Original Article

PD-L1 expression in malignant pleural effusion samples and its correlation with oncogene mutations in non-small cell lung cancer

Zhengbo Song[1], Guoping Cheng[2], Yiping Zhang[1]

[1]Department of Medical Oncology, [2]Department of Pathology, Zhejiang Cancer Hospital, Hangzhou 310022, China

Contributions: (I) Conception and design: Z Song; (II) Administrative support: Y Zhang; (III) Provision of study materials or patients: Z Song; (IV) Collection and assembly of data: G Cheng; (V) Data analysis and interpretation: Z Song; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Zhengbo Song, MD. Department of Chemotherapy, Zhejiang Cancer Hospital, Guangji Road No. 38, Hangzhou 310022, China. Email: songzhengbo83@163.com.

Background: Programmed death ligand 1 (PD-L1) tumor proportion score (TPS) is currently widely used for selection of immune therapies in non-small cell lung cancer (NSCLC). Most of samples for PD-L1 expression were obtained from tumor tissue. However, the feasible of malignant pleural effusion (MPE) cytological samples for PD-L1 detection is poorly reported. And the correlation between oncogene mutations and PD-L1 expression based on high-throughput sequencing is rarely studied.

Methods: NSCLC MPE cytological samples and partially paired tumor tissue from our institution analyzed for PD-L1 immunohistochemistry (IHC) using the clone SP263 pharmDx kit and evaluated genomic aberrations in all patients using next generation sequencing (NGS).

Results: One hundred and twenty-three MPE cell blocks and 29 paired tumor tissue were successfully tested for PD-L1 expression. PD-L1 TPS of ≥50% were seen in 18.7% (23/123) of all samples. The accordance of PD-L1 expression in tumor tissue and MPE samples was 86.2% (50% as cut-off value). PD-L1 TPS ≥50% tumors were significantly associated with *EGFR* wild-type (P=0.007), but, no correlation between other genes and PD-L1 expression. A trend of longer overall survival (OS) was observed in patients with PD-L1 TPS <50% than those TPS ≥50% (20.0 *vs.* 13.8 months, P=0.057). No difference of tumor mutational burden (TMB) was observed between patients with PD-L1 ≥50% and <50% (8.2/MB and 7.7/MB, P=0.47).

Conclusions: Our results suggest that cytological material is feasible for PD-L1 IHC analysis. Gene alterations could partially contribute to select the samples that with different PD-L1 expression. No correlation between the PD-L1 expression and TMB.

Keywords: Tumor proportion score (TPS); malignant pleural effusion (MPE); programmed death ligand 1 (PD-L1); oncogene mutations

Submitted Sep 22, 2019. Accepted for publication Oct 26, 2019.
doi: 10.21037/jtd.2020.02.06
View this article at: http://dx.doi.org/10.21037/jtd.2020.02.06

Introduction

Patients of advanced non-small cell lung cancer (NSCLC) harbored *EGFR*, *ALK* and *ROS1* improved the overall survival (OS) and quality of life after the molecular targeted therapy (1-6). However, the survival in patients with wild-type of gene alternations was not improved recently. The anti-programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) immune checkpoint inhibitors, have been approved for systemic therapy in advanced NSCLC for remarkable efficacy compared with chemotherapy, especially in patients with PD-L1 tumor proportion scores (TPSs) of ≥50% (7-9). However, many questions are not well answered currently (10). PD-L1 detection was mostly based on tumor tissue in previous studies. It is not well known that whether the cytological samples could be used for PD-L1 detection. The relationship between *EGFR/ALK*

mutations and PD-L1 expression was clearly investigated, while, the data based on high-throughput sequencing was scarce.

In present study, 123 malignant pleural effusion (MPE) samples were retrospectively analyzed for PD-L1 expression. Meanwhile, all of the samples were detected gene alterations with next generation sequencing (NGS) containing 416 genes. We aim to expound the feasibility of MPE samples for PD-L1 detection and investigate the correlation between oncogene mutations and PD-L1 expression.

Methods

Sample selection

Patients were enrolled in the study between Aug 2015 and Dec 2016. Eligible patients were aged at least 18 years and had advanced, non-squamous NSCLC with pleural effusion. All of the pleural effusion samples were confirmed as malignant by cytological smears. At the time of enrollment, the patients had not received targeted inhibitors. Patient exclusion criteria included squamous cell lung cancer, small cell lung cancer, or other metastatic malignancies tumor to the lung. Diagnosis of the tumors was performed by institutional pathologists with the accordance of the 2015 WHO classification. The study was approved by Zhejiang Cancer Hospital Ethics Committee (IRB2014-03-032). Written informed consent was obtained from all participants.

