, which included *EGFR*, *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*), *v-raf murine sarcoma viral oncogene homolog B* (*BRAF*), *human epidermal growth factor receptor 2* (*HER2*), *anaplastic lymphoma kinase* (*ALK*) and *ROS proto-oncogene 1* (*ROS1*). *EGFR*, *KRAS*, *BRAF* and *HER2* mutations were tested using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which has been validated as a standard method to detect these driver gene mutations in our previous studies and implemented in Taiwan clinical practice (16-20). Genomic DNA (gDNA) was extracted for serial biochemical reactions. For gDNA extraction, QIAamp DNA formalin-fixed paraffin-embedded (FFPE) Tissue Kit (cat. no. 56404; Qiagen GmbH) was used according to the manufacturer's instructions. Briefly, gDNA from three sections of 10 μm thick FFPE was extraction following deparaffination, lysing, heating, washing and eluting. The Typlex/iPlex PRO kit (cat. no. 10217; Agena Bioscience, Inc.) was utilized for biochemical reactions according to the manufacturer's instructions for the MassARRAY® kit system (cat. no. 10411; Agena Bioscience, Inc.). Briefly, PCR was used to amplify the region containing *EGFR* mutations, and then single nucleotide extension was performed using detection probes, followed by MALTI-TOF MS analysis. *EGFR* mutations could be distinguished from wild-type genes due to the mass difference of an incorporated single nucleotide. For PCR amplification, a final volume of 5 μl reaction mixture containing 10 ng gDNA, 0.5 units HotStar Taq polymerase (cat. no. 203203; Qiagen GmbH), 500 mM dNTPs, 100 nM forward primers, 100 nM reverse primers, 1X of HotStar buffer (diluted from 10X) and 1.625 mM $MgCl_2$ was used. The following thermocycling conditions were used

for PCR: Initial denaturation at 94˚C for 15 min, followed by 45 touch-down amplification cycles consisting of 15 cycles of 94˚C for 20 sec, annealing at 61˚C for 30 sec and 72˚C for 60 sec with another 30 cycles of 94˚C for 20 sec, annealing at 57˚C for 30 sec and 72˚C for 60 sec. Shrimp alkaline phosphatase (SAP) treatment for dNTP neutralization was carried out as follows: 0.5 units SAP with 1X SAP buffer (diluted from 10X concentrated) were prepared in a final 2 μl mixture. It was added into the PCR product for 40 min at 37˚C incubation and then inactivated at 80˚C for 5 min. The last step was to probe for single nucleotide extensions using a Typlex™/iPlex PRO kit (cat. no. 10217; Agena Bioscience, Inc.) containing 0.0205 μl Sequnase, 0.1 μl termination mix, 0.2 μl 10X Typlex buffer and multiplex extension primers (Table SI) at a final concentration of 7-14 μM in a 2 μl reagent mix. The following thermocycling conditions were used: 94˚C for 30 sec, followed by a 40-cycle extension reaction (each cycle contained five rounds of 94˚C for 20 sec, 80˚C for 5 sec, and 60˚C for 5 sec). After SpectroClean Resin clean up (using 6 mg resin for each reaction in the 384-well plate and rotating at room temperature for 20 min to eliminate salt contamination), samples were loaded onto a SpectroCHIP® matrix (Agena Bioscience, Inc.) using a Nanodispenser and then analyzed using Autoflex® MALDI-TOF MS (Bruker Corporation). Data were collected and analyzed using the Typer4 software (Ver. 4.0.53; Agena Bioscience, Inc.). The PCR primers and probes used in the present study are provided in Table SI and the representative *EGFR* mutation spectra detected using MALDI-TOF MS are shown in Fig. S1. The *ALK* fusion mutation was assessed using a fully automated VENTANA ALK (D5F3) CDx Assay (cat. no. 790-4796, Roche Diagnostics, Ltd.) for immunohistochemical staining (IHC) using the pre-diluted anti-ALK (D5F3) rabbit monoclonal primary antibody (cat. no. 3633; Cell Signaling Technology, Inc.). Based on the diagnostic procedure suggested by VENTA ALK (D5F3) CDx Assay, the biopsy was fixed by 10% formalin at room temperature overnight. After water resin and dehydration by gradient alcohol from 75-100%, the biopsy was embedded in paraffin. A 4 μm thick FFPE section was required for automatic IHC assay. The *ROS1* fusion mutation was investigated using fluorescent *in situ* hybridization as previously described (Fig. S2) (16-18,21). All the aforementioned methods for driver gene mutation detection were included in the written informed consent approved by the IRB of Taichung Veterans General Hospital.

