Clinics in Oncology

Research Article
Published: 08 Mar, 2019

The Prevalence of PD-L1 Expression in Lung Cancer

*Yu-Chen Chang[1,2], Ping-Chih Hsu[3], Shih-Hung Li[3], Shih-Hua Weng[3], Chih-Hsi Kuo[2,3], Tai-Yun Yang[3] and Chien-Ying Liu[2,3]**

[1]Department of Internal Medicine, Chang Gung Memorial Hospital, Taipei, Taiwan

[2]Department of Medicine, Chang Gung University, Taoyuan, Taiwan

[3]Department of Thoracic Medicine, Division of Pulmonary Oncology, Chang Gung Memorial Hospital, Taiwan

Abstract

Background: To find out the factors which influence the expression rate of PD-L1 by routine clinical parameters and simplified the therapeutic strategies for immune checkpoint inhibitor treatment, we profiled the relationships between of PD-L1 expression rate with clinical parameters (gender, smoking status, body weight, clinical) and some biomarkers for targeted therapy (EGFR, ALK).

Methods: We enrolled in 500 patients with lung cancer diagnosis and further biopsy specimens, including 371 Adenocarcinoma (AD), 57 Squamous Cell Carcinoma (SCC), 9 small cell carcinoma, and 63 other Non-Small Cell Lung Cancer (NSCLC), and analyzed by using immuno histochemistry and cancer molecular study.

Result: We found that 21.0% of total specimens (105 cases) showed strong positive PD-L1 expression on Tumor Cells (TC), and 45.8% (229 cases) for weak positive expression rate. We also found that there's no significance difference in most parameters or biomarkers except clinical stage in adenocarcinoma, which showed apparent lower expression rate than higher infiltration stages.

Conclusion: We concluded that our trial can provide some evidences which shows different PD-L1 expression situation in different stages, especially in the subtype of AD and SCC, which may become a good reference for physicians to make strategies to treat lung cancer.

Introduction

Since Lung Cancer (LC) causes the most casualties and morbidity cases worldwide, the efficacy of therapies against tumor become important issue [1]. However, most of the patients are told the diagnosis at the higher stage, which means the approaches of curative method, may not be the first choice [2]. Therefore, the immunotherapy for tumor immune reaction as second line therapy has been tested and proved its advances in Overall Survival (OS) and objective response rate than traditional palliative chemotherapy in recent years [2,3].

Since the immunogenic checkpoints was found in LC, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1), it seems to be possible to control the progression of tumor by immune reaction. After antigen of the tumor was recognized by Antigen Presenting Cells (APC), the immune response may be triggered by T cells. However, the microenvironment with checkpoints, such as CTLA-4 and PD-1/PD-L1 may suppress the immune reaction. As a result, the antibody that blocks the immune checkpoints seems to be the way to treat tumor [4]. Some analysis even found the situation between the anti-PD-L1 treatments would make some effect on tumors which present high percentage of PD-L1 [5]. As a result, such as ipilimumab, anti-CTLA-4 antibodies, was approved the usage in melanoma, and nivolumab and pembrolizumab, both are anti-PD-1 IgG, was permitted for first-line or second-line treatment for Non-Small Cell Lung Cancer (NSCLC) [2,4]. In recent study, the anti-PD-L1 atezolizumab was even added for first-line chemotherapy in metastatic non squamous NSCLC [6].

However, there is still a controversial issue for predicting the prognosis and benefits for immunotherapy, which would cause the difficulties on the selection on patients and drugs usage [7]. As a result, the criterion for the time and the situation in which the physicians conduct the immunotherapy is still in doubt, which leads the difference in the time and situation for introducing the therapy, and causes the totally different outcomes and survival time for each patient.

Recently, some clinician tried to find out the relationship between the expression of PD-L1 with the subtypes or the stage of LC, or other tumor markers and lung cancer driver genes for

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Correspondence:
Chien-Ying Liu, Department of Thoracic Medicine, Division of Pulmonary Oncology, Chang Gung Memorial Hospital, Taiwan, Tel: 886-3-3281200; Fax:886-3-3287787; E-mail: cyliu01@cgmh.org.tw

Received Date: *16 Feb 2019*
Accepted Date: *04 Mar 2019*
Published Date: *08 Mar 2019*

Citation:
Chang Y-C, Hsu P-C, Li S-H, Weng S-H, Kuo C-H, Yang T-Y, et al. The Prevalence of PD-L1 Expression in Lung Cancer. Clin Oncol. 2019; 4: 1591.