Preparation of cell block and tumor PD-L1 analysis

About 50-mL fluid specimens were centrifuged at 2,500–3,000 rpm for 5 min. Cell sediments were then harvested, fixed with 3 times the volume of 10% neutral-buffered formalin for 60 min, wrapped in filter paper, and processed in an automatic tissue processor. The samples were embedded in paraffin and sectioned at a thickness of 4–5 mm.

Ventana independently stained all cases using PD-L1 IHC assay platforms. At Ventana, sections were stained with anti-PD-L1 (SP263, Roche) rabbit monoclonal primary antibody and a matched rabbit immunoglobulin G-negative control with an OptiView DAB IHC Detection Kit on the BenchMark ULTRA automated staining platform. Three pathologists were independently evaluated all PD-L1 immunostained slides.

NGS analysis

Cell blocks were obtained and shipped to the central testing laboratory by required conditions. The tests were performed in Nanjing Geneseeq Technology Inc., China. Briefly, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples. Purified DNA was qualified by Nanodrop2000 (Thermo Fisher Scientific) and quantified by Qubit 3.0 using the dsDNA HS Assay Kit (Life Technologies) according to the manufacturer's recommendations. Sequencing libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems) with an optimized manufacturer's protocol. Customized xGen lockdown probes (Integrated DNA Technologies) targeting 416 cancer-relevant genes were used for hybridization enrichment (*Table S1*). The capture reaction was performed with the NimbleGen SeqCap EZ Hybridization and Wash Kit (Roche) and Dynabeads M-270 (Life Technologies) with optimized manufacturers' protocols. Genomic fusions were identified by FACTERA with default parameters. Copy number variations (CNVs) were detected using ADTEx (http://adtex.sourceforge.net) with default parameters. Somatic CNVs were identified using paired normal/tumor samples for each exon.

TMB was defined as the number of somatic, coding, base substitution, and indel mutations per megabase of genome examined. For our panel TMB calculation, all base substitutions, including non-synonymous and synonymous alterations, and indels in the coding region of targeted genes were considered with the exception of known hotspot mutations in oncogenic driver genes and truncations in tumor suppressors. Synonymous mutations were counted in order to reduce sampling noise and known driver mutations were excluded as they are over-represented in the panel, as previously described (11).

Statistical methods

Fisher's exact test was used to compare categorical variables. All P values reported are two-sided, and tests were conducted at the 0.05 significance level. The relationship between different groups was analyzed with chi-square tests. Progression-free survival (PFS) with targeted therapy was defined as the time from initiation targeted treatment to documented progression or death from any cause. PFS was plotted by the Kaplan-Meier method. All analyses were performed using SPSS® version 18.0 (SPSS Inc., Chicago, IL, USA). The last follow-up date was May 31, 2018.

Table 1 Clinicopathological characteristics of study participants

Variable	Number (%)
Gender	
Male	65 (52.8)
Female	58 (47.2)
Age (years)	
≥65	46 (37.4)
<65	77 (62.6)
Smoking history	
Yes	51 (41.5)
No	72 (58.5)
Performance status	
0–1	106 (86.2)
2	17 (13.8)
Metastatic status	
M1a	73 (59.3)
M1b	50 (40.7)
Histology	
Adenocarcinoma	119 (96.7)
Non-adenocarcinoma	4 (3.3)
History of chemotherapy	
Yes	37 (30.1)
No	86 (69.9)

The median follow-up time was 20.2 months (range, 3.0–29.5 months). No patients were lost to follow-up.

Results

Baseline clinical and pathologic characteristics

Of the 123 patients analyzed, 65 were male and 58 of female with median age of 59 years old (range, 33 to 81 years old). Most of patients were with histology of adenocarcinoma (119 of 123). Fifty-one patients had smoking history and 72 were never smokers. The details of clinical and pathologic characteristics in present study were listed in *Table 1*.

PD-L1 expression in MPE samples and paired tumor tissues

Totally, 48 (39.0%) were with PD-L1 negative, followed by 1–5% (n=28), and PD-L1 TPSs of 5–49% (n=24). Twenty-three were with proportion of PD-L1 TPS of ≥50%. PD-L1 TPS ≥50% was seen significantly more frequently in smokers as compared to never smokers (P=0.01) and males (P=0.025). While not associated with patient tumor stage (P=0.53), age (P=0.85) and performance status (P=0.33) (*Table S2*).

Twenty-nine patients were obtained the paired tumor tissue and with PD-L1 IHC detection (*Figure 1*). Among the 29 samples, 14 had PD-L1 TPS ≥1% in tumor tissue, and 11 in paired MPE samples, with agreement statistics of 69.0% (20/29) (*Table 2*). When 50% as cut off value, the accordance between MPE samples and tumor tissue was 86.2% (25/29) (*Table 3*). The details of comparison between MPE samples and tumor tissue was presented in *Tables 2-4*.