Statistical analysis. Univariate analyses of the association between the status of tumor progression and the characteristics of patients were carried out using Fisher's exact test and logistic regression model. The Kaplan-Meier method was used to analyze the survival time of patients. Differences in survival time were analyzed using the log-rank test. The logistic regression model and Cox proportional hazard model were used for multivariate analyses of the prognostic factors of disease progression status and survival outcomes. Each variable was independently subjected to analysis using the logistic regression model and Cox proportional hazard model, followed by selection of statistically significant variables for subsequent analysis. To evaluate survival outcomes, progression-free survival (PFS) was defined as the length of time from operation to disease progression or mortality due to any cause, overall survival (OS) was defined as the length of time from operation to mortality due to any cause and post-progression survival was defined as the length of time from documented tumor progression to mortality due to any cause among patients that experienced disease progression. Driver gene-targeted treatment indicated patients receiving targeted therapy corresponding to the driver gene mutations detected; for instance, EGFR-TKI for *EGFR* mutation and ALK inhibitor for *ALK* fusion, respectively (5). All statistical analyses were carried out using SPSS (version 15.0; SPSS, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients and demographic data. Between August 2011 and December 2020, 2,162 patients with lung cancer underwent surgical resection for curative purposes. Of them, a total of 65 patients (3.0%) had non-R0 resection and were included in the present study for analysis (Table I). The median follow-up time was 36.2 months (95% CI, 14.3-58.0). The median age was 64 years (range, 35-87). Of the patient cohort, 24 patients were female (36.9%) and 27 patients were non-smokers (41.5%). Baseline ECOG PS was 0-1 in 61 patients (93.8%). Adenocarcinoma (60.0%) and squamous cell carcinoma (23.1%) were the most common histological types. In terms of types of operation, 2 (3.1%), 50 (76.9%) and 13 (20.0%) patients underwent pneumonectomy, lobectomy and wedge resection, respectively. Mediastinal lymph node dissection was performed in 61 patients (93.8%). A total of 42 patients (64.6%) had received adjuvant chemotherapy, while 26 patients (40.0%) had received adjuvant radiotherapy.

Pathological features and disease progression patterns. An analysis of the pathological features and disease progression patterns are presented in Table II. Regarding the resection margin status, 60 patients (92.3%) had R1 status, while 5 patients (7.7%) had R2 status. The involved surgical margins were predominantly in the parenchymal margin (33 patients; 50.8%) and bronchial margin (21 patients; 32.3%). Of the patient cohort, 6 patients (9.2%) exhibited positive surgical margins in the vascular areas. The involvement of the bronchial and vascular margin was identified in 5 patients (7.7%; Table SII). Angiolymphatic invasion was revealed in 43 patients (66.2%), perineural invasion was revealed in 23 patients (35.4%), extranodal involvement was revealed in 18 patients (27.7%) and spread through air space (STAS) invasion was revealed in 12 patients (18.5%). In terms of visceral pleural invasion status, 33 (50.8%), 14 (21.5%), 9 (13.8%) and 9 (13.8%) patients were revealed to be PL0, PL1, PL2 and PL3, respectively (Table II).

The pathological stages were reported as 0-I, II and IIIA-B in 16 (24.6%), 15 (23.1%) and 34 (52.3%) patients, respectively (Table I). Regarding driver gene mutation status, 21 patients harbored an *EGFR* mutation, while 1 patient harbored an *ALK* fusion. The *EGFR* mutation spectrum included 10 patients with an exon 19 deletion, 7 patients with an exon 21 L858R mutation, 2 patients with an exon 18 G719X mutation, 1 patient with an exon 20 insertion and 1 patient with an exon 19 deletion plus an exon 20 T790M compound mutation. The driver gene mutation status of the remaining 43 patients (55.2%) was negative or unknown (Table I). In total, 39 patients (60.0%) had disease progression and 3 patients

Table I. Patient characteristics and demographic data.