Table 1: Patients' clinical information.

Characteristics	Total	Strong:	Weak:	Negative:	χ^2 and/or
		PD-L1 (>50%)	PD-L1 (1~50%)	PD-L1 (0~ <1%)	p-value
Patients, No. (%)	500	105	229	166	
	-100%	(%)	(%)	(%)	
Gender (male / female)	279 / 221	63 / 42	131 / 98	85 / 81	χ^2=2.356
					p=0.340
Age (mean ± SD)	64.0 ± 12.5	64.0 ± 12.8	63.9 ± 12.8	63.7 ± 12.0	p=0.712
Body Mass Index (BMI)	23.5 ± 4.0	23.9 ± 4.6	23.6 ± 3.9	23.2 ± 3.8	p=0.340
Body weight					
>=50 kg	404	88	181	135	χ^2=1.286
<50 kg	73	12	33	28	p=0.526
Smoking status					
Never smokers	278	53	130	95	χ^2=1.915
Former + Concurrent	206	50	90	66	p=0.384
Unknown	16	2	9	5	
Stage of disease at diagnosis					
I~II	73	6	27	40	χ^2=26.39
III	109	34	45	30	p<0.0001*
IV	311	63	155	93	
Stage of T					
1-2	132	22	55	55	χ^2=7.0228
3-4	299	70	142	87	p=0.030
Unknown	69	13	32	24	
Stage of N					
0-1	141	13	61	67	χ^2=27.05
2-3	333	88	156	89	p<0.0001*
Unknown	26	4	12	10	
Stage of M					
0	180	40	70	70	χ^2=6.178
1+	310	63	155	92	p=0.046*
Unknown	10	2	4	4	
ECOG PS at diagnosis					
0~1	372	78	169	125	χ^2=17.73
2	45	9	18	18	p=0.023
3	28	8	15	5	
4	6	3	3	0	
Histology					
Adeno	371	65	173	133	χ^2=25.78
Squa	57	17	26	14	p<0.001*
other-NSCLC	63	23	28	12	
SCLC	9	0	2	7	
EGFR mutation at diagnosis					
Exon 19 deletion	90	14	43	33	χ^2=7.310
Exon 21 L858R	71	12	30	29	p=0.478
Exon 20 T790M	8	2	5	1	(if for mutation only:
Others*	42	8	20	14	χ^2=4.181
WT	189	45	90	54	p=0.124)

Unknown	100	24	41	35	
ALK rearrangement at diagnosis					
+ve	22	5	12	5	χ^2=0.887
-ve	322	66	152	104	p=0.644
Unknown	156	34	65	57	
Mets at diagnosis					
Pleural effusion	124	27	60	37	χ^2=0.881
					p=0.644
Brain metastasis	50	13	24	13	χ^2=1.634
					p=0.442
Liver metastasis	57	13	29	15	χ^2=1.457
					p=0.483
Bone metastasis	159	29	79	51	χ^2=1.647
					p=0.439
Adrenal gland metastasis	38	13	16	9	χ^2=4.757
					p=0.093
Methods of study					
Dako 22C3	500	105	229	166	

Abbreviations: No.: Number; SD: Standard Deviation; AD: Adenocarcinoma, SCC: squamous cell carcinoma; SCLC: Small Cell Lung Cancer

NSCLC, such as Anaplastic Lymphoma Kinase (ALK) rearrangement or Epidermal Growth Factor Receptor (EGFR) mutation [5]. Some recent studies for evaluating if the expression of PD-L1 in tumor correlates with these mutations were conducted, which showed that the higher expression rate of PD-L1 in tumor was seemed to have poorer prognosis, but the relationship between the mutations for these oncogenes with the expression rate for PD-L1 in tumor remain unidentified [8,9].

In order to gaining more understanding of the correlation between the presenting rate for PD-L1 in LC with the mutations for oncogenes which are related to LC, we conducted a survey for identifying if this clinic pathological feature exists.

