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Programmed cell death ligand 1 expression in esophageal squamous cell carcinoma: a comparative analysis of three different assays

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As one of the most aggressive and lethal malignant tumors, the 5-year survival rate of oesophageal cancer is less than 20%.[1] There are two main pathological subtypes of esophageal cancer: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma.[1] In China, more than 95% of esophageal cancer is ESCC. Encouragingly, cancer immunotherapy has entered a new era recently with the discovery of drugs that interfere with specific immune checkpoints. Moreover, due to the good effect of immunotherapy in squamous cell carcinoma, it may be a new strategy for ESCC treatment in the future.

The expression of programmed cell death-ligand 1 (PD-L1) was identified as a predictive diagnostic marker for the selection of patients who might benefit from anti-PD-1 axis drugs. At present, there are many qualitative detection antibodies against PD-L1 that can be used to evaluate the expression of PD-L1. The only companion diagnostic test approved by the USA Food and Drug Administration for pembrolizumab in non-small cell lung cancer (NSCLC) is the PD-L1 immunohistochemistry (IHC) 22C3 PharmDx kit (Agilent Technologies, Inc., Santa Clara, CA, USA), which was developed for testing on the Dako Autostainer (Agilent Technologies, Inc., Santa Clara, CA, USA).[2] Another diagnostic test, Dako antibody 28-8 (Abcam, Cambridge, United Kingdom) was also approved as a complementary assay for NSCLC. In addition, a third antibody developed for the Ventana BenchMark platform (Roche, Basel, Switzerland), namely, the SP263 PharmDx assay (Roche, Basel, Switzerland), was approved by the Conformité Européene in treatment decisions for NSCLC.[3] In current studies of immunotherapy for ESCC, there is no widely accepted antibody for the detection of PD-L1, which affects the consistency of these studies to a certain extent. Therefore, comparing the analytical performance and comparability of these methods in ESCC is of great significance for the standardization of immunotherapy for ESCC.

Based on the previous study,[4] the 22C3 assay seems the most important antibody to see PD-L1 status of ESCC. Here, in this study, we took 22C3 as the standard assay and compared the PD-L1 expression results of IHC 22C3 with those of Dako's 28-8 and Ventana's SP263 to assess their effectiveness as a screening tool in the diagnostic routine of ESCC.

A total of 324 consecutive patients who underwent curative esophagectomy with R0 resection for histologically verified ESCC between December 2005 and June 2013 at the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences were included in the present study. The current study was conducted in accordance with the *Declaration of Helsinki*. The Clinical Research Ethics Committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences approved this study (No. NCC2976). Patients who received pre-operative chemotherapy/radiotherapy and with distant metastasis were excluded. The pathological classification of the primary tumor and the degree of lymph node metastasis were assessed according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (eighth edition). All ESCC tissue samples were stained with hematoxylin and eosin and confirmed by two pathologists independently. All 324 samples of a respective

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tumor region were extracted from areas harboring a high tumor/stroma ratio. A series of 4-μm thick sections were cut and transferred to adhesive slides according to the manufacturer's instructions.

The tissue microarrays (TMAs) were performed with three validated assays according to the manufacturer's instructions: staining for PD-L1 22C3 assay (Agilent, Santa Clara, CA, USA) and 28-8 (Abcam, ab205921, Cambridge, United Kingdom) was performed on the Dako Autostainer Link 48 platform (Dako, Carpinteria, CA, USA). Staining for PD-L1 SP263 assay (Roche, Basel, Switzerland) was performed on the Ventana Benchmark XT Stainer (Roche). Two pathologists who were blinded to the clinical data assessed the samples independently, and disagreements were resolved by a third experienced pathologist. In the present study, for the combined positive score (CPS), the results were divided into three groups using <1, 1 to 49, and 50 to 100 positive cells.

Statistical analyses were performed using SPSS 18.0 software (IBM Corporation, Armonk, NY, USA). The overall percent agreement (OPA), positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were calculated to evaluate the clinical performance of the assays.