Characteristic	No. of patients (n=65)
Median age, years (range)	64 (35-87)
Sex, n (%)	
Female	24 (36.9)
Male	41 (63.1)
Smoking status, n (%)	
Non-smokers	27 (41.5)
Smokers	38 (38.5)
ECOG PS, n (%)	
0-1	61 (93.8)
2	4 (6.2)
Histological types, n (%)	
Adenocarcinoma	39 (60.0)
Squamous cell carcinoma	15 (23.1)
Adenosquamous cell carcinoma	6 (9.2)
Others[a]	5 (7.7)
Pathological stage, n (%)	
0-I	16 (24.6)
II	15 (23.1)
IIIA-B	34 (52.3)
Operation type, n (%)	
Pneumonectomy	2 (3.1)
Lobectomy	50 (76.9)
Wedge resection	13 (20.0)
Lymph node dissection, n (%)	
Yes	61 (93.8)
No	4 (6.2)
Driver gene mutation status, n (%)	
Yes[b]	22 (33.8)
No or unknown	43 (55.2)
Adjuvant chemotherapy, n (%)	
Yes	42 (64.6)
No	23 (35.4)
Adjuvant radiotherapy, n (%)	
Yes	26 (40.0)
No	39 (60.0)

[a]Includes 2 cases of large cell carcinoma, 2 cases of lymphoepithelial carcinoma and 1 case of adenoid cystic carcinoma. [b]Includes 21 *epidermal growth factor receptor* mutations and one *anaplastic lymphoma kinase* fusion. ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Table II. Pathological features and disease progression patterns of patients with early-stage non-small cell lung cancer and non-R0 resection after surgery.

Pathological features	No. of patients (n=65)
Resection margin, n (%)	
R1	60 (92.3)
R2	5 (7.7)
Angiolymphatic invasion, n (%)	
Yes	43 (66.2)
No	22 (33.8)
Perineural invasion, n (%)	
Yes	23 (35.4)
No	42 (64.6)
Extranodal involvement, n (%)	
Yes	18 (27.7)
No	47 (72.3)
Spread through air space, n (%)	
Yes	12 (18.5)
No	7 (10.8)
Unknown	46 (70.8)
Visceral pleural, n (%)	
PL0	33 (50.8)
PL1	14 (21.5)
PL2	9 (13.8)
PL3	9 (13.8)
Disease progression, n (%)	
No	23 (35.4)
Yes	39 (60.0
Intrathoracic	17 (26.2)
Extrathoracic	22 (33.8)
Mortality[a]	3 (4.6)

[a]Mortality of 2 patients was due to infection and the mortality of 1 patient was due to another type of advanced oral cancer.

(4.6%) died. Among patients with disease progression and patients who succumbed, a total of 20 (47.6%) patients received driver-gene targeted therapy following disease progression.

Association between patient characteristics and disease progression. Results of a univariate analysis of the association between patient characteristics and disease progression are shown in Table III. Disease progression was more likely in patients with pathological stage II-IIIB compared with patients with stage 0-I (73.5 vs. 37.5%, respectively; P=0.015). Positive angiolymphatic invasion was associated with an increased risk of disease progression (76.7 vs. 40.9%, respectively; P=0.006). The risk of disease progression for patients with positive perineural invasion and STAS was markedly increased. Rates of disease progression were similar between different histological types. Furthermore, patients with known driver gene mutations had a significantly increased risk of disease progression compared with patients with a negative or unknown status (95.5 vs. 48.8%, respectively; P<0.001).

An increased risk of disease progression was revealed for patients that underwent lobectomy or pneumonectomy, compared with patients that underwent wedge resection (71.2 vs. 38.5%, respectively; P=0.049). There was also a numerically higher risk of disease progression in patients who received adjuvant chemotherapy compared with individuals who did not (P=0.057). The risk of disease progression was

Table III. Univariate analysis of the association between the characteristics of patients and disease progression.