Patients and Methods

Subjects

We collected 500 patients who diagnosed lung cancer by histology in Linkou Chang Gung Memorial Hospital (LCGMH, Taoyuan, Taiwan) in February 2013 to October 2017. Prior written informed consent was also conducted from all patients, and protocol was also approved by ethics committee in LCGMH (IRB number: 201601397B0). After we collected these samples, these samples would be fixed by formalin, embedded by para film and stained by Hematoxylin and Eosin (H&E). They would be reviewed by pathologists to confirm the diagnosis of LC for all the samples.

To measure the difference of the expression of PD-L1 in tumor cells between different clinical parameters for lung cancer, the diagnosed age, the gender of the patient, and the history for smoking, which would be recorded on the admission or outpatient notes, were also included and calculated. The clinical stage for lung cancer was also measured by radiologist or nuclear medicine physicians after reviewing the images of tumors, and made the diagnosis based on 7th edition of AJCC staging system, which is based on the size, the extension or the location of the tumor (T), the lymph node invasions (N), and distant metastasis (M), and we recorded it for parameter comparison [10]. All the related data will be presented at Table 1.

Methods

PD-L1 staining was performed by using a mouse anti-human PD-L1 monoclonal antibody, which was provided by Dako 22c3 protocol (PD-L1 IHC 22C3 pharmDx monoclonal mouse clone by Dako Inc.), and Immuno Histo Chemistry (IHC) procedure was followed by suggested staining and interpreting procedure. Human EFGR gene mutation Fluorescence Polymerase Chain (PCR) Diagnostic Kit was used (Scorpion & ARMS by QIAGEN Inc.) for detection of EGFR gene mutation, and all the procedures were followed by manufacturer's instructions. The rabbit monoclonal anti-ALK IHC kit (VENTANA ALK (D5F3) CDx Assay by Ventana Inc.) was used for ALK gene rearrangement by the producer's suggestions. After we measured, we recorded related information at the Table 2.

Statistical analysis

After all the data was collected, including the histologic categories subtype of LC which is examined by pathologists, the mutation for EGFR and ALK by protocols, the percentage of the presence for PD-L1 by IHC protocol and so on. We would also review the stage of each patient with their cancer by PET analysis or other cancer scan methods. After we all the data was collected, we would make the comparison between the different PD-L1 expression rate with other pathological or biochemical findings by ANOVA or Chi-square test (Yate's correction was needed when expected count or count in any cell is less than 5) for binary clinical characteristics or Kruskal-Wallis rank sum test (K-W test) for categorical clinical characteristics. We used the IBM SPSS Statistics to measure the odds ratio for each clinical or biochemical data to see the relationships. We also used it the analyze the cohort at the Figure 1.

Results

Expression of PD-L1 on tumor cells on different patients

We evaluated the PD-L1 expression on different tumor samples from 500 patients and all the related data is listed at Table 1. We collected patients with NSCLC diagnosis, including 371 Adenocarcinoma (AD), 57 Squamous Cell Carcinoma (SCC), 63

Table 2: Biomarkers' characterization on 223 NSCLC patients with PD-L1 expression.

	No expression		Expression		Total
	PD-L1 <1%	Total for positive	Weak (1-50%)	Strong (>50%)	
Histology					
AD	133 (35.8%)	238 (64.2%)	173 (46.6%)	65 (17.6%)	371
SCC	14 (24.6%)	43 (75.4%)	26 (45.6%)	17 (19.8%)	57
Other	12 (19.0%)	51 (79.0%)	28 (44.4%)	23 (34.6%)	63
SCLC	7 (77.8%)	2 (22.2%)	2 (22.2%)	0 (0%)	9
Total	166 (33.2%)	334 (66.8%)	229 (45.8%)	105 (21.0%)	500
EGFR mutation					
Mutated	77(36.5%)	134(63.5%)	98(46.4%)	36(17.1%)	211
Exon 19 deletion	33 (15.6%)	57 (27.0%)	43 (20.4%)	14 (6.6%)	90
Exon 21 L858R	29 (13.7%)	42 (19.9%)	30 (14.2%)	12 (5.7%)	71
Exon 20 T790M	1 (0.6%)	7 (3.3%)	5 (2.4%)	2 (0.9%)	8
Others*	14 (6.6%)	28 (13.3%)	20 (9.4%)	8 (3.9%)	42
WT	54 (28.6%)	135 (71.4%)	90 (47.6%)	45 (23.8%)	189
Unknown	35 (35.0%)	65(65.0%)	41 (41.0%)	24 (24.0%)	100
ALK rearrangement					
+ve	5 (22.7%)	17 (77.3%)	12 (54.6%)	5 (22.7%)	22
-ve	104 (32.3%)	218 (67.7%)	152 (47.2%)	66 (20.5%)	322
Unknown	57 (36.5%)	99 (63.5%)	65 (41.7%)	34 (21.8%)	156

