Journal of the National Cancer Center 4 (2024) 162–168

Contents lists available at ScienceDirect

Journal of the National Cancer Center

journal homepage: www.elsevier.com/locate/jncc

Short Communication

Concordance between four PD-L1 immunohistochemical assays and 22C3 pharmDx assay in esophageal squamous cell carcinoma in a multicenter study

Liyan Xue[1,†], Yuan Li[2,3,4,†], Lili Jiang[5,†], Chao Liu[6,†], Na Cheng[1,†], Changyuan Guo[1], Yan Jin[2,3,4], Ping Zhou[5], Xuemin Xue[1], Yue Wang[2,3,4], Weiya Wang[5], Yanhui Liu[4,*], Jianming Ying[1,*]

[1] Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[2] Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China
[3] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China
[4] Institute of Thoracic Oncology, Fudan University, Shanghai, China
[5] Department of Pathology, West China Hospital, Sichuan University, Chengdu, China
[6] Department of Pathology, Guangdong General Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, China

ARTICLE INFO

Keywords:
Consistency evaluation
Immunotherapy
Esophageal squamous cell carcinoma
PD-L1

ABSTRACT

Background: The prediction of response to immunotherapy mostly depends on the programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) status, and the 22C3 pharmDx assay has been approved in esophageal squamous cell carcinoma (ESCC). However, the widespread use of the 22C3 pharmDx assay is limited due to its availability. Thus, alternative PD-L1 assays are needed. We aimed to investigate the analytical and clinical diagnostic performances of four PD-L1 assays and to compare their concordances with the 22C3 pharmDx assay.

Methods: The PD-L1 22C3 pharmDx assay was performed on the Dako Autostainer Link 48 platform, three testing assays (PD-L1 E1L3N XP antibody [Ab], PD-L1 BP6099 Ab and PD-L1 CST E1L3N Ab) on the Leica BOND-MAX/III platform, and one testing assay (PD-L1 MXR006 Ab) on the Roche VENTANA Benchmark Ultra platform. A total of 218 ESCC cases from four centers were included in this retrospective study. Professionals from each center stained and read the IHC slides independently and determined the combined positive score (CPS) and the tumor proportion score (TPS).

Results: Regarding analytical performance, the four testing assays demonstrated good correlations with the 22C3 pharmDx assay when evaluated by the TPS or CPS ($\rho > 0.8$ for all four assays). Regarding diagnostic performance (CPS $\geq$ 10 was used as the cutoff), the four testing assays showed moderate concordances with the 22C3 pharmDx assay (kappa > 0.7 for all four assays). The overall percent agreements between each testing assay and the 22C3 pharmDx assay was at least 87.2 %.

Conclusion: This study provides insight into the potential interchangeability of the four PD-L1 assays with the 22C3 pharmDx assay.

1. Introduction

Immunotherapy with immune checkpoint inhibitors, including programmed cell death protein 1 (PD-1) and programmed cell death-ligand 1 (PD-L1) inhibitors, has become an important part of the treatment for advanced cancers. Immunohistochemistry (IHC) expression of PD-L1 has been recognized as a critical marker to predict immunotherapy response.[1] The US Food and Drug Administration (FDA) has approved four PD-L1 IHC assays (22C3, 28-8, SP263 and SP142) as companions or complementary diagnostics. However, each approved assay must be used on its associated IHC platform, detection system and scoring algorithm, which has brought large complexity to clinical and pathological applications. Therefore, comparative studies on the PD-L1 assays have arisen in recent years.[2-10] The representative Blueprint Projects have investigated the interchangeability of the four FDA-approved assays regarding their analytical and clinical diagnostic performances in

* Corresponding authors.
E-mail addresses: yanh_liu@163.com (Y. Liu), jmying@cicams.ac.cn (J. Ying).
† These authors contributed equally to this work.

https://doi.org/10.1016/j.jncc.2023.11.003
Received 19 May 2023; Received in revised form 16 November 2023; Accepted 26 November 2023

non-small cell lung carcinoma, and the results have shown high concordance and interchangeability of the 22C3, 28-8 and SP263 assays when scoring tumor cells.[2,4]

There have also been numerous comparative studies on non-FDA-approved assays. These assays can be made de novo or from any change in the protocol for an FDA-approved IHC assay. Notably, a meta-analysis of 57 comparative studies demonstrated that properly designed assays may perform equally well to the original FDA-approved assays and even better than other analytically comparable FDA-approved assays.[11] For example, the non-FDA-approved PD-L1 assay (the 22C3 assay performed on the VENTANA platform) showed good consistency,[12] and even higher diagnostic accuracy than the FDA-approved 28-8 and SP263 assays when compared to the standard 22C3 pharmDx assay (Dako platform) .[11,13,14] The E1L3N assays used in some clinical trials and laboratory studies have also achieved high concordance with the 22C3 IHC pharmDx assay in several comparative studies.[9,10,15-17] For example, Hodgson et al. compared the analytical and clinical diagnostic performances between the E1L3N and FDA-approved 22C3, SP263 and SP142 assays, and the results showed remarkable concordance.[9]

With respect to esophageal squamous cell carcinoma (ESCC), the phase III KEYNOTE 181 trial showed that pembrolizumab prolonged overall survival compared with chemotherapy and when used as a second-line therapy in advanced ESCC patients, with a PD-L1 combined positive score (CPS) ≥ 10. Based on this trial, the FDA approved the use of pembrolizumab as a second-line monotherapy for recurrent and/or metastatic ESCC in patients with a PD-L1 CPS ≥ 10 using the 22C3 pharmDx assay.[18] Furthermore, the KEYNOTE 590 trial showed that pembrolizumab plus chemotherapy used as first-line therapy could improve overall survival in ESCC patients with a CPS ≥ 10. However, the widespread use of the PD-L1 22C3 pharmDx assay is limited, and more alternatives are desired.

