PD-L1 over-expression and survival in patients with non-small cell lung cancer: a meta-analysis

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Background: Observational studies on the prognostic role of programmed cell death-ligand 1 (PD-L1) in non-small cell lung cancer (NSCLC) are controversial.

Methods: To clarify the impact of PD-L1 in NSCLC survival, we performed this meta-analysis that included eligible studies. The combined hazard ratios (HR) and their corresponding 95% confidence intervals (CIs) were calculated in terms of overall survival.

Results: A total of five studies with 877 patients were evaluable for this meta-analysis. Our results suggested that PD-L1 overexpression had a poor impact on survival of patients with NSCLC, the HR (95% CI) was 1.43 (1.24-1.63) overall, 1.51 (1.24-1.7954) in Asian patients, 1.35 (1.08-1.63) in non-Asian patients. Moreover, there was no heterogeneity between the studies.

Conclusions: PD-L1 overexpression indicates a poor prognosis for patients with NSCLC.

Keywords: Programmed cell death-ligand 1 (PD-L1); prognosis; lung cancer; meta-analysis

Introduction

Lung cancer remains the most lethal cancer worldwide, despite improvements in diagnostic and therapeutic techniques. Its incidence has not peaked in many parts of world, particularly in China, which has become a major public health challenge all the world (1). The prognosis for lung cancer patients is generally poor, with an overall 5-year survival rate of approximately 15%, and it has shown little improvement in recent decades (2,3). Several independent prognostic factors for survival have been identified: performance status (PS), disease stage, age, sex and amount of weight lost (4). Some of these factors are useful when choosing treatment options for an individual, principally disease stage and PS. However, the discriminant value of most potential prognostic biological markers is insufficient to predict the optimal therapeutic course for an individual (5,6).

The first duplication of programmed cell death-1 (PD-1) (B7-H1) was created based on its DNA sequence (7). PD-1, an immune checkpoint which is expressed on the surface of T, B and NK cells, is a surface-receptor member of the B7-CD28 superfamily (8). The key role of the PD-1 pathway plays in blunting the T cell immune function was confirmed for the first time in PD-1 knockout mice (9). Cells that express PD-1 evade T cell immunity via mechanisms such as exhaustion, apoptosis and anergy, and thereby defend tumor cells from cytolysis (10). Programmed cell death-ligand 1 (PD-L1), the major ligand for PD-1, is a cell surface protein in the B7 family which is found in tumor specimens from non-small cell lung cancer (NSCLC) patients (11). The association between PD-L1 overexpression and survival in lung cancer patients has been studied for several years. However, no consensus has been reached; conflicting results have been reported from different laboratories. We therefore carried out a meta-analysis of data from published studies to quantitatively review the effect of PD-L1 overexpression in tumor tissue on survival in patients with NSCLC.

www.tlcr.org *Transl Lung Cancer Res* 2015;4(2):203-208

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Table 1 Main characteristics and results of the eligible studies

First author-year	Patients source	Histology	Stage	N pts	Method	Positive (%)	HR estimation	Survival results
Zhang 2014	China	AC	I-III	143	IHC	NA	HR and 95% CI 1.98 (1.01-3.89)	Poor
Velcheti 2013 Yale cohort	USA	NSCLC	I-IV	155	IHC	36.1	Survival curves 1.43 (1.12-2.14)	Poor
Velcheti 2013 Greek cohort	Greece	NSCLC	I-IV	303	IHC	24.8	Survival curves 1.32 (1.09-1.75)	Poor
Chen YB 2012	China	NSCLC	I-III	120	IHC	57.5	HR and 95% CI 2.95 (1.63-4.38)	Poor
Ma W 2011	China	NSCLC	I-IV	47	IHC	48.9	Survival curves 1.48 (1.15-1.97)	Poor
Wu 2011	China	NSCLC	I-III	109	IHC	53.2	Survival curves 1.39 (1.08-1.87)	Poor

N pts, number of patients; HR, hazard ratio; AC, adenocarcinoma; IHC, immunohistochemistry; NA, not applicable; CI, confidence interval; NSCLC, non-small cell lung cancer.

Materials and methods

Search strategy and study selection

The electronic databases PubMed and China National Knowledge Infrastructure (CNKI) were searched for studies to include in our meta-analysis. An upper date limit of January 31, 2015 was applied; we used no lower date limit. Searches included the terms "Programmed cell death-ligand 1", "PD-L1", "B7-H1" and "prognosis". We also reviewed the Cochrane Library for relevant articles. The references reported in the identified studies were also used to complete the search.

Studies eligible for inclusion in this meta-analysis met the following criteria: (I) measure PD-L1 expression in the primary lung cancer tissue with immunohistochemistry (IHC) or other methods; (II) provide information on survival (studies investigating response rates only were excluded); (III) have a follow up time exceeding 5 years; and (IV) when the same author reported results obtained from the same patient population in more than one publication, only the most recent report, or the most complete one, was included in the analysis. Two reviewers (PZ and ZZ) independently determined study eligibility. Disagreements were resolved by consensus.