AD: Adenocarcinoma; SCC: Squamous Cell Carcinoma, SCLC: Small Cell Lung Cancer

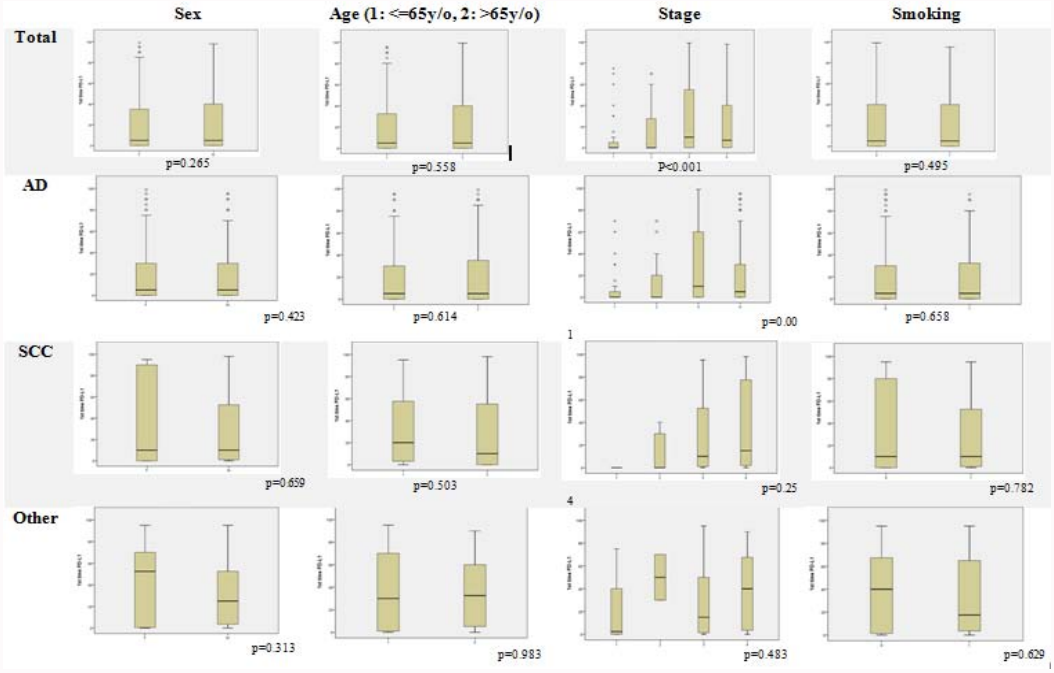

Figure 1: Association between PD-L1 expression on tumor cells and other clinical parameters in the whole patient cohort, Adenocarcinoma (AD), Squamous Cell Carcinoma (SCC), and other type of subgroups.

undifferentiated or unidentified NSCLC, and 9 small cell lung cancer (SCLC). PD-L1 expression was showed at any intensity in 334 cases (66.8%), which could be divided to two groups: 229 cases (45.8%) for low expression for expression rate between 1% to 50%, and 105 cases (21.0%) for high expression for expression rate over 50%. The clinical stage for NSCLC was clearly defined and used in this paper according to 7th edition of AJCC staging system based on the size, the extension or the location of the tumor (T), the lymph node invasions (N), and distant metastasis (M), and we recorded it for parameter comparison [10]. We use K-W test to check if there're some significant differences for PD-L1 expression between each subgroup for clinical characteristics, we discover that there're several significant difference for the rate between AD with other subgroups (p=0.002 with SCC subgroup, p<0.001 with other unidentified NSCLC, and p=0.002