The median age of the patients was 59 years (34–78 years), and 23.8% were women [Supplementary Table 1, http://links.lww.com/CM9/A666]. Figure 1 illustrates the immunohistochemical staining in a representative ESCC sample from the TMAs. Different staining scores for the same tumor were compared, with relatively high inter-observational consistency (agreement higher than 90%).

Regarding the comparison between 28-8 and 22C3, at the "1" cutoff, 77.5% (251/324) and 77.2% (250/324) of patients were negative (CPS <1), respectively; 19.1% (62/324) and 19.4% (63/324) had CPS values of 1 to 49, respectively; and 3.4% (11/324) and 3.1% (10/324) were strongly positive (CPS ≥50), respectively. The actual numbers of cases for the comparison between 28-8 and 22C3 are shown in Supplementary Table 2, http://links.lww.com/CM9/A666 according to the thresholds <1, 1 to 49, and ≥50. The OPA between 28-8 and 22C3 was 93.5% at the "1" cutoff and 99.1% at the "50" cutoff. At the "1" cutoff, the sensitivity and specificity were 85.1% and 96.0%, with a PPV and NPV of 86.3% and 95.6%, respectively, and an AUC of 0.906. At the "50" cutoff, the sensitivity and specificity were 90.0% and 99.4%, with a PPV and NPV of 81.8% and 99.7%, respectively, and the AUC was 0.947.

The actual numbers of concordance cases for the SP263 and 22C3 assays are shown in Supplementary Table 3, http://links.lww.com/CM9/A666. At the "1" cutoff, the OPA was 81.5%, with a difference in 18.5% of the samples. The sensitivity and specificity were 79.7% and 82.0%, respectively, with a PPV and NPV of 56.7% and 93.2%, respectively, and an AUC of 0.809. At the "50" cutoff, the OPA was 96.0%, with a sensitivity of 80.0%