Data extraction and quality assessment

The final articles included were assessed independently by two reviewers (PZ and ZZ). Data retrieved from the reports included first author, publication year, patient source, histology, disease stage, test method, PD-L1 positive and survival data (*Table 1*). If data from any of the above

categories were not reported in the primary study, items were treated as "not applicable". We did not contact the author of the primary study to request the information.

Statistical methods

For the quantitative aggregation of the survival results, hazard ratios (HR) and their 95% confidence intervals (CIs) were combined to give the effective value. When these statistical variables were not given explicitly in an article, they were calculated from available numerical data using methods reported by Parmar *et al.* (12).

Heterogeneity of the individual HRs was calculated with Chi-squared tests according to Peto's method (13). Meanwhile, heterogeneity test with I^2 statistic and Q statistic was performed. All the studies included were categorized by patient race, histology, disease stage. Individual meta-analysis was conducted in each subgroup. If HRs were found to have fine homogeneity, a fixed effect model was used for secondary analysis; if not, a random-effect model was used. In this meta-analysis, DerSimonian-Laird random effects analysis (14) was used to estimate the effect of PD-L1 overexpression on survival. By convention, an observed HR >1 implies worse survival for the group with PD-L1 overexpression. The impact of PD-L1 on survival was considered to be statistically significant if the 95% CI did not overlap with 1. Horizontal lines represent 95% CIs. Each box represents the HR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (HR =1.0).
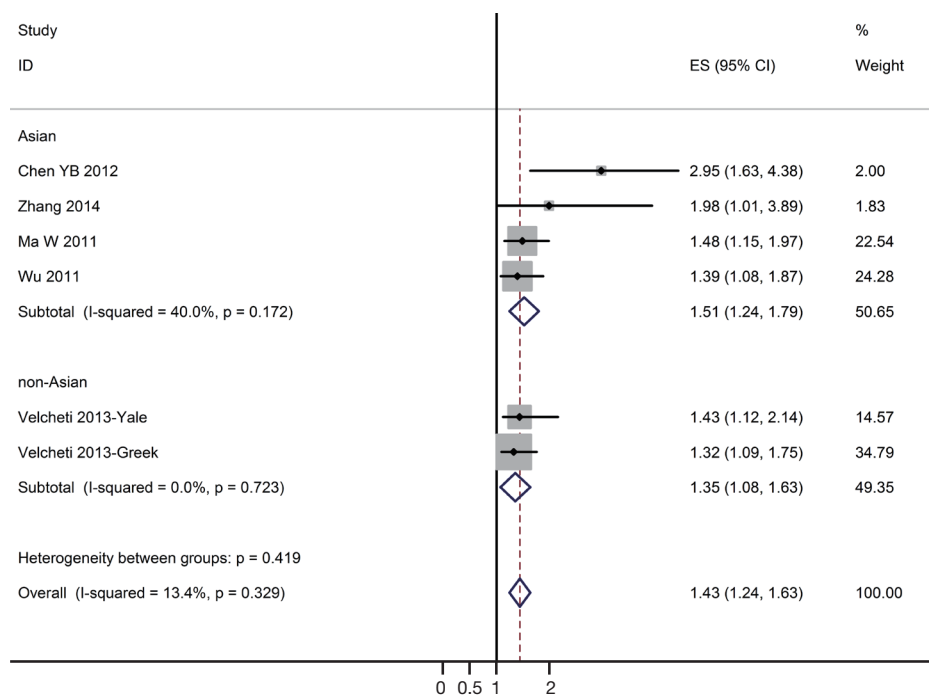
Figure 1 Meta-analysis (forest plot) of the five evaluable studies assessing PD-L1 in NSCLC stratified by ethnic source. PD-L1, programmed cell death-ligand 1; NSCLC, non-small cell lung cancer.

Evidence of publication bias was sought using the methods of Egger *et al*. (15) and of Begg *et al*. (16). Moreover, contour-enhanced funnel plot (17) was performed to aid in interpreting the funnel plot. If studies appear to be missing in areas of low statistical significance, then it is possible that the asymmetry is due to publication bias. If studies appear to be missing in areas of high statistical significance, then publication bias is a less likely cause of the funnel asymmetry. Intercept significance was determined by the *t*-test suggested by Egger (P<0.05 was considered representative of statistically significant publication bias). All calculations were performed using STATA version 11.0 (Stata Corporation, College Station, TX, USA).

Results

Study selection and characteristics

Five studies (18-22) published between 2011 and 2014 were eligible for this systematic review with meta-analysis. All reported the prognostic value of PD-L1 status for survival in NSCLC patients. The total number of patients included was 877 ranging from 47 to 303 patients per study (median 175). The major characteristics of the five eligible

publications are reported in *Table 1*.

These publications followed several different patient cohorts. The NSCLC studies considered either all lung cancer subtypes (n=4) and adenocarcinomas (n=1). All five studies used IHC to evaluate PD-L1 expression in NSCLC. Among all the five studies evaluating PD-L1 expression in NSCLC, four studies (419 patients: 47.8%) were performed in Asian populations, and the remaining one study (458 patients: 52.2%) followed European or American patients. The proportion of patients exhibiting PD-L1 overexpression in individual studies ranged from 24.8% to 57.5%.