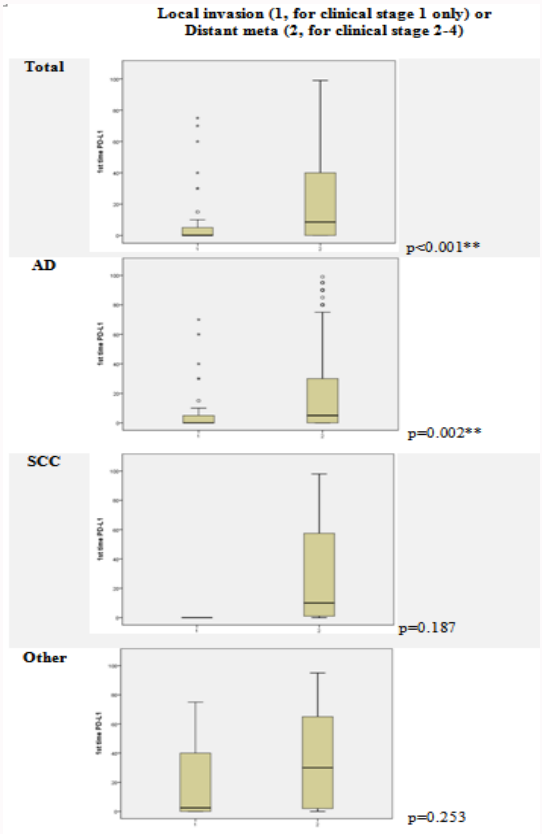

Figure 1: (Cont.) Association between PD-L1 expression on tumor cells and other clinical parameters in the whole patient cohort, Adenocarcinoma (AD), Squamous Cell Carcinoma (SCC), and other type of subgroups.

with SCLC), SCC and SCLC (p=0.002), and unidentified NSCLC with SCLC subgroups (p<0.001). But there's no significant difference between SCC group and other NSCLC group (p=0.366). And we also found that there was no obvious difference in most of the parameters in three separate groups for PD-L1 expression rate in tumor cells (Strong for expression rate over 50%, and weak for expression rate at 1~50%, and negative for rate at less than 1%), except for clinical stage of the tumor cells invasion in T (p=0.030), N (p<0.0001), M (p=0.0046) and ECOG when diagnosis is made (p=0.023) (Table 1).

The correlation between tumor cell PD-L1 expression and patients' clinical data for LC was analyzed in whole cohort in Figure 1. We discovered that there's no significant differences in the age of the patients (p=0.558 in total), the existence of the history of smoking (p=0.495 in total), the diagnosed clinical stage of LC in SCC and others (p=0.254 in SCC, and =0.483 in others), or the gender of the patients (p=0.265 in total), except the significantly different for lower PD-L1 expression rate in the clinical stage for stage 1 in AD when compared to other clinical tumor invasion stages (p=0.01, p=0.02, and 0.025, respectively). To realize more about the influence of the clinical features of distant metastasis for PD-L1 expression as the difference in clinical stages, we divided all the stages into two groups: local invasion for stage 1 only, and distant metastasis for stage 2 to 4, and tried to find out the significant differences for the immune checkpoints expression rate, and we also used 1% of PD-L1 expression rate as cut-off point. It showed that only the difference in AD group has significant disparity (p=0.002, 95% OR=1.655-6.105) for PD-L1 expression. However, when we use the different method for dividing clinical invasion group- that is, we defined stage 1 and 2 for local invasion, and stage 3 and 4 for distant metastasis- it showed the significant expression difference for AD (p<0.001, 95% OR=1.558-4.847) and SCC subgroups (p=0.042, 95% OR=1.312-51.258). Interestingly, when we tried to analyze the expression rate differences for different distant metastasis site, we revealed that most of the distant metastasis site may not be related to the expression of PD-L1 rate in tumor, except for the lymph node invasion in SCC (p=0.026) and other NSCLC subgroup (p=0.021), brain metastasis in SCC (p=0.021). And some distant metastasis site may result in some differences which have borderline significance, such as liver invasion in SCC (p=0.100), adrenal invasion in AD (p=0.088).

We also analyzed the biochemical characteristics on lung cancer cells for PD-L1 expression, and we found that there's a border line significant difference the existence for EGFR mutation and the positive finding for PD-L1 expression (OR=0.696, p=0.091, for 1% expression rate as cutline), but no similar result was found on ALK rearrangement (OR=1.622, p=0.645, for 1% expression rate as cutline). And we also found the similar result for comparing each different type of EGFR mutation (p=0.124), which were all recorded in Table 1, Table 2.