Our present study aimed to compare the analytical and clinical diagnostic performances of the four PD-L1 assays (PD-L1 E1L3N XP antibody [Ab], PD-L1 MXR006 Ab, PD-L1 BP6099 Ab, PD-L1 CST E1L3N Ab) and the 22C3 pharmDx assay in ESCC and illustrate their interchangeabilities.

2. Materials and methods

2.1. Specimens

This retrospective multicenter study included 218 ESCC patients who underwent endoscopic biopsy or esophagectomy between June 2020 and June 2021. Among them, 69 biopsy samples were obtained from the Cancer Hospital, Chinese Academy of Medical Sciences, 50 biopsy samples were from Guangdong General Hospital, Guangdong Academy of Medical Sciences, 24 biopsy samples and 25 surgical samples were from the West China Hospital, Sichuan University, and 50 surgical samples were from the Fudan University Shanghai Cancer Hospital.

2.2. IHC staining and interpretation of PD-L1

For the pilot trial, 10 biopsy samples were selected and serially sliced in the Cancer Hospital, Chinese Academy of Medical Sciences (the main center) and then stained and scored independently for the PD-L1 22C3 pharmDx assay in four centers. The results were used to assess the consistency of IHC staining and interpretation among the four centers. These 10 cases were not included in the formal study.

The formal study included a total of 218 specimens from four centers. Pathologists from each of the four centers stained and assessed the PD-L1 22C3 pharmDx assay and four testing assays independently using their own specimens. The Dako PD-L1 22C3 pharmDx assay (Dako) was used as a reference assay on the Dako Autostainer Link 48 Platform (Dako). The other four testing assays included three (PD-L1 E1L3N XP Ab, PD-L1 BP6099 Ab and PD-L1 CST E1L3N Ab) on the Leica BOND-MAX/III platform and one (PD-L1 MXR006 Ab) on the Roche VENTANA Benchmark Ultra platform. Detailed information on these four testing PD-L1 assays is shown in Supplementary Table 1, and detailed IHC staining protocols was shown in Supplementary Table 2.

PD-L1 expression was assessed to determine the tumor proportion score (TPS) and CPS by two pathologists from each center who were certified to perform PD-L1 scoring. During the assessment, we rigorously adhered to the double-blind principle, wherein the pathologists were unaware of the assay and case information, thereby facilitating the most authentic and objective evaluation feasible. All sections had at least 100 viable tumor cells. The TPS was defined as the number of positive tumor cells divided by the total number of viable tumor cells (continuous variable; 0 to 100%); the CPS was defined as the number of positive tumor cells, tumor-associated lymphocytes and macrophages divided by the total number of viable tumor cells and multiplied by 100 (continuous variable; 0 to 100). A clinically relevant cutoff (CPS ≥ 10) was used. The mean scores of the two pathologists were recorded. Samples with largely different scores were evaluated again by both pathologists until a consensus was reached.

2.3. Statistical analysis

SPSS 23.0 (IBM Corp., Armonk, NY, United States) and R software for Windows version 3.6.3 were used to analyze the data and to plot the figures. To assess the similarity in staining and scoring between the 22C3 pharmDx assay and the four different testing assays, bubble plots and pairwise Spearman's rank correlation coefficients (ρ) were generated pairwise between assays for TPS and CPS. Agreement across positivity thresholds was assessed using weighted Cohen's kappa (kappa values 0.40 to 0.69 indicated weak agreement, 0.70 to 0.79 indicated moderate agreement, 0.80 to 0.89 indicated strong agreement, and ≥ 0.9 indicated nearly perfect agreement).[4] Pairwise calculations for the overall percent agreement (OPA), positive percent agreement and negative percent agreement between the 22C3 pharmDx assay and four different testing assays were performed.

3. Results

3.1. Analytical comparison of four PD-L1 testing assays

3.1.1. PD-L1 staining to determine the CPS and TPS

Representative IHC images for the five assays used in this study are shown in Fig. 1. The CPS and TPS for 218 cases using five assays are shown in Fig. 2. The four testing assays showed good correlation with the 22C3 pharmDx assay across the samples with respect to both the CPS and TPS. The distributions of the CPS and TPS for each assay among the 218 cases are shown in Fig. 3. In general, the distribution of the CPS and TPS was similar between the four testing assays and the 22C3 pharmDx assay, although the number of samples with a CPS < 1 was slightly higher with the 22C3 pharmDx assay than with the other four testing assays.

3.1.2. Pairwise comparisons of PD-L1 staining according to the CPS and TPS

The pairwise comparisons between the four different testing assays and the 22C3 pharmDx assay are shown in Fig. 4. Each bubble plot represents a comparison between the 22C3 pharmDx assay (indicated on the x-axis) and one testing assay (shown on the y-axis). Each testing assay demonstrated good correlation with the 22C3 pharmDx assay for both the CPS and TPS (ρ > 0.8 for all cases).