Characteristic	No. of disease progression or mortality	P-value[a]	OR (95% CI)	P-value[b]
Age, n (%)		>0.999		0.615
<65 years	21 (61.8)		Reference	
≥65 years	21 (67.7)		1.30 (0.47-3.61)	
Sex, n (%)		>0.999		0.791
Female	16 (66.7)		Reference	
Male	26 (63.4)		0.87 (0.30-2.50)	
Smoking status, n (%)		>0.999		0.814
Non-smoker	17 (63.0)		Reference	
Smoker	25 (65.8)		1.13 (0.40-3.16)	
ECOG PS, n (%)		0.178		0.999
0-1	38 (62.3)		Reference	
2	4 (100.0)		977787404.9 (0.00-NE)	
Histological types, n (%)		>0.999		0.916
Adenocarcinoma	25 (64.1)		Reference	
Others	17 (65.3)		1.06 (0.37-2.99)	
Pathological stage, n (%)		0.015		0.012
Stage 0-I	6 (37.5)		Reference	
Stage II-IIIB	36 (73.5)		4.61 (1.40-15.15)	
Operation type, n (%)		0.049		0.034
Lobectomy or pneumonectomy	37 (71.2)		Reference	
Wedge resection	5 (38.5)		0.25 (0.07-0.90)	
Angiolymphatic invasion, n (%)		0.006		0.006
Yes	33 (76.7)		Reference	
No	9 (40.9)		0.21 (0.07-0.63)	
Perineural invasion, n (%)		0.109		0.094
Yes	18 (78.3)		Reference	
No	24 (57.1)		0.37 (0.12-1.19)	
Extranodal invasion, n (%)		0.565		0.430
Yes	13 (72.2)		Reference	
No	29 (61.7)		0.62 (0.19-2.03)	
Spread through air space, n (%)		0.082		
Yes	7 (58.3)		Reference	
No	2 (28.6)		0.29 (0.04-2.11)	0.220
Unknown	33 (71.7)		1.18 (0.49-6.75)	0.375
Pleural involvement, n (%)		0.606		0.493
Yes	22 (62.5)		Reference	
No	20 (60.6)		0.70 (0.25-3.89)	
Adjuvant chemotherapy, n (%)		0.057		0.039
Yes	31 (73.8)		Reference	
No	11 (47.8)		0.33 (0.04-0.95)	
Adjuvant radiotherapy, n (%)		0.602		0.526
Yes	18 (69.2)		Reference	
No	24 (61.5)		0.71 (0.25-2.04)	
Driver gene mutation status, n (%)		<0.001		0.004
Yes	21 (95.5)		Reference	
No or unknown	21 (48.8)		0.05 (0.01-0.37)	

[a]Fisher's exact test. [b]Logistic regression model. OR, odds ratio; ECOG, Eastern Cooperative Oncology Group; PS, performance status; NE, not estimatable.

Table IV. Multivariate analysis of disease progression, progression-free survival and post-progression survival.

A, Disease progression

Factor	Adjusted OR/HR	95% CI	P-value[a]
Stage II-IIIB vs. 0-I	4.95	1.12-22.22	0.035
Driver gene mutation status, yes vs. no or unknown	24.08	2.77-209.01	0.004

B, Progression-free survival

Factor	Adjusted OR/HR	95% CI	P-value[b]
ECOG PS 2 vs. 0-1	3.49	1.10-11.03	0.033
Stage II-IIIB vs. 0-I	2.55	1.06-6.17	0.037
Driver gene mutation status, yes vs. no or unknown	3.28	1.55-6.94	0.002

C, Post-progression survival

Factor	Adjusted OR/HR	95% CI	P-value[b]
Driver gene-targeted therapy, yes vs. no	0.38	0.16-0.91	0.030

[a]Logistic regression model. [b]Cox proportional hazard model. OR for disease progression, and HR for progression-free survival and post-progression survival. OR, odds ratio; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

similar between patients that received adjuvant radiotherapy and patients that did not (P=0.602).

There was a significant association between the tumor stages and patient treatments. Compared with patients with stage 0-I tumors, an increased number of patients with stage II-IIIB tumors underwent lobectomy or pneumonectomy (89.8 vs. 50.0%, respectively; P=0.002) and adjuvant chemotherapy (77.6 vs. 25.0%, respectively; P<0.001), which demonstrated the association of tumor stages on the treatment decision. Furthermore, stage II-IIIB tumors were associated with an increased risk of angiolymphatic invasion (81.6 vs. 18.8%, respectively; P<0.001). However, the rate of positive driver gene mutations was not significantly different between patients with stage II-IIIB and 0-I tumors (36.7 vs. 25.5%, respectively; P=0.546).