Discussion

As the importance of immune checkpoint inhibitor treatments, such as anti-PD-L1 (nivolumab, pembrolizumab, atezolizumab, etc.) or anti-CTLA-4 (ipilimumab, etc.), was emphasized recently in several NSCLC clinical therapy trials [1,6,11-14], which all showed significant and obvious ability for anti-tumor, some parameters used for estimating the situation in tumor invasion and response to these treatments, including PD-L1 expression, smoking status, mutations or dislocations for oncogenes, or neoantigen burden [8,15-18].

Since some trials revealed that these efficacies for treatments correlate to high PD-L1 expression in tumor cells [8,12,17,19], some research teams started finding the clinical data and trying to make the relationship between these data with the condition of PD-L1 presentation rate, including subtypes of lung cancer, the smoking status, gender, or the stage of tumor invasion [14,20-23]. In our study, we revealed that some of these parameters, especially for the clinical stage for lymph nodes invasion and local invasion, and the site of metastasis, such as lymph node or brain metastasis in SCC, would have some significant difference between different degree of PD-L1 expression rate, which is quite dissimilar from other studies, which showed that not only the TNM stage and sites of metastasis but also smoking status, gender, histology or tumor size would make an influence on PD-L1 expression [5,8,9,22-26]. Fortunately, there is still some cohort's support our results, which also revealed the degree of tumor invasion or nodal metastasis, especially in adenocarcinoma type of lung cancer, would cause some effects of decreasing PD-L1 expression [8,9,23,26,27]. We think it would result from the different lung cancer subtype would cause different PD-L1 expression, especially for SCLC to other subtypes, and AD to SCC, which is quite like Dr. Jiang's and Dr. Shimoji's conclusion [8,28], even though the trial only included AD and SCC in Dr. Jiang's results, but unlike Dr. Brody's cohort study, which showed no difference in histological category [5]. On the other hand, due to the toleration effect for tumor development when react to contiguous immune cells, the expression of PD-L1 seem to become a good weapon to defense for immune attack, and easier for tumor to grow and metastasis rather than local restriction as a result, the result seems reasonable for high

PD-L1expression in metastatic or higher stage of tumor invasion, and may correlate for poor prognosis [29-31]. In addition, in one recent research, it was unveiled that microRNA-33a, a small segment of non coding RNA which may influence the presenting of PD-L1, had higher concentration in tumors with lower clinical stage [32]. In another study, the researchers found the PD-L1 positive circulating tumor cells, which may also prove the metastatic ability for these high PD-L1 expression rate tumor cells [33]. However, unlike other trials, we didn't find any relationship between gender and smoking status. What's more, the bias of size of the trial compared with other trials or meta-analysis cohorts, the bias for enrollment of this trial, the different method for specimens retrieving (whether they were taken by surgical resection or biopsy by needles), or some oncogenes mutation [34] and microenvironment of immune system adjacent to the tumor [34,35] couldn't be ruled out for this result.

And we tried to find out the correlation of the oncogene mutation for lung cancer with this immune checkpoint as well, we discovered that there're no significant finding for this relationship due to not obvious differences, which showed some similarities for ALK may poses no significance to PD-L1, while some distinction from other similar trials exist for EGFR to PD-L1 expression [9,25,30,36]. Unfortunately, due to the exclusion for immune cells activities or some other common mutation, such as Kirsten rat sarcoma viral oncogene homolog (KRAS), which is proven may cause the different degree of PD-L1 expression [30,37], for the routine working for lung cancer survey in our hospital, we couldn't preclude the possibility of the influence of different immune microenvironment for these tumor or other oncogenes mutation for representation of PD-L1.

In conclusion, we found that there's only clinical stage for lung cancer influence the expression of PD-L1, especially for AD. Though it's a large trial which also enroll 362 patients, we still got some limitation for driver genes mutation or miro-environments for these tumor cells when reacting to immune system. The result may also apply to therapeutic or diagnostic strategies, which may simplify and make the checkpoint inhibitor treatments more effective.

Acknowledgements

The authors wish to thank Ms. Li-Chuan Tsen and Ms. Feng-Ya Shih in the Department of Nursing and Ms. Shi-Jen Chen in the Cancer Registry Center of Chang-Gung Memorial Hospital for their assistance in recording and retrieving of data of the lung cancer patients. This work was partially funded by the Chang-Gung Memorial Hospital (CMRPG-3D0281, -3D0282, -3D0283, and CMRPG 3E2071 for Dr. C. Y. Liu).

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