3.2. Clinical diagnostic performance comparison

Continuous CPSs were stratified by a cutoff of ≥ 10, which is used in clinical practice. Differences in PD-L1 positivity among the five assays

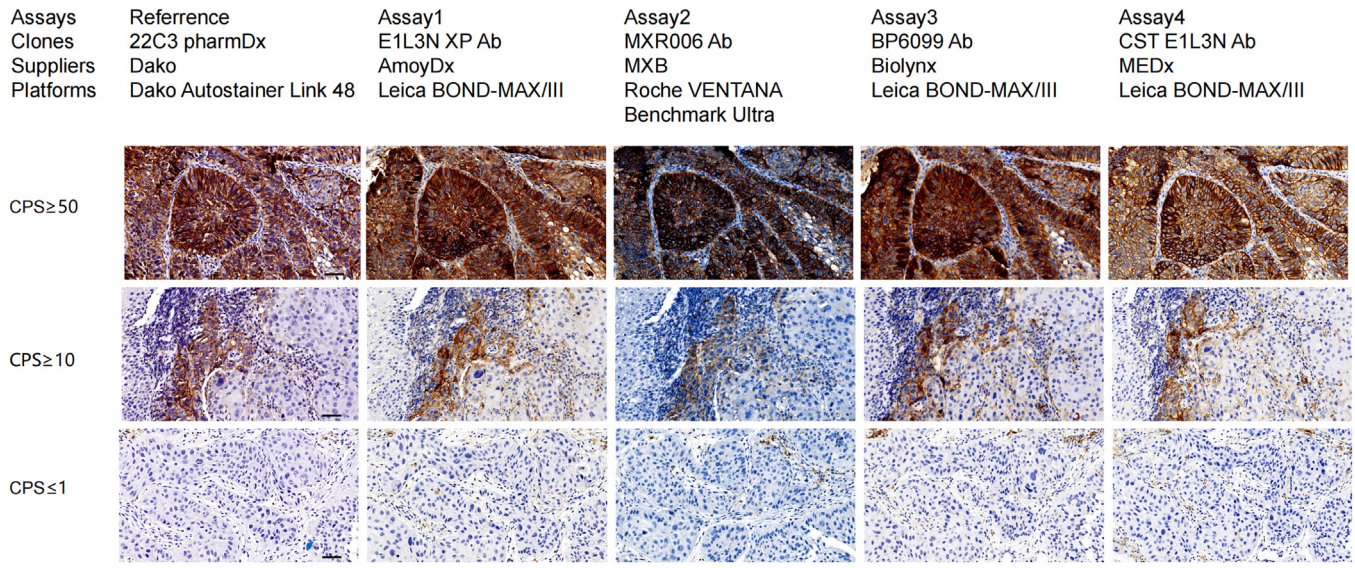

Fig 1. Examples of representative PD-L1 immunohistochemical images with the 22C3 pharmDx assay and four PD-L1 assays at 200× magnification. Assay1: PD-L1 E1L3N XP Ab; Assay2: PD-L1 MXR006 Ab; Assay3: PD-L1 BP6099 Ab; Assay4: PD-L1 CST E1L3N Ab. Scale bar, 50 μm. Ab, antibody.

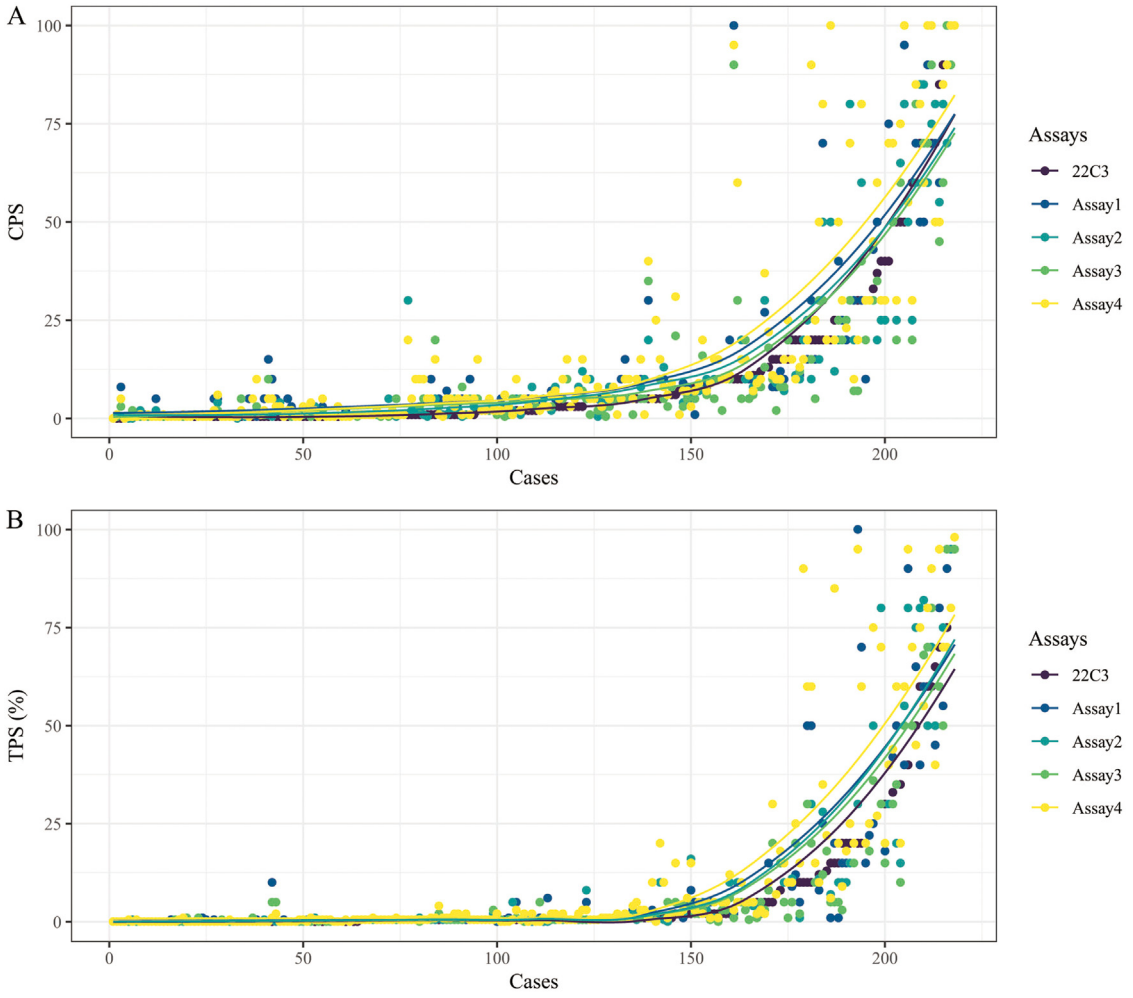

Fig 2. Analytical comparison of the CPS and TPS by case, for each assay. Data points represent the mean score from two pathologists for each assay in each case. Assay1: PD-L1 E1L3N XP Ab, Assay2: PD-L1 MXR006 Ab, Assay3: PD-L1 BP6099 Ab, Assay4: PD-L1 CST E1L3N Ab. Ab, antibody; CPS, combined positive score; TPS, tumor proportion score.