Survival outcomes and impact of driver gene mutation status. The impact of driver gene mutation status on PFS and OS of the overall study cohort was assessed (Fig. 1). Patients harboring known driver gene mutations (11.4 months; 95% CI, 7.1-15.7) were associated with a significantly shorter PFS compared with patients with negative or unknown driver gene mutation status (20.9 months; 95% CI, 0.0-53.0; P=0.008). The OS was not reached for patients with negative or unknown driver gene mutation status compared with patients with known driver gene mutations (58.8 months; 95% CI, 47.2-112.0; P=0.689).

The influence of driver gene-targeted therapy on post-progression survival and OS of the cohort of patients that had disease progression was also evaluated (Fig. 2). Patients that received driver gene-targeted therapy were associated with a significantly longer post-progression survival (40.5 months; 95% CI, 5.2-75.7) compared with patients that did not receive driver gene-targeted therapy (11.5 months; 95% CI, 0.1-22.9; P=0.019). Additionally, patients that received driver gene-targeted therapy were associated with a significantly longer OS (58.8 months; 95% CI, 19.0-98.6) compared with patients that did not receive driver gene-targeted therapy (23.6 months; 95% CI, 21.9-25.3; P=0.020).

Multivariate analysis of disease progression and survival outcomes. Results of the multivariate analyses are summarized in Table IV. Stage II-IIIB tumor stage was associated with a significantly increased risk of disease progression (adjusted OR, 4.95; 95% CI, 1.12-22.22; P=0.035). The presence of a driver gene mutation was also independently associated with a significantly increased risk of disease progression (adjusted OR, 24.08; 95% CI, 2.77-209.01; P=0.004).

Reduced PFS times were associated with an ECOG PS of two (adjusted HR, 3.49; 95% CI, 1.10-11.03; P=0.033), stage II-IIIB tumors (adjusted HR, 2.55; 95% CI, 1.06-6.17; P=0.037) and the presence of a driver gene mutation (adjusted HR, 3.28; 95% CI, 1.55-6.94; P=0.002).

The patients that underwent driver gene-targeted therapy due to disease progression were associated with a significantly longer post-progression survival time (adjusted HR, 0.38; 95% CI, 0.16-0.91; P=0.030).

Discussion

The outcomes for patients with lung cancer have improved over the past number of decades, which is attributed to the advances in novel treatments and the early diagnosis of lung cancer. The current strategy for treatment is personalized therapy, which particularly benefits patients with advanced