NGS results

All results of the comparative analyses are presented in *Figure 2*. Overall, *EGFR* mutations were with most frequent (55.3%), followed with *TP53* mutation (51.2%). Sixteen patients were found to harbor *KRAS* mutations, *ALK* rearrangement were observed in 11 patients. There was no *ROS1* rearrangement, MET amplification and exon 14 skipping among the 123 samples.

Association between PD-L1 expression and oncogene aberrations

Of the 68 patients with *EGFR* mutations, 10.3% of PD-L1 TPS ≥50%, while, the percentage of PD-L1 TPS ≥50% was 29.1% *EGFR* wild-type (P=0.007). Of 11 patients with *ALK* rearrangement, 9 had PD-L1 TPS <50%, as compared with only two tumors with PD-L1 TPS ≥50% (P=0.72). More patients with PD-L1 TPS ≥50% in *KRAS* mutations than wild-type samples (25.0% *vs.* 17.8%, P=0.73) (*Table 5* and *Figure S1*). The median TMB in samples with PD-L1 ≥50% and <50% was 8.2/MB and 7.7/MB, respectively (P=0.47).

PD-L1 expression and clinical treatment

Forty-seven patients with *EGFR* mutations received EGFR-TKIs treatment. The median PFS was 10.2 months (95% CI: 9.1–11.7 months). A trend of longer PFS was observed in patients with PD-L1 TPS ≥50% (11.7 *vs.* 9.7 months, P=0.17). Forty-four patients received first-line platinum-based chemotherapy, including 25 with pemetrexed and 19

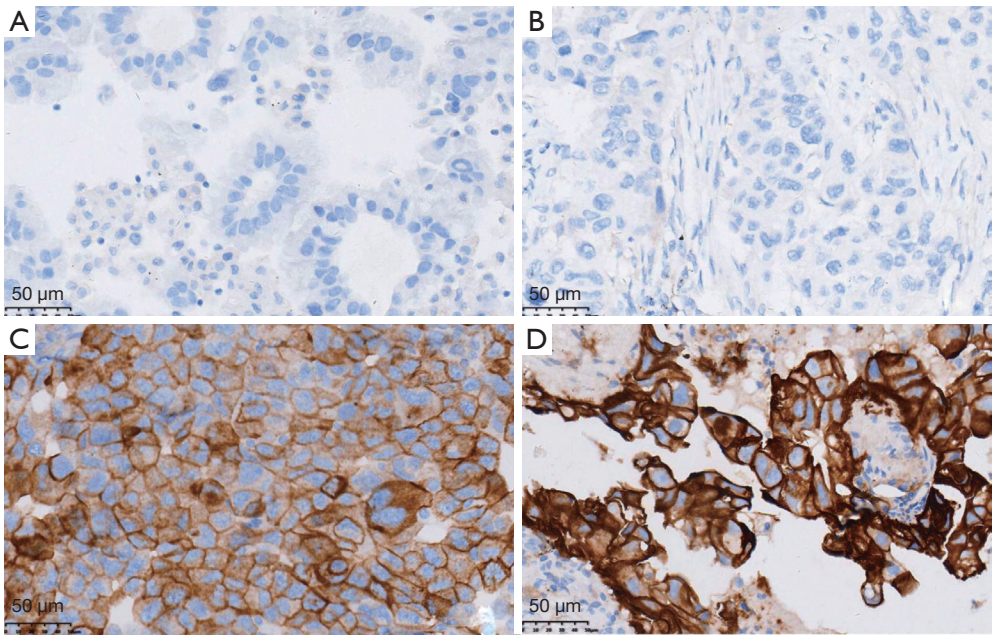

Figure 1 PD-L1 expression in MPE samples (A,C) and paired tumor tissue (B,D) (A and B: TPS =0%; C and D: TPS =100%; IHC, ×400). PD-L1, programmed death ligand 1; MPE, malignant pleural effusion; TPS, tumor proportion score; IHC, immunohistochemistry.

Table 2 PD-L1 expression in tumor tissue and paired MPE samples (a cut-off value of 1%)

MPE	Tumor tissue		Total
	Positive	Negative	
Positive	8	3	11 (37.9)
Negative	6	12	18 (62.1)
Total	14 (48.3)	15 (51.7)	29

PD-L1, programmed death ligand 1; MPE, malignant pleural effusion.

Table 3 PD-L1 expression in tumor tissue and paired MPE samples (a cut-off value of 50%)

MPE	Tumor tissue		Total
	Positive	Negative	
Positive	5	1	6 (20.7)
Negative	3	20	23 (79.3)
Total	8 (27.6)	21 (72.4)	29

PD-L1, programmed death ligand 1; MPE, malignant pleural effusion.