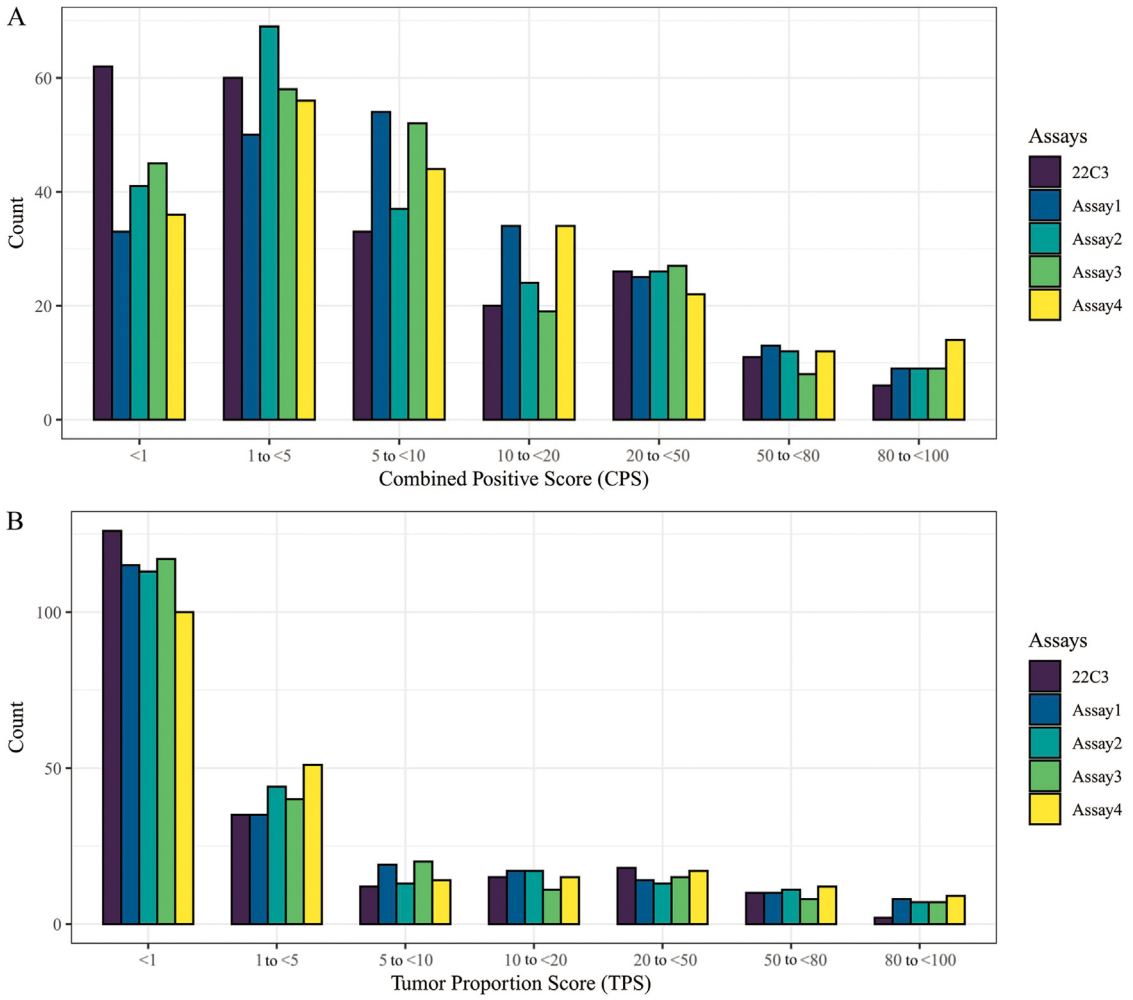

Fig 3. A detailed description of the distribution of PD-L1 expression across five assays for the combined positive score and tumor proportion score. Assay1: PD-L1 E1L3N XP Ab, Assay2: PD-L1 MXR006 Ab, Assay3: PD-L1 BP6099 Ab, Assay4: PD-L1 CST E1L3N Ab. Ab, antibody.

