DOI: https://doi.org/10.4038/jdp.v20i2.7908 *Journal of Diagnostic Pathology 2025;20(2):7-11.*

Audit

PD-L1 immunohistochemistry in oesophageal, gastric and gastro-oesophageal junction adenocarcinoma: comparison of combined positive score across 22C3, and SP263 assays

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Submitted on 28.06.2025. Accepted for publication on 30.12.2025.

Abstract

Introduction and objectives: Programmed death-ligand-1 (PD-L1) is a potential target for immune checkpoint inhibitors in various cancers. The current study aimed to evaluate the concordance of combined positive score (CPS) across 22C3, and SP263 assays when interpreting PD-L1 immunohistochemistry on oesophageal, gastric and gastro-oesophageal junction (GOJ) adenocarcinoma.

Methodology: Twenty-two biopsies and eight resection specimens of oesophageal, gastric and GOJ adenocarcinoma were evaluated for PD-L1 SP263 expression at the Department of Pathology, Royal Surrey Hospital, over a period of four years (January 2021 to December 2024). These specimens were re-evaluated for PD-L1 22C3 assay at Poundbury Cancer Institute by an independent pathologist who was blinded to the PD-L1 SP263 assay results. The two results were compared for concordance.

Results: There was a very strong positive correlation between the SP263 and 22C3 scores of the two assays (Pearson correlation co-efficiency; r=0.89). Discordance was observed in categorising tumours at cut offs of CPS ≥5 and CPS ≥10, with the discordance being more at a cut off of CPS ≥5.

Discussion and conclusion: There are three major commercially available immunohistochemistry assays to quantify PD-L1 expression in cancer cells; SP263, 22C3 and 28-8. A CPS ≥1 is currently used to classify a tumour as PD-L1 positive. According to NICE guidelines, oesophageal or GOJ adenocarcinomas with a PD-L1 CPS ≥5 are eligible for treatment with nivolumab, while a CPS ≥10 qualifies for pembrolizumab therapy. This study demonstrated analytical concordance between the SP263 and 22C3 assays when CPS scoring was applied, consistent with previously published data.

Keywords: PD-L1immunohistochemistry, gastric carcinoma, 22C3, SP263

Introduction

Immune checkpoint inhibitors (ICIs) of programmed death 1 receptor (PD-1)/programmed death ligand 1 (PD-L1) have reformed cancer immunotherapy due to their potential to improve patient outcomes and survival (1). PD-L1 expressed on cancer cells binds to the PD-1 receptor on T-cells, suppressing their proliferation and activation, thereby enabling the cancer cells to evade the immune response. Blocking of PD-L1 and PD-1 binding prevents cancer cells evading the immune system, which is the primary target of immunotherapy (1).

PD-L1 protein expression in cancer cells is determined by immunohistochemistry (IHC). Increased PD-L1 expression in tumour cells by IHC has shown to correlate with improved

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clinical outcomes with PD-L1 inhibitor therapy (1).

There are three main commercially available IHC assays used to quantify PD-L1 expression in cancer cells: the Ventana PD-L1 SP263 assay (SP263), the Dako PD-L1 IHC 22C3 pharmDx assay (22C3), and the Dako PD-L1 IHC 28-8 pharmDx assay (28-8) (2).

Combined positive score (CPS) is a cell counting based approach that calculates the ratio of the total number of PD-L1– positive cells including tumour cells (TCs), mononuclear lymphocytes and macrophages to the total number of viable TCs, multiplied by 100 (2).

$$\text{CPS} = \frac{\text{Number of PDL1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{Total number of viable tumor cells}} \times 100$$

For an evaluation to be performed, a minimum of 100 viable TCs must be present on the slide stained for PD-L1 (2).

The International Collaboration of Cancer Reporting (ICCR) dataset for carcinoma of stomach recommends that PD-L1 positivity is determined by CPS of ≥1 by an FDA-approved companion diagnostic test such as the PD-L1 SP263 assay (3). According to NICE guidelines, patients with gastric, gastro-oesophageal junction (GOJ), or oesophageal adenocarcinoma demonstrating a PD-L1 combined positive score (CPS) of ≥5 are eligible for treatment with nivolumab, while those with a CPS of ≥10 may be considered for pembrolizumab therapy (4). Studies have compared the expression of PD-L1 IHC between 22C3 and SP263 assays, in gastric and GOJ adenocarcinoma and have shown analytical concordance in PD-L1 testing between these two major PD-L1 assays when CPS is applied (1,5).