Table 4 PD-L1 expression in tumor tissue and paired MPE samples (a cut-off value of 10%)

MPE	Tumor tissue		Total
	Positive	Negative	
Positive	8	1	9 (31.0)
Negative	4	16	20 (69.0)
Total	12 (41.4)	17 (58.6)	29

PD-L1, programmed death ligand 1; MPE, malignant pleural effusion.

of other regimens. No PFS difference was found between different regimens (7.0 *vs.* 6.5 months, P=0.97).

The median OS of all patients was 18.4 months (95% CI: 14.9–21.8 months). A trend of longer OS was observed in patients with PD-L1 TPS <50% than those TPS ≥50% (20.0 *vs.* 13.8 months, P=0.057) (*Figure 3*). No survival difference was observed in *EGFR/ALK* positive patients with PD-L1 TPS <50% than those TPS ≥50% (21.0 *vs.* 20.5 months, P=0.21); However, a shorter survival was existed in *EGFR/ALK* negative patients with PD-L1 TPS <50% than those

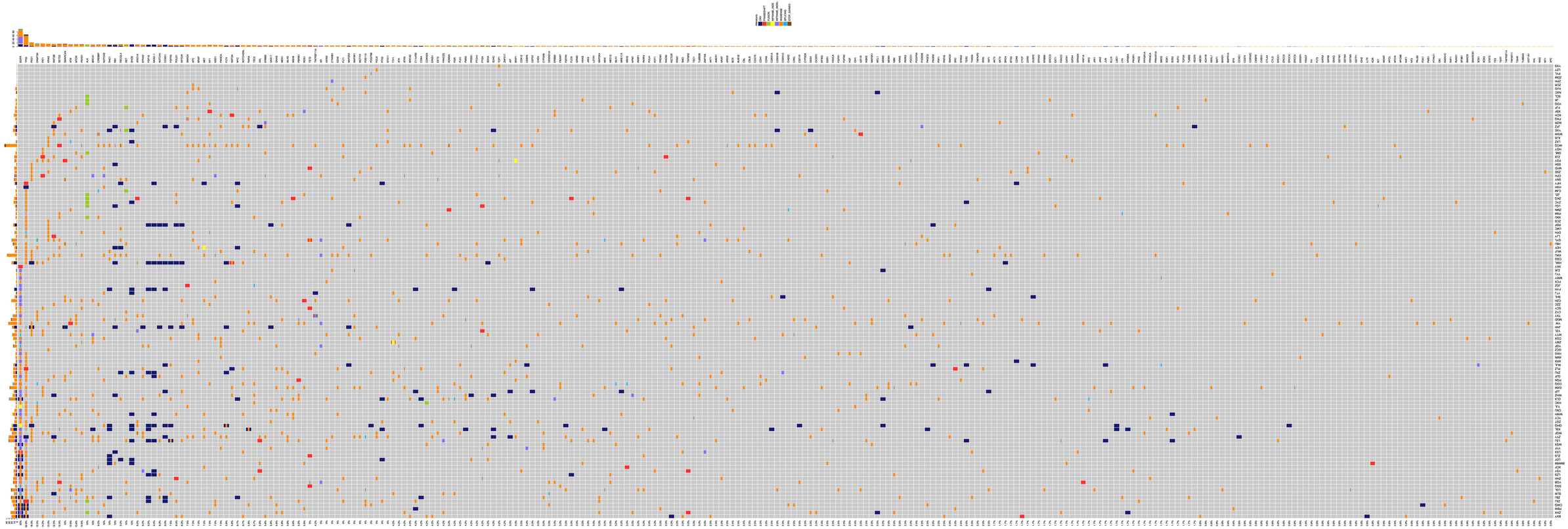

Figure 2 All of the gene alternations in 123 samples.

Table 5 Correlation between common oncogene mutations or rearrangement and PD-L1 over-expression

Gene	Mutation	Wild-type	PD-L1 TPS ≥50% in mutation	PD-L1 TPS ≥50% in wild-type	P
EGFR	68	55	10.3% (7/68)	29.1% (16/55)	0.007
ALK	11	112	18.2% (2/11)	18.8% (21/112)	0.72
KRAS	16	107	25.0% (4/16)	17.8% (19/107)	0.73
TP53	63	60	20.6% (13/63)	16.7% (10/60)	0.57
KRAS/TP53	7	116	28.6% (2/7)	18.1% (21/116)	0.49
RET	3	120	33.3% (1/3)	18.3% (22/120)	0.93
BRAF	9	114	44.4% (4/9)	16.7% (19/114)	0.11
ERBB2	7	116	0.0% (0/7)	19.8% (23/116)	0.42
PIK3CA	9	114	0.0% (0/9)	20.2% (23/114)	0.29
STK11	6	117	16.7% (1/6)	18.8% (22/117)	0.68

PD-L1, programmed death ligand 1; TPS, tumor proportion score.