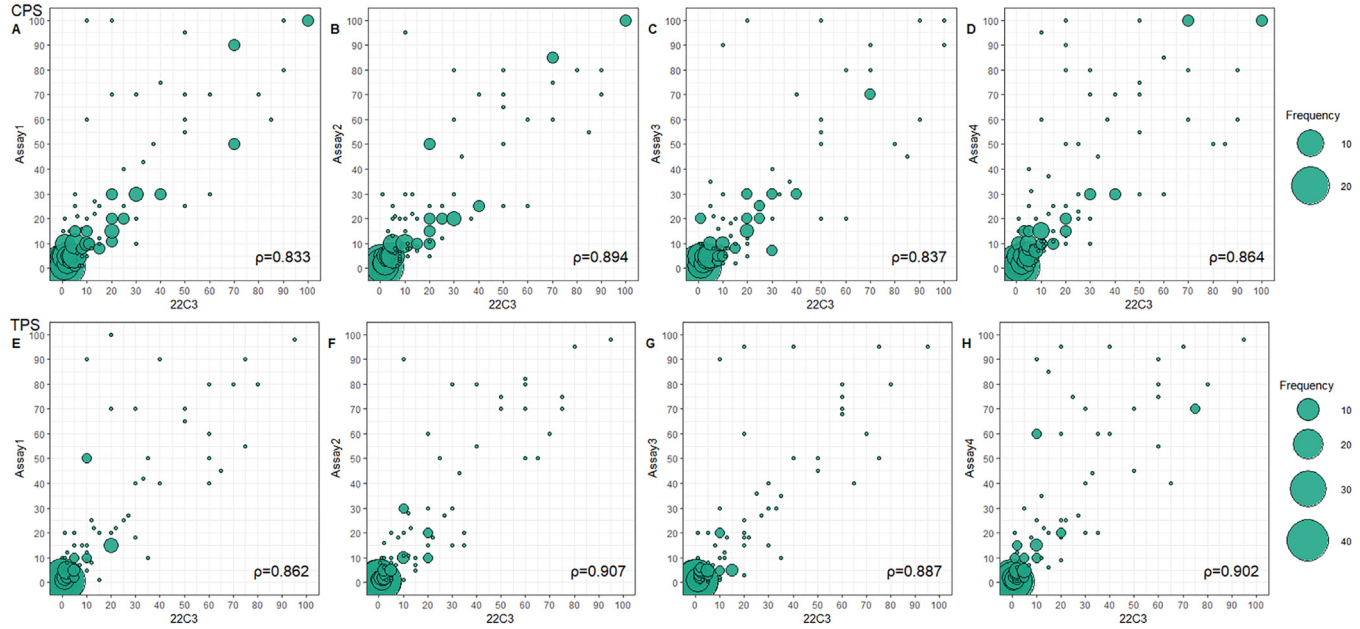

Fig 4. Pairwise comparisons between each of the four testing assays and the 22C3 pharmDx assay for the CPS and TPS and their corresponding pairwise Spearman's rank correlation coefficients (ρ). Assay1: PD-L1 E1L3N XP Ab, Assay2: PD-L1 MXR006 Ab, Assay3: PD-L1 BP6099 Ab, Assay4: PD-L1 CST E1L3N Ab. Ab, antibody; CPS, combined positive score; TPS, tumor proportion score.

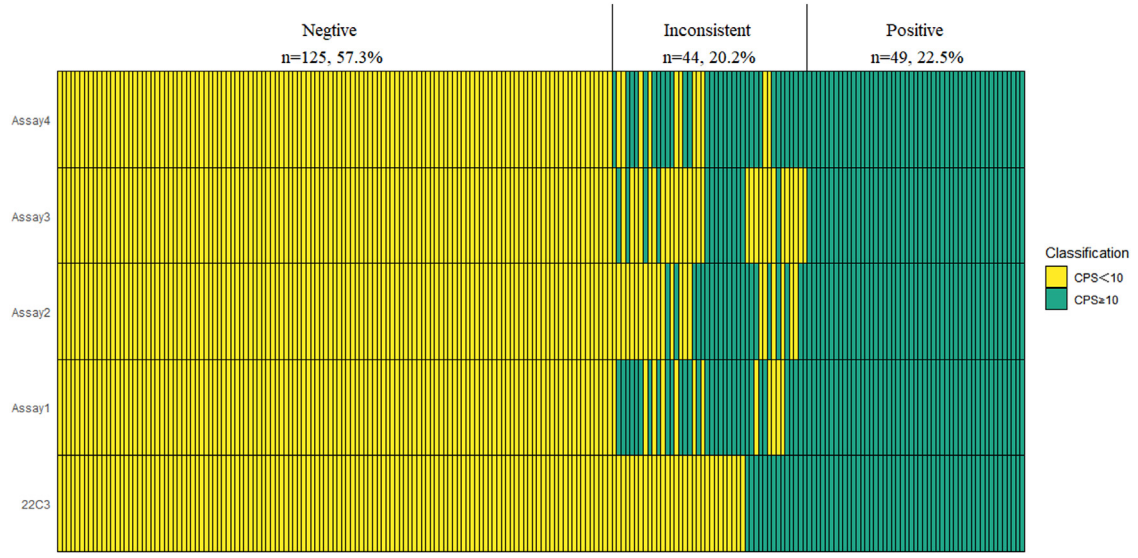

Fig 5. A heatmap used to visualize concordance between the five PD-L1 assays in the 218 cases. Positive cases were defined as those with a CPS ≥ 10. Assay1: PD-L1 E1L3N XP Ab, Assay2: PD-L1 MXR006 Ab, Assay3: PD-L1 BP6099 Ab, Assay4: PD-L1 CST E1L3N Ab. Ab, antibody. CPS, combined positive score.