The objective of this audit was to assess the concordance of the CPS across 22C3 and SP263 assays when interpreting PD-L1 IHC on gastric, oesophageal and GOJ adenocarcinoma.

Methods

Thirty specimens comprising twenty-two biopsies and eight resections of oesophageal, GOJ and gastric adenocarcinoma evaluated for PD-L1 status at Department of Cellular Pathology, Royal Surrey Hospital, over a period of four years (January 2021 to December 2024) were included in the audit. The specimens were evaluated for PD-L1 SP263 assay at the Department of Cellular Pathology, Royal Surrey Hospital and re-evaluated by 22C3 assay at the Poundbury Cancer Institute. PD-L1 22C3 results were reported independently by a trained pathologist at Poundbury Cancer Institute who was blinded to the PD-L1 SP263 assay results. The audit received approval from the Chief of Service, Audit Department, Royal Surrey Hospital (No: DCSS-CA-24-25-110). Demographic and pathological data were obtained from Winpath data base in Department of Cellular Pathology, Royal Surrey Hospital.

Results

Thirty patients were tested for PD L1 status in this cohort. The mean age was 72.2 years. Twenty-four (80%) patients were male. The Pearson correlation co-efficiency (r) between SP263 and 22C3 scores was 0.89 which indicates a very strong positive correlation between two assays (Figure 1).

In gastric or GOJ adenocarcinoma PD-L1 expression is determined by CPS of ≥1. A CPS <1 is considered as no PD-L1 expression. In this cohort three cases (3/30, 10%) showed no PD-L1 expression on both SP263 and 22C3 assays with a 100% concordance.

The cases that showed PD-L1 expression with a CPS ≥1 were further categorised as ≥5 and ≥10 according to NICE guideline recommendations to check the eligibility for nivolumab (CPS ≥5) and pembrolizumab (CPS ≥10) treatment with platinum-and fluoropyrimidine-based chemotherapy for oesophageal, gastric and gastro-oesophageal junction adenocarcinoma (1).

A total of six cases were identified in the category of CPS 1 to <5 on the SP263 assay. Four cases showed concordance on the 22C3 assay. Of the four discordant cases, two cases showed a CPS value of 5 at borderline cut-off when stained with SP263 assay. In the other