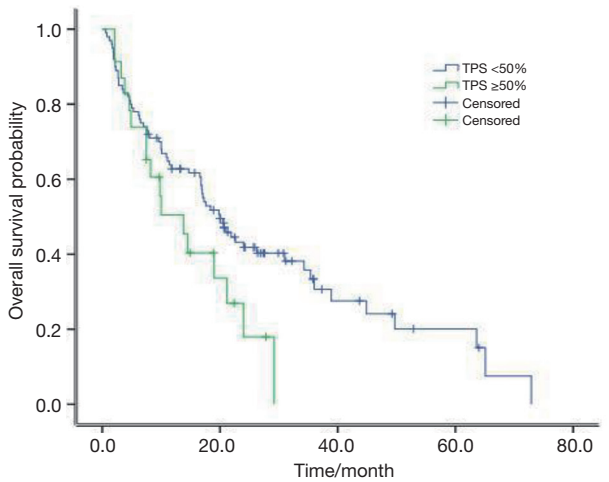

Figure 3 Overall survivals comparison in patients with different PD-L1 expression. PD-L1, programmed death ligand 1.

TPS ≥50% (15.5 *vs.* 12.7 months, P=0.025).

Discussion

A high accordance of PD-L1 expression was found between tumor tissue and cytological samples in present study. Further, we investigated the relationship between gene alternations and PD-L1 expression based on high-throughput sequencing. Our results demonstrated PD-L1 expression was associated with some oncogene aberrations.

Two platforms are currently applied in clinical practice for PD-L1 IHC detection, including DAKO and VENTANA (12-14). Patients with PD-L1 TPS of ≥50% were reported to benefit more from pembrolizumab treatment than chemotherapy in KEYNOTE024 study (7). And the PD-L1 TPS of ≥50% were reported in 20% to 30% of advanced NSCLC (7-9). The difference percentage may contribute to different antibodies in different trials. The Blueprint PD-L1 IHC Assay Comparison Project revealed that three antibodies (22C3, 28-8, SP263) were closely aligned on tumor cell staining, but different from SP142 (15). In present study, The PD-L1 TPS of ≥50% were found in 18.7% patients, which was a similar percentage compared with previous studies (16-18). And a high correlation between staining on cytological cell block material and histological specimens was observed. Our results indicated the feasibility of MPE samples for PD-L1 detection.

PD-L1 TPS of ≥50% in *EGFR* mutation patients were reported with 11% in Gainor *et al.* study (18). However, lung cancer patients harboring *EGFR* mutations are associated with lower response to PD-1/PD-L1 inhibitors (usually lower than 5% in previous studies). Low rates of concurrent PD-L1 expression and $CD8^+$ tumor-infiltrating lymphocytes (TILs) may underlie these results. In present study, 10.3% *EGFR*-mutated samples were with PD-L1 TPS of ≥50%, in contrast, 29.1% of patients with *EGFR* wild type were with PD-L1 TPS of ≥50% which consistence with previous study. In another study, Dong *et al.* found that TP53 mutation significantly activated T-effector and interferon-γ signature. And *TP53/KRAS* co-mutated subgroup manifested exclusive increased expression

of *PD-L1* mutation burden (19). The reason may due to these two genes altered a group of genes involved in cell cycle regulating, DNA replication and damage repair, which results to a favorable efficacy to immune treatment. However, it is not clear for the correlation between *TP53/KRAS* and *PD-L1* expression in Dong *et al.* study. In present study, no significant different was found between *TP53/KRAS* mutation. The small number patients may cause the bias.

As a retrospective nature, our study has several limitations. First, only 29 patients were with paired tumor tissue, hence, the accordance between tumor tissue and MPE sample could not fully validated in present study. Second, only the antibody of SP263 was used to examine the PD-L1 expression, which would be preferred for using another antibody to validate the results. In addition, although the 25% of TPS was recommended as cut-off value for durvalumab study (20). However, in the MYSTIC study, the data showed no more benefit for durvalumab than chemotherapy (21). Hence, 50% may be a preferable cut-off value regardless different antibody. For the purpose of comparison with other antibody, the 50% TPS was used in present study. Thirdly, no PD-1/PD-L1 inhibitors are approved in China, our data could not be examined in clinics. In additions, NGS in present study was not based on whole exome sequencing, the results based on 416 genes may not well represent the real TMB level, the relationship between PD-L1 and TMB needs to be investigated in future study. Last but not least, only 123 patients were collected in present study, hence, the correlation between rare oncogene mutations and PD-L1 expression was not fully investigate and the results might be affected.

In summary, our data suggests that MPE samples is feasible for PD-L1 IHC analysis. The PD-L1 levels of MPE cell blocks were comparable with paired tumor tissues, however, heterogeneity was found between these two media. Gene alterations based on NGS of MPE samples could contribute to select the samples that with different PD-L1 expression.

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (grant no: 81802276).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd.2020.02.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by Zhejiang Cancer Hospital Ethics Committee (IRB2014-03-032). Written informed consent was obtained from all participants.