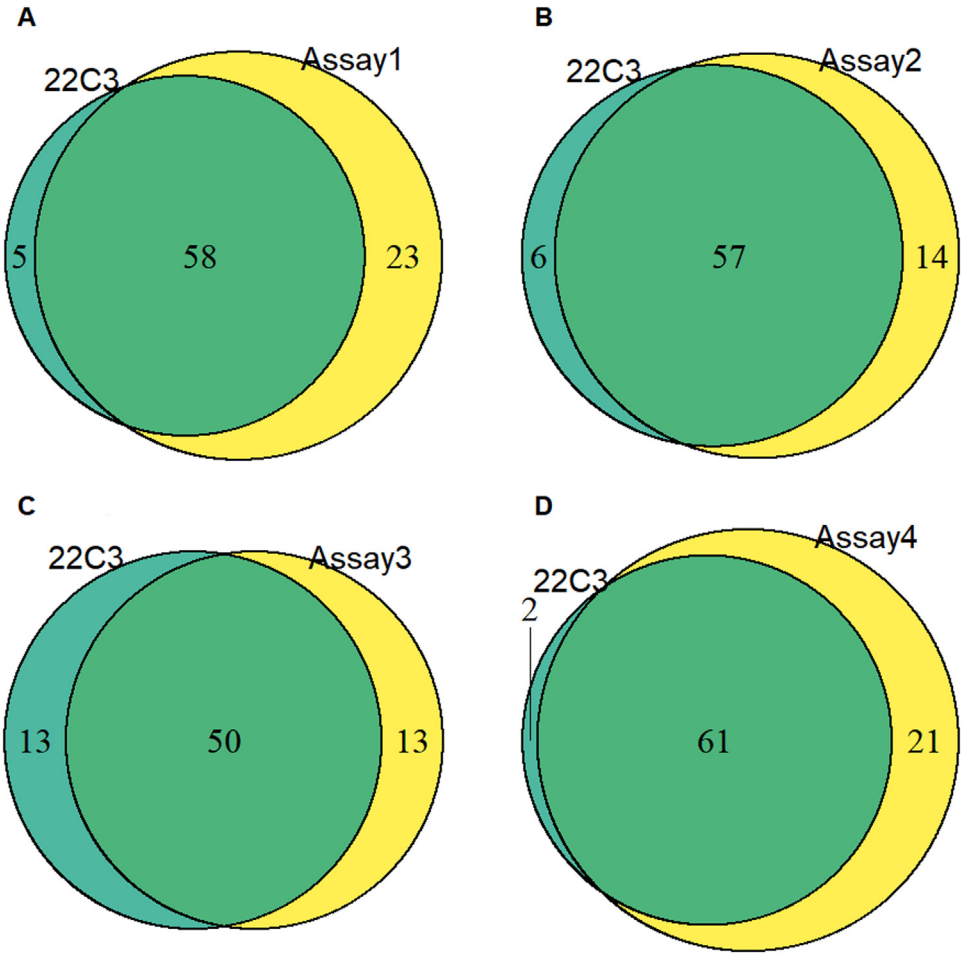

Fig 6. A Venn diagram showing the concordances of positive cases (CPS ≥ 10) between each testing assay and the 22C3 pharmDx assay (the positive cases evaluated with the 22C3 assay are shown in the circle on the left, and the positive cases evaluated by the four testing assays are shown in the circle on the right). Assay1: PD-L1 E1L3N XP Ab, Assay2: PD-L1 MXR006 Ab, Assay3: PD-L1 BP6099 Ab, Assay4: PD-L1 CST E1L3N Ab. Ab, antibody. CPS, combined positive score.

can be visualized in Fig. 5. When evaluated by all five assays, 49/218 (22.5%) cases were classified as all positive, 125/218 (57.3%) cases were classified as all negative; additionally, 44/218 (20.2%) patients showed discordance in their positive status among the five assays. The specific concordance for the positive cases between each testing assay and the 22C3 pharmDx assay is shown in Fig. 6.

Cohen's kappa statistic showed moderate agreement between each testing assay and the 22C3 pharmDx assay, with kappa values ranging from 0.710 to 0.785 for all samples (Supplementary Table 3). The OPA, positive percent agreement and negative percent agreement for the total, surgical and biopsy samples are also shown in Supplementary Table 3. The OPAs for the total 218 cases between each testing assay and the

22C3 pharmDx assay were 87.2%, 90.1%, 88.0% and 89.4%, respectively. There was no significant difference in concordance between each testing assay and the 22C3 pharmDx assay for either surgical or biopsy samples.

4. Discussion

Due to the complexity of the PD-L1 assay testing procedure, a large number of comparative studies have been performed in recent years. Previous comparative studies mostly compared the FDA-approved PD-L1 assays in non-small cell lung cancer, head and neck squamous cell carcinoma, and urothelial carcinoma or gastric adenocarcinoma.[2-10] The assays included in our comparative study were four non-FDA-approved PD-L1 assays, and they were compared with the 22C3 pharmDx assay in ESCC samples.

Regarding the comparison of analytical performance, our results showed good correlation ($\rho > 0.8$ for all four assays) between each PD-L1 assay and the 22C3 pharmDx assay, and the results were similar to those in some previous studies. For example, Zajac M. et al. showed a good linear analytical association among four FDA-approved PD-L1 assays when evaluating immunocytes in urothelial carcinoma ($\rho = 0.91$ between the 22C3 pharmDx and SP263 assays, $\rho = 0.88$ between the 28-8 pharmDx and SP263 assays, and $\rho = 0.87$ between the SP142 and SP263 assays).[19]

Regarding the comparison of clinical diagnostic performance, we also showed moderate concordance (kappa > 0.7 for all four assays), and the OPA between each testing assay and the 22C3 pharmDx assay was at least 87.2%. However, the concordances varied in previous comparison studies, which may be the result of using different PD-L1 assays and sample types (surgical, biopsy, or tissue microarray [TMA] specimens) in various types of cancer. Ahn S. et al. showed strong concordance between the 22C3 pharmDx and 28-8 pharmDx assays in gastric adenocarcinoma (kappa = 0.927 and 0.899 at CPS = 1 and CPS = 10 cutoff values, respectively).[6] Munari E. et al. manifested a moderate concordance between the 22C3 pharmDx and SP263 assays in urothelial carcinoma TMA samples (kappa = 0.77 and OPA = 89.6%). Nevertheless, Kim SW. et al. reported weak concordance when comparing the 22C3 pharmDx assay and the SP263 assay in gastric adenocarcinoma samples (kappa = 0.311 and OPA = 61% in biopsy specimens at a CPS = 1 cutoff value).[5] Notably, Cerbelli B. et al. showed similar results to ours in the same histotype of cancer: squamous cell carcinoma. The concordances were moderate when they compared the 22C3 pharmDx assay and the SP263 assays in head and neck squamous cell carcinoma (kappa = 0.891 and OPA = 98% at a CPS = 1 cutoff value, and kappa = 0.808 and OPA = 90% at a CPS = 20 cutoff value).[7] Based on these findings, our non-FDA-approved PD-L1 assays may achieve considerable consistence as FDA-approved PD-L1 assays did.