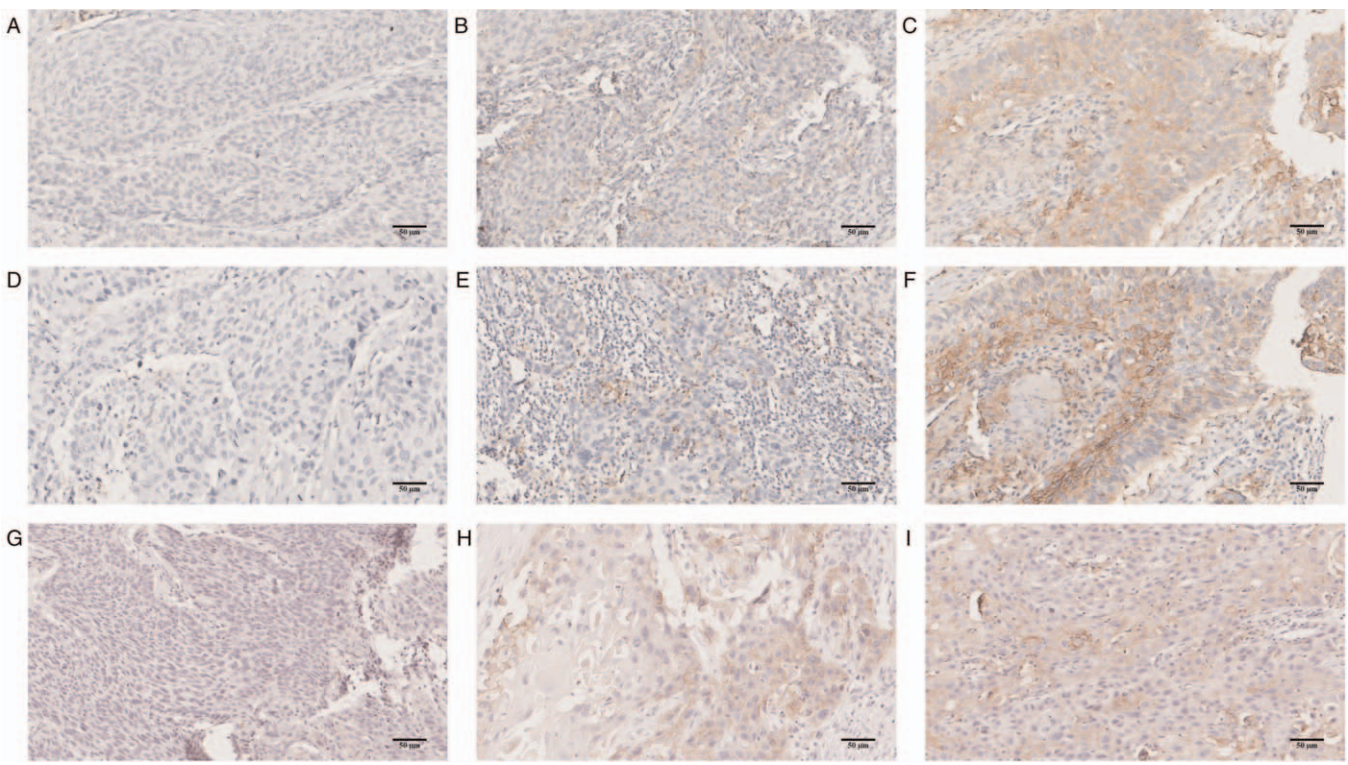

Figure 1: Representative examples of PD-L1 expression in esophageal squamous cell carcinoma using three different antibodies: 28-8, SP263, and 22C3. Examples of CPS thresholds <1, 1 to 49, and ≥50 for Abcam 28-8 antibody are shown in panels A, B, and C, respectively; examples for the Ventana antibody (SP263) are shown in panels D, E, and F, respectively; and examples for the Dako antibody (22C3) are shown in panels G, H, and I. CPS: Combined positive score; PD-L1: Programmed cell death-ligand 1.

and a specificity of 96.5%. The PPV and NPV were 42.1% and 99.3%, respectively, and the AUC was 0.882.

Overall, we found that 28-8 and 22C3 IHC staining scores were highly consistent. For SP263, the CPS tended to be higher than those of the other two assays, with strong and intense membrane staining.

To the best of our knowledge, this is the first study performed to assess the concordance of the PD-L1 IHC 22C3 assay with the SP263 and 28-8 assays in the TMA of ESCC. A previous study of NSCLC suggested that the PD-L1 clone 28-8 and 22C3 displayed strong correlation across samples.[5] In our study, similar results were observed in the CPSs of ESCC when comparing 22C3 with 28-8 or SP263 at 1 and 50 cutoffs. Thus, the detection of PD-L1 expression with the 28-8 assay may be appropriately used in place of the 22C3 assay for the purposes of guiding therapy with anti-PD-1/PD-L1 in ESCC.

Medical centers seldom have two or more automatic detection systems, so it is difficult to run two different staining platforms simultaneously on limited samples. In addition, different antibodies have different antigen epitopes, which affect the consistency of IHC staining intensity. Therefore, antibodies with high sensitivity used for PD-L1 detection may be beneficial in reducing false negatives in small biopsy specimens. In agreement with the study of Hendry *et al*,[5] the CPSs of the 28-8 assay were similar to those of 22C3. However, SP263 assay showed a higher rate of PD-L1-positive cases (both in 1 and 50 cutoffs) when compared with 22C3, which may be explained by the high-intensity staining of SP263 on the cell membrane. The precise reason for this discrepancy is not yet clear and the definition of positivity will depend on the clinical situation and the intended treatment. At the same time, considering that there is no similar study in esophageal cancer at present, the tumor heterogeneity of esophageal cancer and lung cancer cannot be neglected. In the present study, however, the expression of PD-L1 was affected by the use of TMAs rather than whole sections, according to a previous study.[6] It is worth noting that tumors may heterogeneously express PD-L1, which may lead to an overestimation or underestimation of the true PD-L1 levels. Although our results demonstrate that the 28-8 assay shows high agreement with the 22C3 assay, the efficacy of these antibodies in detecting the expression of PD-L1 for the purposes of guiding therapy with PD1/PD-L1 inhibitors in ESCC still needs more research.

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Conflicts of interest

None.

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