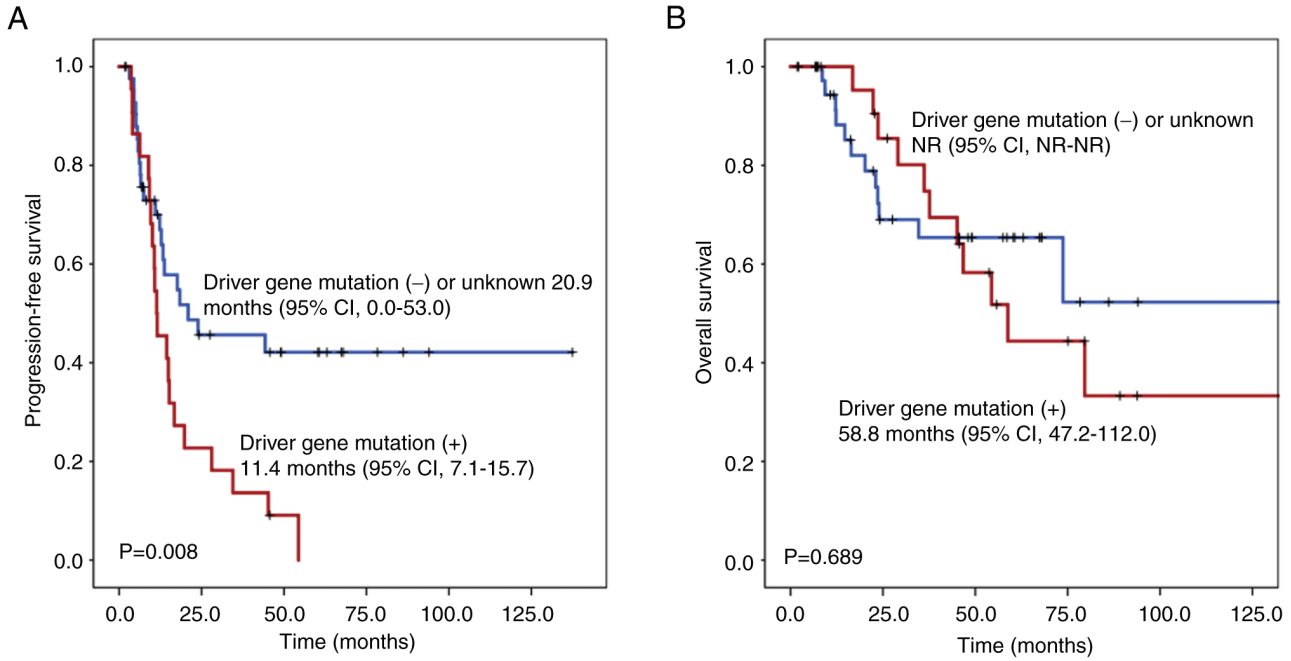

Figure 1. Survival time of overall population. Analysis of the driver gene mutation status on (A) progression-free survival, which was significantly shorter in patients with positive driver gene mutation compared with those with negative or unknown driver gene mutation, and (B) overall survival, which were not significantly different between the two groups of patients in the overall population. NR, not reached; CI, confidence interval; +, positive.

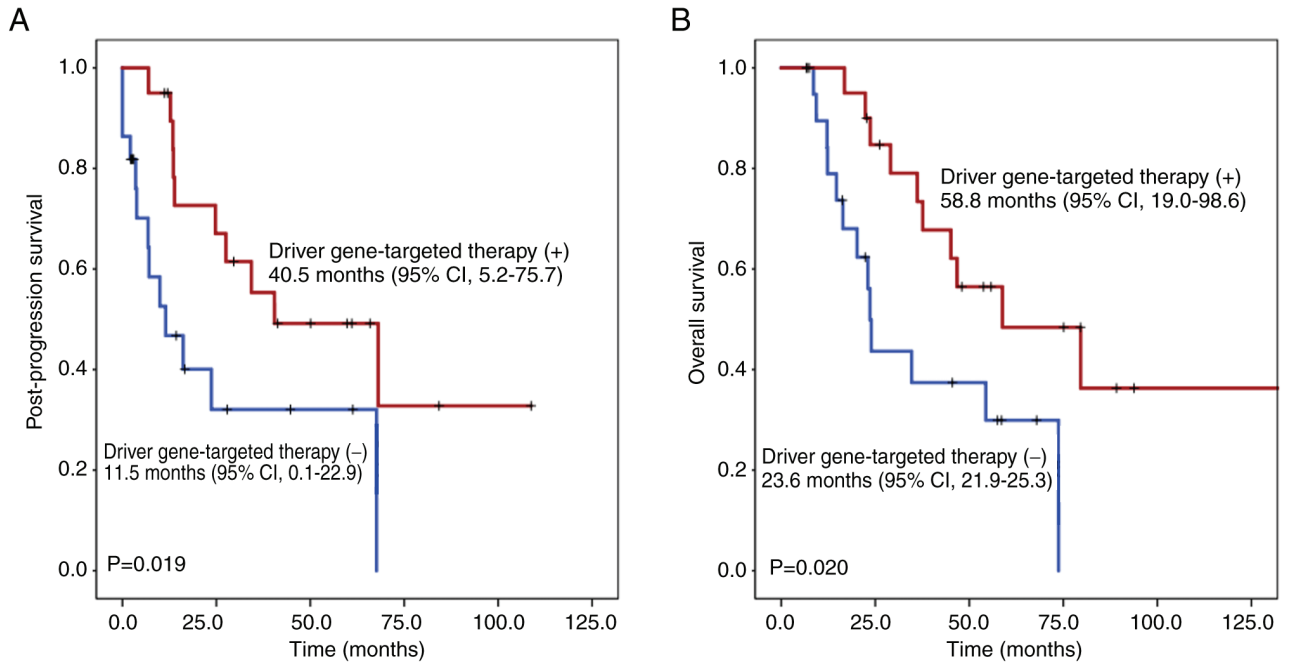

Figure 2. Survival time of patients with disease progression. Analysis of the driver gene-targeted therapy on (A) post-progression survival and (B) overall survival for patients with disease progression. CI, confidence interval; +, positive; - negative.