Non-FDA-approved PD-L1 assays cannot be directly used in clinical diagnosis and treatment, but some, such as the E1L3N assay, are widely used in the laboratory. Moreover, the E1L3N assay was included in a considerable number of previous comparison studies, which showed moderate to strong concordance between the E1L3N and the FDA-approved PD-L1 assays.[9,10,15,17,20,21] Tretiakova M. et al. showed that the kappa value was only slightly lower between the E1L3N and 22C3 pharmDx assays (kappa = 0.81) than between the 22C3 pharmDx and 28-8 pharmDx assays (kappa = 0.84) in bladder carcinomas.[10] Hodgson A. et al. compared the consistency among the E1L3N assay and three FDA-approved PD-L1 assays (22C3, SP263 and SP142) in urothelial carcinoma of the bladder, and the results showed strong concordance between the E1L3N and 22C3 pharmDx assays (kappa = 0.866) when evaluating immunocytes, and the concordance was higher than those found between any two of the three FDA-approved PD-L1 assays (kappa = 0.722, 0.567, 0.519, respectively).[9] In addition, Rimm DL. et al. showed a higher consistency between the E1L3N and 28-8 pharmDx assays than between the 22C3 pharmDx and 28-8 pharmDx assays in non-small cell lung carcinoma.[17] Similar to previous studies, we also showed moderate concordance between the E1L3N and 22C3 pharmDx assays in ESCC (kappa = 0.712 and 0.764 for assay1 and assay4, respectively). Noteworthy, previous studies have shown that E1L3N, compared to the 22C3 pharmDx assay, may be more appropriate for earlier samples, since the glycan part of the 22C3 epitope is not stable over time.[22,23] Although two other PD-L1 assays were evaluated in comparative studies for the first time, they also showed moderate concordance (kappa = 0.785 and 0.710) when compared with the 22C3 pharmDx assay. All of the above studies demonstrate that some non-FDA-approved assays can achieve good agreement with FDA-approved assays, indicating their potential interchangeability.

The samples in our study consisted of 75 surgical specimens and 143 biopsy specimens, which may reflect the real distribution of clinical samples. For one thing, the small biopsy tissues may not be representative of the whole tumor due to tumor heterogeneity. Previous studies have showed weak to moderate concordances between paired biopsy and surgical specimens in head and neck squamous cell carcinoma.[24] However, such paired comparative studies of surgical and biopsy specimens from ESCC have not been conducted, which would be of great value and needs further evaluation. For another, the only specimens available for biomarker assessment are biopsy specimens in advanced ESCC patients with unresectable tumors. Notably, when comparing the concordance between each testing assay and the 22C3 pharmDx assay using the surgical specimens or biopsy specimens separately, no significant differences were found (Supplementary Table 3). Many previous studies have demonstrated similar results. Xu et al. analyzed the concordance between the E1L3N and 22C3 pharmDx assays in non-small cell lung carcinoma, and the results showed that the OPA between the two assays was 97.8% in surgical specimens and 93.9% in biopsy specimens.[20] Kim et al. also showed similar concordances between different assays when using surgical specimens or biopsy specimens in gastric adenocarcinomas even when evaluating with different cutoffs.[5] Thus, the interchangeability of different assays may not be significantly affected by specimen types.

There are also some limitations in our study. First, the lack of therapeutic and prognostic information hindered us to further investigate the predictive ability of each assay regarding immunological efficacy, as well as determine the most optimal cutoff value, as has been done in a previous study.[25] Therefore, further investigations are needed to explore their abilities to predict immunotherapy efficacy. Second, the stratification analysis on biopsy samples and surgical samples may be affected by their staining and interpretation in different centers, although the platforms and IHC staining procedures were standardized and the pilot trial showed relatively good concordances among four centers. Additional research is warranted in cases where multiple centers offer both biopsy and surgical samples. Third, subjective variabilities may exist among pathologists during their CPS evaluating process.[15,26,27] However, our study was designed to evaluated the inter-assay consistency, while the evaluation of inter-observer consistency was not incorporated.

5. Conclusions

In conclusion, our multicentric study showed good correlation in analytical performance and moderate concordance in clinical diagnostic performance between four PD-L1 assays and the 22C3 pharmDx assay in ESCC. This study provides an insight into the potential interchangeability of these four assays, but further studies are needed to confirm our findings.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

This study was approved by the independent ethics committees at the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (approval number: 21/200-2871), the Guangdong General Hospital, Guangdong Academy of Medical Sciences, the Fudan University Shanghai Cancer Center, and the West China Hospital, Sichuan University, and was performed in accordance with the Declaration of Helsinki.

Data availability

The datasets used and/or analyzed in the current study are available from the corresponding authors on reasonable request.

Acknowledgments

The authors would like to thank Xiuyun Liu, Xinlan Luo, Xu Cai, and Wenhua Jiang for their assistance in the immunohistochemical staining involved in the current study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jncc.2023.11.003.

Author contributions

Y.L. and J.Y. developed the study concept and design. L.X., Y.L., L.J., C.L. and N.C. developed the methodology and wrote, reviewed and revised the paper. C.G., Y.J., P.Z., X.L., X.X., Y.W. and W.W. provided acquisition, analysis and interpretation of the data. All authors read and approved the final paper.