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. 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.
3. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
4. Wu YL, Zhou C, Liam CK, et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. Ann Oncol 2015;26:1883-9.
5. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
6. Shi YK, Wang L, Han BH, et al. First-line icotinib versus cisplatin/pemetrexed plus pemetrexed maintenance therapy

for patients with advanced EGFR mutation-positive lung adenocarcinoma (CONVINCE): a phase 3, open-label, randomized study. Ann Oncol 2017;28:2443-50.
7. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016;375:1823-33.
8. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387:1837-46.
9. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34.
10. Sacher AG, Gandhi L. Biomarkers for the Clinical Use of PD-1/PD-L1 Inhibitors in Non-Small-Cell Lung Cancer: A Review. JAMA Oncol 2016;2:1217-22.
11. Rimm DL, Han G, Taube JM, et al. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. JAMA Oncol 2017;3:1051-8.
12. Kerr KM, Nicolson MC. Non-Small Cell Lung Cancer, PD-L1, and the Pathologist. Arch Pathol Lab Med 2016;140:249-54.
13. Sholl LM, Aisner DL, Allen TC, et al. Programmed Death Ligand-1 Immunohistochemistry--A New Challenge for Pathologists: A Perspective From Members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2016;140:341-4.
14. Hirsch FR, Mcelhinny A, Stanforth D, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. J Thorac Oncol 2017;12:208-22.
15. Ilie M, Khambata-Ford S, Copie-Bergman C, et al. Use of the 22C3 anti-PD-L1 antibody to determine PD-L1 expression in multiple automated immunohistochemistry platforms. PLoS One 2017;12:e0183023.
16. Rangachari D, VanderLaan PA, Shea M, et al. Correlation between Classic Driver Oncogene Mutations in EGFR, ALK, or ROS1 and 22C3-PD-L1 ≥50% Expression in Lung Adenocarcinoma. J Thorac Oncol 2017;12:878-83.
17. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540-50.
18. Gainor JF, Shaw AT, Sequist LV, et al. EGFR Mutations and ALK Rearrangements Are Associated with Low Response Rates to PD-1 Pathway Blockade in Non-Small Cell Lung Cancer: A Retrospective Analysis. Clin Cancer Res 2016;22:4585-93.
19. Dong ZY, Zhong WZ, Zhang XC, et al. Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. Clin Cancer Res 2017;23:3012-24.
20. Powles T, O'donnell PH, Massard C, et al. Efficacy and Safety of Durvalumab in Locally Advanced or Metastatic Urothelial Carcinoma: Updated Results From a Phase 1/2 Open-label Study. JAMA Oncol 2017;3:e172411.
21. Planchard D, Yokoi T, Mccleod MJ, et al. A Phase III Study of Durvalumab (MEDI4736) With or Without Tremelimumab for Previously Treated Patients With Advanced NSCLC: Rationale and Protocol Design of the ARCTIC Study. Clin Lung Cancer 2016;17:232-236.e1.

Cite this article as: Song Z, Cheng G, Zhang Y. PD-L1 expression in malignant pleural effusion samples and its correlation with oncogene mutations in non-small cell lung cancer. J Thorac Dis 2020;12(4):1385-1392. doi: 10.21037/jtd.2020.02.06