stage lung cancer. In such a scenario, both pathological classification and biomarker assessment serve important roles in the decision of treatment regimens (4,22,23). Low dose computed tomographic screening both increases the identification of lung cancer cases and reduces mortality due to lung cancer among high-risk populations (24,25). A retrospective study from Taiwan also suggests a positive association between the diagnostic shift from late to early-stage and improved outcomes for patients with lung cancer (26). For patients diagnosed with early-stage lung cancer, complete resection is the mainstream form of therapy as it provides the opportunity to cure this disease. However, a small proportion of patients may have an incomplete lung tumor resection, which is hypothesized to both increase the risk of disease progression and worsen patient outcomes (12). The appropriate treatment of these patients is currently unclear. The present study revealed the

characteristics and outcomes of a cohort of patients (obtained over a 10-year period) with early-stage NSCLC and non-R0 resection, and investigated the importance of driver gene mutation detection and driver gene-targeted therapy.

The treatment options for patients that have incomplete resection include re-resection and adjuvant chemotherapy and radiotherapy (5,14). However, the evidence to prescribe these treatments is limited as previous studies indicate inconsistent results (5,14,26). Osarogiagbon *et al* (27) analyzed the National Cancer Database from 2004 to 2011, and reveal that 4.7% of 112,998 patients with NSCLC had incomplete resection. Of the 4.7% of patients, adjuvant chemotherapy increases the 5-year survival rate across all stages, although radiotherapy is associated with reduced 5-year survival rate in patients diagnosed with stage I disease. A population-based cohort study carried out in the Netherlands, including 427 patients with incompletely resected lung cancer out of a total of 8,528 patients that underwent surgical treatment between 2015 and 2018, suggests that adjuvant chemotherapy, but not radiotherapy, may improve OS (28). By contrast, a study by Park *et al* (29) reports that chemotherapy does not affect the disease progression pattern or survival time of patients following the incomplete resection of NSCLC. However, to the best of the authors' knowledge, previous studies have not analyzed the impact of driver gene mutation status and the role of targeted therapy.

EGFR mutations and *ALK* fusions are prognostic factors of higher disease recurrence rates after surgery for patients with early-stage lung cancer. A study by Ito *et al* (30), including 877 patients with resected lung cancer, evaluated the prognostic impact of *EGFR* mutations and suggests that the presence of *EGFR* mutations are associated with an increased 5-year recurrence rate and a decreased 5-year recurrence-free survival, compared with healthy patients. Additionally, a previous study by Park *et al* (31), including 659 patients with resected NSCLC, also reveals that *EGFR* mutations are associated with an increased risk of recurrence and distant metastasis. Similarly, previous studies by Fujibayashi *et al* (32) and Shin *et al* (33) both suggest a reduced recurrence-free survival in patients with resected stage IA lung adenocarcinoma with an *ALK* fusion. However, all these previous studies evaluated patients with complete resection; therefore, this may suggest different outcomes for patients with non-R0 resection, as these patients do not have a cancer-free status after surgery. Without prompt treatment, some patients with *EGFR* mutations may experience a hyper-progressive disease, which may lead to a rapid tumor progression and a shorter survival time (34). In the present study, *EGFR* mutations were the most common driver gene mutations revealed. Following an adjustment for clinicopathological features of the patients, the presence of driver gene mutations remained an independent predictor of both an increased risk of disease progression and a decreased PFS. However, in patients experiencing disease progression, driver-gene targeted therapy improved post-progression survival time, which may explain the similar OS between patients with and without driver gene mutations.