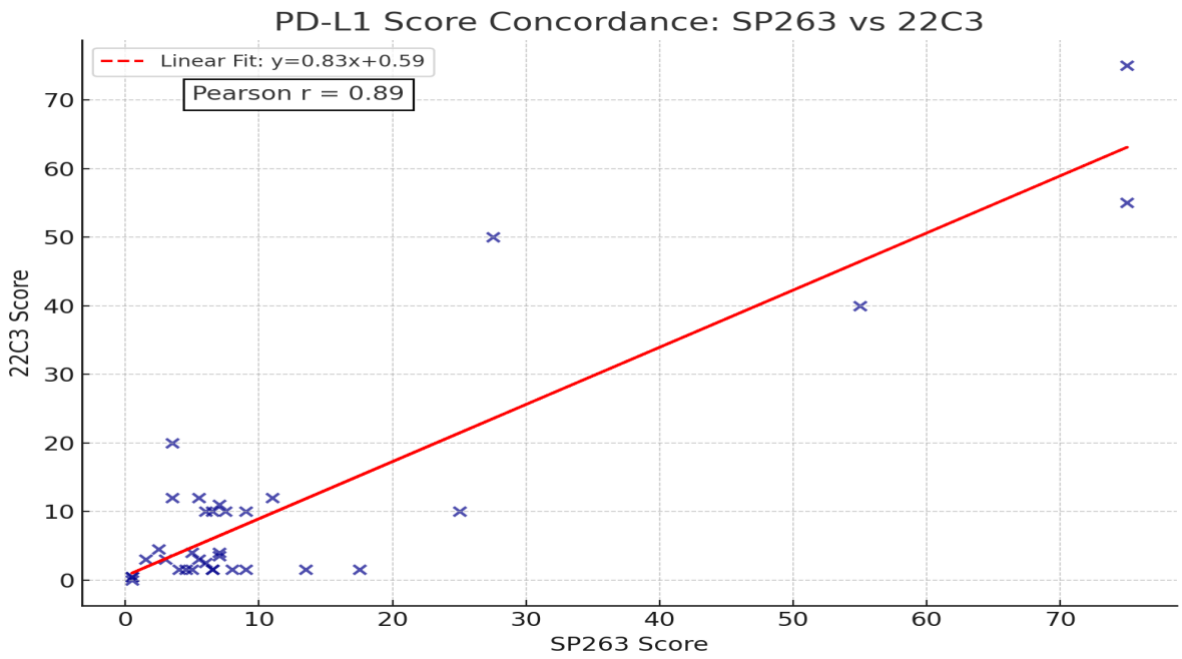

Figure 1 : Concordance between PD-L1 SP263 and 22C3 scores

two cases the re-cut section stained with 22C3 assay showed higher value for CPS than SP263 assay (Table 1). In both these occasions the reason for the discrepancy was inter-observer variability when counting of CPS.

The category ≥5 on SP263 included a total number of eleven cases (Table 1). In seven cases CPS value was at a borderline cut off of either 5 or 10 suggesting inter-observer variability as the reason for the discrepancy. In three cases the discordance was attributed to tissue loss due to recutting of sections for 22C3 assay and discrepancy in intensity of staining of two assays.

The category of ≥10 included total number of 10 cases and seven cases showed concordance (Table 1).

Discussion

A PD-L1 IHC assay is considered to be the most effective and widely used diagnostic test for anti–PD-1/PD-L1 inhibitors (1). There are three commercially available PD-L1 assays used in current clinical practice. This study observed a very strong concordance when analysing PD-L1 IHC using SP263 and 22C3 assay in oesophageal, gastric and GOJ adenocarcinoma. Klempner et al assessed the correlation between SP263(Ventana), 28-8(Dako), and 22C3(Dako) assays using the CPS scoring algorithms and showed a good analytical concordance across the range of PD-L1 expression (1). Park Y et al studied PD-L1 testing in gastric cancer with SP263 and 22C3 assays and demonstrated a high concordance of CPS at a cut-off of ≥1 and ≥10 (1).

Categorising PD-L1 as ≥5 needs more care than categorising as ≥1 or ≥10. The discordance experienced in category ≥5 could have been minimised by increasing the size of the sample tested by using resection specimens with more viable tumour than biopsies The observed discrepancies in PD-L1 categorization at the ≥5 threshold between the two pathologists may be explained by inter-observer variability in hotspot selection, in interpretation of staining intensity in viable tumour cells deemed positive, and in evaluation of background lymphocytes and macrophages within the selected area.

Recommendations

Factors contributing to discordance included interobserver variability and technical issues in section preparation and staining resulting in heterogeneity of IHC staining. Selecting of

Table 1: Demographic data and combined positive score across 22C3, and SP263 assays