Supplementary

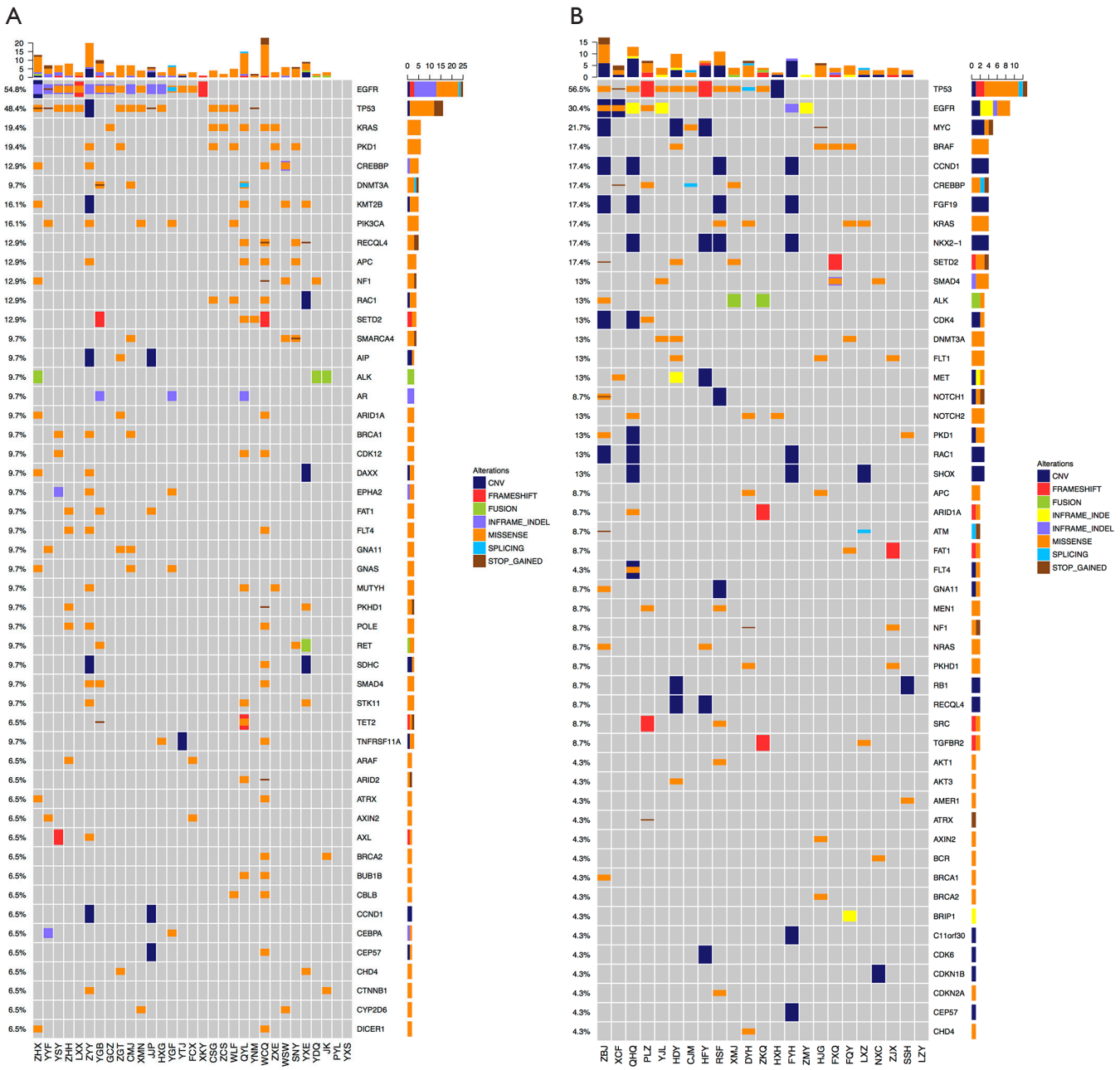

Figure S1 Top 50 genes in patients with PD-L1 TPS <50% (A) and with PD-L1 TPS ≥50% (B). PD-L1, programmed death ligand 1; TPS, tumor proportion score.

Table S1 List of 416 cancer-related target genes with NGS detection

ABCB1 (MDR1)
ABCC2 (MRP2)
ADH1B
AFF1
AFF4
AIP
AKT1
AKT2
AKT3
ALDH2
ALK
AMER1
APC
AR
ARAF
ARID1A
ARID2
ARID5B
ASXL1
ATF1
ATIC
ATM
ATR
ATRX
AURKA
AURKB
AXIN2
AXL
BAIAP2L1
BAK1
BAP1
BARD1
BCL2
BCL2L11 (BIM)
BIRC3
BLM
BMPR1A
BRAF
BRCA1
BRCA2
BRD4
BRIP1
BTG2
BTK
BUB1B
c11orf30
CBL
CBLB
CCND1
CCNE1
CD274 (PD-L1)
CD74
CDA
CDC73
CDH1
CDK10
CDK12
CDK4
CDK6
CDK8
CDKN1A
CDKN1B
CDKN1C
CDKN2A
CDKN2B
CDKN2C
CEBPA
CEP57
CHD4
CHEK1
CHEK2
CLIP1
CLTC
COL1A1

Table S1 (*continued*)

CREB1
CREBBP
CRKL
CSF1R
CTCF
CTLA4
CTNNB1
CXCR4
CYLD
CYP19A1
CYP2A6
*CYP2B6*6*
*CYP2C19*2*
*CYP2C9*3*
*CYP2D6*3*
*CYP2D6*4*
*CYP2D6*5*
*CYP2D6*6*
*CYP2D6*7*
*CYP2D6*11*
*CYP2D6*12*
*CYP2D6*14*
*CYP3A4*4*
*CYP3A5*1*
*CYP3A5*3*
DAXX
DCTN1
DDIT3
DDR2
DENND1A
DHFR
DICER1
DNMT3A
DPYD
DUSP2
EGFR
EML4
EP300
EPAS1
EPCAM
EPHA2
EPHA3
EPS15
ERBB2 (HER2)
ERBB3
ERBB4
ERC1
ERCC1
ERCC2
ERCC3
ERCC4
ERCC5
ERG
ESR1
ETV1
ETV4
ETV6
EWSR1
EXT1
EXT2
EZH2
EZR
FANCA
FANCC
FANCD2
FANCE
FANCF
FANCG
FANCL
FAT1
FBX1
FBXW7
FEV
FGF19
FGFR1