In the present study, patients that received lobectomy or pneumonectomy, as well as patients that underwent adjuvant chemotherapy were associated with an increased risk of disease progression, following incomplete resection of the lung tumor. However, tumor stage is the most important factor when determining postoperative treatment (5). Current guidelines suggest adjuvant chemotherapy for patients with stage II disease or higher, and that sub-lobar resection may be only suitable for patients with a single lung tumor that is >2 cm (5,14). A positive association between the tumor stage and therapeutic options was aforementioned in the present study; hence, an increased risk of progression may be attributed to tumor stage, but not the postoperative treatments. However, there was not a significant effect of tumor stage on driver gene mutation status, which supported the independent role of driver gene mutation status in predicting disease progression.

The Lung Cancer Mutation Consortium study prospectively enrolled patients with advanced stage lung adenocarcinoma and assessed 10 driver gene mutations (34). The results demonstrate that patients harboring driver gene mutations with corresponding targeted therapy have notably improved outcomes (35). Regarding patients with completely resected early-stage NSCLC, ADAURA (11) and ALINA (9) clinical trials both suggest adjuvant osimertinib and alectinib may increase the progression-free survival time of patients with *EGFR* mutations and *ALK* fusions, respectively (9,11). Currently, early initiation of molecular testing and biomarker assessment are recommended by clinical practice guidelines (36). Although the appropriate treatment for disease progression following incomplete lung cancer resection has not yet been established, numerous patients with this condition may require systemic treatment, for example, chemotherapy. In the present patient cohort, the assessment of driver gene mutations and the use of corresponding targeted therapy could improve patient outcomes and improve the survival time. The data from the present study suggested that driver gene-targeted therapy was independently associated with an improved post-progression survival. Therefore, due to the increased risk of disease progression, early comprehensive analysis of driver gene mutation status for patients with incomplete resection of lung cancer may be suggested.

In addition to chemotherapy, immunotherapy and targeted therapy (in either a neoadjuvant or adjuvant setting) both improve the outcomes of patients with early-stage resectable NSCLC (9-11). However, these studies evaluated patients with complete resection of lung tumors. Theoretically, immunotherapy and targeted therapy may potentially benefit patients with non-R0 resection lung cancer. However, prospective studies are still required in order to establish the appropriate treatment for these patients in the future.

A major limitation of the present study was that it was retrospective, which may have led to bias as the operations included, and the data collected from patients were not planned ahead of time. With the recent advances in surgical techniques (37), incomplete resection only occurs in a small subset of patients (12). The present study involved a cohort from a 10-year period from in Taichung Veterans General Hospital (Taichung, Taiwan), with a 3-year follow-up. Although the data were collected retrospectively, the present study attempted to ensure the validity of the characteristics, diagnosis as well as treatment course of each patient, genetic alterations and the outcome evaluation. Compared with previous studies (27-29), the importance of driver gene mutation detection and driver gene-targeted therapy was further

investigated. In the era of precision medicine for lung cancer, sorting patients according to both their clinicopathological features and results of biomarker assessment remains important in order to prescribe personalized treatment, and may also be beneficial for patients with incompletely resected lung cancer (5). Further studies are needed to investigate why patients with driver gene mutations have an increased risk of disease progression and to evaluate whether targeted therapy as treatment following a non-R0 resection could improve the outcomes of patients. In addition to the possibility of bias, it is difficult to establish a cause-and-effect relationship between the intervention and patient outcome in a retrospective study. Therefore, future prospective studies using a larger cohort may provide an improved algorithm in the biomarker assessment and management of patients with lung cancer and incomplete resection.

Although the presence of driver gene mutations were associated with an increased risk of disease progression and a reduced PFS, patients that had disease progression may benefit from driver-gene targeted therapy. Therefore, the results of present study suggested an earlier comprehensive analysis of the driver gene mutation status for patients with incomplete resection of lung cancer in order to identify at-risk individuals and apply the corresponding targeted therapy promptly when they experience disease progression.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The data generated in the present study may be found in Mendeley Data at the following URL: https://data.mendeley.com/datasets/v4jxnb9gt3/1.

Authors' contributions

PYS and JST confirm the authenticity of all the raw data. JST designed this manuscript. PYS, CYC, CHL, YWH, YHH, KHH, JST, GCC and TYY conducted the study and analyzed the data. PYS wrote the manuscript and JST guided the writing. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB nos. CF12019 and CF20175). Written informed consent for clinical data records and genetic testing was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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