Age	Sex	Tumour site	Tumour type	PD-L1 score		Reason for discordance
				SP263	22C3	
54	M	Oesophago-gastrectomy	G3 , diffuse	<1	0.5	Concordant
60	M	Gastric biopsy	G2/G3, intestinal	<1	<1	
51	F	Oesophageal biopsy	G1, intestinal	<1	0	
73	M	Oesophago-gastrectomy	G2, intestinal	4	1 to 2	
78	M	Gastric biopsy	G2/G3, intestinal	3	3	
82	M	Oesophageal biopsy	G2/G3, intestinal	1 to 2	3	
86	M	Gastric cardia biopsy	G2/G3, intestinal	2 to 3	4 to 5	
68	**M**	**Oesophageal biopsy**	**G3, diffuse**	**2 to 5**	**12**	Re-cut section for 22C3 showed higher value
72	**M**	**Oesophageal biopsy**	**G2, intestinal**	**3 to 4**	**20**	
78	**F**	**Pre-pyloric biopsy**	**G2, intestinal**	**5**	**4**	CPS value at a borderline cut off of 5 or 10 suggesting inter-observer variability
51	**M**	**Oesophageal biopsy**	**G3, intestinal**	**5**	**1 to 2**	
74	**F**	**GOJ biopsy**	**G3,diffuse**	**5 to 8**	**10**	
50	**M**	**Gastrectomy**	**G3, mixed**	**8 to 10**	**5**	
74	**M**	**Oesophageal biopsy**	**G2/G3, intestinal**	**5 to 6**	**3 to 4**	
78	**M**	**Oesophageal biopsy**	**G2/G3, intestinal**	**5 to 6**	**10**	
81	**M**	**GOJ biopsy**	**G2/G3, intestinal**	**8**	**10**	
50	**M**	**Gastric biopsy**	**G3, intestinal**	**6 to 8**	**<5**	Tissue loss in recutting sections for 22C3 assay
83	**M**	**Oesophago-gastrectomy**	**G3, mixed**	**6 to 7**	**1 to 2**	
71	**F**	**Oesophageal biopsy**	**G2/G3, intestinal**	**6**	**1 to 2**	
47	**M**	**GOJ biopsy**	**G2, intestinal**	**6 to 8**	**11**	Re-cut section for 22C3 showed higher value
81	M	Oesophageal biopsy	G3, mixed	10 to 12	12	Concordant
78	M	Oesophageal biopsy	G3, intestinal	25	10	
52	F	Gastrectomy	G3, mixed	12 to 15	15 to 20	
80	M	Gastrectomy	G2/G3, intestinal	25 to 30	50	
67	M	Gastric biopsy	G2/G3, intestinal	70 to 80	55	
82	M	Gastric biopsy	G3, intestinal	50 to 60	40	
81	M	Subtotal gastrectomy	G3, diffuse	75	75	
85	**M**	**Oesophageal biopsy**	**G3, intestinal**	**12 to 15**	**1 to 2**	Tissue loss in recutting sections for 22C3 assay
66	**M**	**Gastrectomy**	**G3, intestinal**	**15 to 20**	**1 to 2**	
76	**M**	**Oesophageal biopsy**	**G2, intestinal**	**12 to 15**	**3 to 4**	

biopsies with adequate tumour devoid of necrotic tissue, so that tumour is not depleted when the tissue is recut for further testing sections can minimise technical issues.

Conclusion

The two assays of SP263 and 22C3 used for the assessment of PD-L1 expression in oesophageal, gastric and GOJ adenocarcinoma showed high concordance in CPS. However extra care is required in categorising tumours at CPS cut off values that determine eligibility for therapy.

References

1. Park Y, Koh J, Na HY, et al. PD-L1 Testing in Gastric Cancer by the Combined Positive Score of the 22C3 PharmDx and SP263 Assay with Clinically Relevant Cut-offs. *Cancer Res Treat*. 2020;52(3):661-670. doi:10.4143/crt.2019.718

2. Klempner SJ, Cowden ES, Cytryn SL, et al. PD-L1 Immunohistochemistry in Gastric Cancer: Comparison of Combined Positive Score and Tumor Area Positivity Across 28-8, 22C3, and SP263 Assays. *JCO Precis Oncol*. 2024;8:e2400230. doi:10.1200/PO.24.00230

3. Shi C, Badgwell BD, Grabsch HI, et al. Data Set for Reporting Carcinoma of the Stomach in Gastrectomy. *Arch Pathol Lab Med*. 2022;146(9):1072-1083. doi:10.5858/arpa.2021-0225-OA

4. Allum WH, Blazeby JM, Griffin SM, et al. Guidelines for the management of oesophageal and gastric cancer. *Gut*. 2011;60(11):1449-1472. doi:10.1136/gut.2010.228254

5. Narita Y, Sasaki E, Masuishi T, et al. PD-L1 immunohistochemistry comparison of 22C3 and 28-8 assays for gastric cancer. *J Gastrointest Oncol*. 2021;12(6):2696-2705. doi:10.21037/jgo-21-505