Table S1 (*continued*)

FGFR2
FGFR3
FGFR4
FH
FLCN
FLI1
FLT1 (VEGFR1)
FLT3
FLT4
GATA1
GATA2
GATA3
GATA4
GATA6
GNA11
GNAQ
GNAS
GOLGA5
GOPC
GRIN2A
GRM3
GSTM1
GSTP1
GSTT1
HDAC2
HGF
HIP1
HLA-A
HNF1A
HNF1B
HRAS
HSD3B1
IDH1
IDH2
IGF1R
IGF2
IKBKE
IKZF1
IKZF3
IL7R
INPP4B
INPP5D
IRF2
JAK1
JAK2
JAK3
JUN
KDM5A
KDM6A
KDR (VEGFR2)
KIF5B
KIT
KITLG
KLC1
KLLN
KMT2A
KMT2B
KRAS
KTN1
LHCGR
LMO1
LRIG3
LYN
LZTR1
MAP2K1 (MEK1)
MAP2K2 (MEK2)
MAP2K4
MAP3K1
MAP4K3
MAX
MCL1
MDM2
MDM4
MED12
MEF2B

Table S1 (*continued*)

MEN1
MET
MGMT
MITF
MLH1
MLH3
MLLT1
MLLT10
MLLT3
MLLT4
MPL
MRE11A
MSH2
MSH3
MSH6
MTHFR
MTOR
MUTYH
MYC
MYCL
MYCN
MYD88
NAT1
NBN
NCOA4
NF1
NF2
NFKBIA
NKX2-1
NOTCH1
NOTCH2
NPM1
NQO1
NR4A3
NRAS
NSD1
NTRK1
PAK3
PALB2
PALLD
PARK2
PARP1
PARP2
PAX5
PBRM1
PCDH11Y
PDCD1 (PD1)
PDCD1LG2 (PD-L2)
PDE11A
PDGFRA
PDGFRB
PDK1
PGR
PHOX2B
PIK3C3
PIK3CA
PIK3R1
PIK3R2
PKD1
PKD2
PKHD1
PLAG1
PLK1
PMS1
PMS2
POLD1
POLE
POLH
POT1
POU5F1
PPP2R1A
PRDM1
PRF1
PRKACA
PRKAR1A

Table S1 (*continued*)

PRKCI
PRSS1
PTCH1
PTEN
PTK2
PTPN11
PTPRD
QKI
RAC1
RAD50
RAD51
RAD51C
RAD51D
RAF1
RARA
RB1
RECQL4
RET
RHOA
RICTOR
RNF146
RNF43
ROS1
RPTOR
RRM1
RTEL1
RUNX1
SBDS
SDC4
SDHA
SDHAF2
SDHB
SDHC
SDHD
SEPT9
SERP2
SETBP1
SETD2
SF3B1
SGK1
SH2D1A
SHOX
SLC34A2
SLC7A8
SLX4
SMAD2
SMAD3
SMAD4
SMAD7
SMARCA4
SMARCB1
SMO
SOX2
SPOP
SPRY4
SRC
SRY
STAG2
STAT3
STK11
STMN1
STRN
STT3A
SUFU
TACC1
TACC3
TEK
TEKT4
TERC
TERT
TET2
TFG
TGFBR2
THADA
TMEM127

Table S1 (*continued*)

TMPRSS2
TNFAIP3
TNFRSF11A
TNFRSF14
TNFRSF19
TNFSF11
TOP1
TOP2A
TP53
TPM3
TPM4
*TPMT*2*
*TPMT*3*
*TPMT*4*
*TPMT*5*
*TPMT*6*
*TPMT*7*
*TPMT*10*
TRIM24
TRIM27
TRIM33
TSC1
TSC2
TSHR
TTF1
TUBB3
TYMS
UGT1A1
VEGFA
VHL
WAS
WISP3
WRN
WT1
XPA
XPC
XRCC1
YAP1
ZNF2
ZNF217
ZNF444
ZNF703

NGS, next generation sequencing.

Table S2 Comparison of clinical characteristics of PD-L1 TPS ≥50% versus TPS <50%

Variable	PD-L1 TPS ≥50%	PD-L1 TPS <50%	P
Gender			0.025
Male	17	48	
Female	6	52	
Age, years			0.85
<65	14	63	
≥65	9	37	
Smoking history			0.01
Yes	15	36	
No	8	64	
Metastasis site			0.53
M1a	15	58	
M1b	8	42	
Chemotherapy history			0.33
Yes	5	32	
No	18	68	
Performance status			0.33
0–1	19	92	
2	4	8	

PD-L1, programmed death ligand 1; TPS, tumor proportion score.