References

1. Yi M, Jiao D, Xu H, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Mol Cancer*. 2018;17(1):129.
2. 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(2):208–222.
3. Kerr K, Tsao M, Yatabe Y, et al. OA03.03 phase 2B of blueprint PD-L1 immunohistochemistry assay comparability study. *J Thorac Oncol*. 2018;13(10):S325.
4. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of blueprint phase 2 project. *J Thorac Oncol*. 2018;13(9):1302–1311.
5. Kim SW, Jeong G, Ryu MH, Park YS. Comparison of PD-L1 immunohistochemical assays in advanced gastric adenocarcinomas using endoscopic biopsy and paired resected specimens. *Pathology*. 2021;53(5):586–594.
6. Ahn S, Kim KM. PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28-8 pharmDx assays for responses to immunotherapy. *Mod Pathol*. 2021;34(9):1719–1727.
7. Cerbelli B, Girolami I, Eccher A, et al. Evaluating programmed death-ligand 1 (PD-L1) in head and neck squamous cell carcinoma: concordance between the 22C3 PharmDx assay and the SP263 assay on whole sections from a multicentre study. *Histopathology*. 2022;80(2):397–406.
8. de Ruiter EJ, Mulder FJ, Koomen BM, et al. Comparison of three PD-L1 immunohistochemical assays in head and neck squamous cell carcinoma (HNSCC). *Mod Pathol*. 2021;34(6):1125–1132.
9. Hodgson A, Slodkowska E, Jungbluth A, et al. PD-L1 immunohistochemistry assay concordance in urothelial carcinoma of the bladder and hypopharyngeal squamous cell carcinoma. *Am J Surg Pathol*. 2018;42(8):1059–1066.
10. Tretiakova M, Fulton R, Kocherginsky M, et al. Concordance study of PD-L1 expression in primary and metastatic bladder carcinomas: comparison of four commonly used antibodies and RNA expression. *Mod Pathol*. 2018;31(4):623–632.
11. Torlakovic E, Lim HJ, Adam J, et al. "Interchangeability" of PD-L1 immunohistochemistry assays: a meta-analysis of diagnostic accuracy. *Mod Pathol*. 2020;33(1):4–17.
12. Neuman T, London M, Kania-Almog J, et al. A harmonization study for the use of 22C3 PD-L1 immunohistochemical staining on Ventana's platform. *J Thorac Oncol*. 2016;11(11):1863–1868.
13. Røge R, Vyberg M, Nielsen S. Accurate PD-L1 protocols for non-small cell lung cancer can be developed for automated staining platforms with clone 22C3. *Appl Immunohistochem Mol Morphol*. 2017;25(6):381–385.
14. Ilie M, Juco J, Huang L, Hofman V, Khambata-Ford S, Hofman P. Use of the 22C3 anti-programmed death-ligand 1 antibody to determine programmed death-ligand 1 expression in cytology samples obtained from non-small cell lung cancer patients. *Cancer Cytopathol*. 2018;126(4):264–274.
15. Adam J, Le Stang N, Rouquette I, et al. Multicenter harmonization study for PD-L1 IHC testing in non-small-cell lung cancer. *Ann Oncol*. 2018;29(4):953–958.
16. Ma J, Li J, Qian M, et al. PD-L1 expression and the prognostic significance in gastric cancer: a retrospective comparison of three PD-L1 antibody clones (SP142, 28-8 and E1L3N). *Diagn Pathol*. 2018;13(1):91.
17. 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(8):1051–1058.
18. Kojima T, Shah MA, Muro K, et al. Randomized phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. *J Clin Oncol*. 2020;38(35):4138–4148.
19. Zajac M, Scott M, Ratcliffe M, et al. Concordance among four commercially available, validated programmed cell death ligand-1 assays in urothelial carcinoma. *Diagn Pathol*. 2019;14(1):99.
20. Xu H, Dong X, Zhao H, et al. Clinical evaluation of a laboratory-developed test using clone E1L3N for the detection of PD-L1 expression status in non-small cell lung cancer. *J Clin Lab Anal*. 2021;35(3):e23696.
21. Zhang W, Cao Z, Gao C, et al. High concordance of programmed death-ligand 1 expression with immunohistochemistry detection between antibody clones 22C3 and E1L3N in non-small cell lung cancer biopsy samples. *Transl Cancer Res*. 2020;9(10):5819–5828.
22. Fernandez AI, Gaule P, Rimm DL. Tissue age affects antigenicity and scoring for the 22C3 immunohistochemistry companion diagnostic test. *Mod Pathol*. 2023;36(7):100159.
23. Haragan A, Liebler DC, Das DM, et al. Accelerated instability testing reveals quantitative mass spectrometry overcomes specimen storage limitations associated with PD-L1 immunohistochemistry. *Lab Invest*. 2020;100(6):874–886.
24. Paolino G, Pantanowitz L, Barresi V, et al. PD-L1 evaluation in head and neck squamous cell carcinoma: insights regarding specimens, heterogeneity and therapy. *Pathol Res Pract*. 2021;226:153605.
25. Matsubara Y, Toriyama K, Kadowaki S, et al. The impact of combined PD-L1 positive score on clinical response to nivolumab in patients with advanced esophageal squamous cell carcinoma. *Esophagus*. 2023;20(3):524–532.
26. Robert ME, Rüschoff J, Jasani B, et al. High interobserver variability among pathologists using combined positive score to evaluate PD-L1 expression in gastric, gastroesophageal junction, and esophageal adenocarcinoma. *Mod Pathol*. 2023;36(5):100154.
27. Crosta S, Boldorini R, Bono F, et al. PD-L1 testing and squamous cell carcinoma of the head and neck: a multicenter study on the diagnostic reproducibility of different protocols. *Cancers*. 2021;13(2):292.