Meta-analysis

The results of the meta-analysis are reported in *Figure 1*. Overall, the combined HR for all eligible studies evaluated PD-L1 expression in NSCLC was 1.43 (95% CI: 1.24-1.63), indicating that PD-L1 overexpression was an indicator of poor prognosis for NSCLC patients. Meanwhile, no significant heterogeneity was detected among these studies (I^2=13.4%, P=0.329). When grouped according to the geographic settings of individual studies, the combined HRs of Asian studies and non-Asian studies were 1.51 (95% CI:
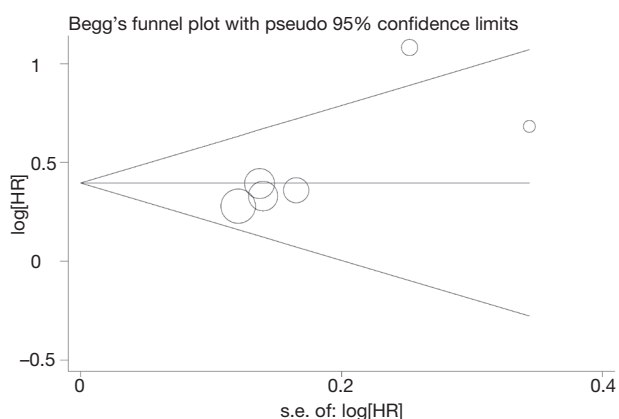
Figure 2 Funnel plot of the five evaluable studies assessing PD-L1 in NSCLC. PD-L1, programmed cell death-ligand 1; NSCLC, non-small cell lung cancer.

1.24-1.7954) and 1.35 (95% CI: 1.08-1.63), respectively (*Figure 1*).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All five eligible studies investigating NSCLC patients yielded a Begg's test score of P=0.274 and an Egger's test score of P=0.183, meanwhile according to the contour-enhanced funnel plot (*Figure 2*), the absence of publication bias was found in all five studies. These results suggest that there is no publication bias.

Discussion

PD-L1 expression leads to a negative antitumor immune response (23). PD-L1 is a ligand for PD-1, which is expressed on the surface of immune cells. By means of the PD-1/PD-L1 pathway, PD-L1 enables cancer cells to evade the host's immune system via the apoptosis of T-cell clones, the inhibition of lymphocyte proliferation and T-cell cytokine secretion (24-26). CD8þT-cells play a major role in cellular responses, including in antitumor immune defense, while tumor-infiltrating lymphocytes (TILs) contribute to good clinical outcomes in many types of cancer (27). Fewer TILs have been found in PD-L1-positive regions compared with PD-L1-negative regions, which means that PD-L1 expression appears to have a negative effect on the host's antitumor response (23).

In the present meta-analysis, we have combined five published studies including 877 patients with NSCLC

to yield summary statistics that indicate that PD-L1 overexpression has a significant correlation with poor survival in NSCLC and adenocarcinoma patients. This correlation was observed in both Asian and non-Asian study populations.

Recently, several systematic reviews (28-36) with meta-analyses on other biological prognostic factors for NSCLC had been reported. P53, microvessel density, HER-2, Ki-67 and RAS might be poor prognostic factors for survival in NSCLC, however, Bcl-2 might be better prognostic factor for survival in NSCLC. In order to clarify the prognostic impact of other biological factors in lung cancer, our group has performed several systematic reviews of the literature with meta-analyses. We found that vascular endothelial growth factor (VEGF) (37), E-cadherin (38) and matrix metalloproteinase 2 (39) might be poor prognostic factor in NSCLC, COX-2 (40) might be poor prognostic factor for stage I NSCLC, the ground glass opacity (GGO) area (41) had a favorable prognostic value of overall survival and relapse-free survival in small lung adenocarcinoma.

However, there are limitations to our study. This meta-analysis was limited to articles published in English and Chinese and could not include studies that were not published due to negative or useless results. Another potential source of bias is related to the method of HR and 95% CI extrapolation. If these statistics were not reported by the authors, we calculated them from the data available in the article. If this was not possible, we extrapolated them from the survival curves, necessarily making assumptions about the censoring process. Data for multivariate survival analysis reported in the article were included in the present systematic review with meta-analysis; if these data were not available, data calculated from survival curves by univariate analysis were included. These results should be confirmed by an adequately designed prospective study. Furthermore, the exact value of PD-L1 overexpression status needs to be determined by appropriate multivariate analysis. Unfortunately, few prospectively designed prognostic studies concerning biomarkers have been reported; thus, our collection of many retrospective studies revealed more significance.

Publication bias (42) is a major concern for all forms of meta-analysis; positive results tend to be accepted by journals, while negative results are often rejected or not even submitted. The present analysis does not support publication bias; the obtained summary statistics likely approximate the actual average. However, it should be noted that our meta-analysis could not completely exclude biases.

For example, the study was restricted to papers published in English and Chinese, which probably introduced bias.

In conclusion, PD-L1 overexpression is associated with a poor prognosis in patients with NSCLC in present meta-analysis. These results should be confirmed by an adequately designed prospective study.

Acknowledgements

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