

PD-L1 Positivity Rates in Head and Neck Squamous Cell Carcinoma in a UK Reference Centre

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Short Report

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Abstract

PD-L1 combined positive score (CPS) testing using the 22C3 PharmDx assay guides pembrolizumab eligibility in recurrent/metastatic head and neck squamous cell carcinoma (HNSCC). We retrospectively audited local CPS results in 98 HNSCC cases (2021–2023) against published reference data from the KEYNOTE-048 trial (CPS ≥ 1 in $\sim 85\%$; CPS ≥ 20 in $\sim 43\%$ of the trial population). CPS ≥ 1 was observed in 90.8% ($p=0.12$ vs reference) and CPS ≥ 20 in 59.2% ($p=0.0015$ vs reference). Oral cavity primaries showed the highest CPS ≥ 20 rate (74.4%); with resections showing a higher rate than biopsies (76.2% vs 54.5%, $p=0.085$). Local CPS ≥ 20 positivity was significantly higher than the KEYNOTE-048 reference population. Re-audit is underway to examine reproducibility.

Key messages

- Local PD-L1 CPS ≥ 20 positivity in HNSCC (59.2%) was significantly higher than the KEYNOTE-048 trial reference population ($\sim 43\%$), while CPS ≥ 1 positivity showed a similar but non-significant trend.
- Local PD-L1-positive populations may differ materially from the trial population underpinning current CPS-based treatment thresholds, relevant to clinicians interpreting CPS results.
- A prospectively defined, fully consecutive re-audit is needed to confirm reproducibility.

Introduction

Programmed death-ligand 1 (PD-L1) combined positive score (CPS) testing using the 22C3 PharmDx assay is the standard biomarker used to select patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) for first-line pembrolizumab-based therapy, following the phase 3 KEYNOTE-048 trial. In that trial's screened population, CPS ≥ 1 was observed in approximately 85% of tumours and CPS ≥ 20 in approximately 43%.^[1]

PD-L1 expression is routinely audited at our centre against benchmarked data to monitor technical assay reliability and pathologist training to ensure a reliable clinical service against national accreditation guidelines.

CPS scoring combines PD-L1 staining in squamous cell carcinoma cells as well mononuclear immune cells (lymphocytes and macrophages). It is inherently subject to inter-observer and pre-analytical variability. Divergence between a local reporting population and the trial population underpinning treatment thresholds has potential implications for patient selection, and may reflect case-mix (e.g. subsite distribution), specimen type, referral pathway selection, or assay/scoring technique. Crucially, the clinical relevance of accurate CPS reporting in HNSCC now extends beyond the recurrent/metastatic setting: the phase 3 KEYNOTE-689 trial demonstrated a statistically significant improvement in event-free survival with perioperative pembrolizumab in resectable stage III/IVA HNSCC, leading to FDA approval in June 2025.^[2] As immunotherapy indications expand into earlier-stage disease, the volume

PD-L1 CPS testing in HNSCC are likely to increase further, with complex referral pathways and pathology networks potentially complicating or delaying reporting. It is, therefore, important to ensure assay reliability across reference centres. This audit was undertaken to establish baseline local CPS distribution and inform whether standardisation or re-audit is warranted.

Methods

Design and setting

Retrospective single-centre audit conducted in the Cellular Pathology Department, University Hospital Southampton NHS Foundation Trust. The cases included primary biopsies and resections from local patients, as well as cases referred from the regional cancer network for PD-L1 testing.

Population

HNSCC specimens (biopsies and resections) reported with a PD-L1 CPS result using the 22C3 pharmDx assay between 2021 and 2023 ($n = 98$) were identified from departmental reporting records.

Comparator standard

CPS ≥ 1 and CPS ≥ 20 positivity rates in the KEYNOTE-048 screened population (approximately 85% and 43% respectively).[1]

Data collected

CPS value, tumour subsite (as recorded at reporting), and specimen type (biopsy vs resection). Subsites were grouped into standard anatomical categories (oral cavity, oropharynx, larynx, hypopharynx, nasopharynx, and nodal/metastatic presentations).

Analysis

Proportions positive at CPS ≥ 1 and CPS ≥ 20 were calculated overall, by subsite, and by specimen type. Local proportions were compared with the KEYNOTE-048 reference proportions using exact one-sample binomial tests. Biopsy and resection positivity rates were compared using Fisher's exact test.

Results

Of 98 cases reviewed, 77 (78.6%) were biopsy specimens and 21 (21.4%) were resections. Overall, CPS ≥ 1 was recorded in 89/98 cases (90.8%) and CPS ≥ 20 in 58/98 cases (59.2%); median CPS across the series was 20. Compared with the KEYNOTE-048 reference proportions, the local CPS ≥ 1 rate was numerically but not statistically significantly higher (exact one-sample binomial test, $p = 0.12$), while the local CPS ≥ 20 rate was significantly higher ($p = 0.0015$).

Positivity varied by subsite (Table 1). Oral cavity primaries showed universal CPS ≥ 1 positivity (100%) and the highest CPS ≥ 20 rate (74.4%), with the highest median CPS (40) of any subsite group. Laryngeal and hypopharyngeal primaries showed lower CPS ≥ 20 rates (41.7% and 0% respectively), though hypopharyngeal numbers were small (n = 2).

By specimen type (Table 2), CPS ≥ 1 positivity was similar between biopsies (89.6%) and resections (95.2%; Fisher's exact $p = 0.68$). CPS ≥ 20 positivity was higher in resections (76.2%) than biopsies (54.5%), a difference that approached but did not reach statistical significance ($p = 0.085$). Median CPS was also higher in resections (50) than biopsies (20).

Table 1
CPS positivity by tumour subsite (n = 98)

Tumour subsite	n	CPS ≥ 1 (%)	CPS ≥ 20 (%)	Median CPS
Oral cavity	39	39 (100.0)	29 (74.4)	40
Oropharynx	18	16 (88.9)	10 (55.6)	20
Larynx	12	11 (91.7)	5 (41.7)	10
Hypopharynx	2	1 (50.0)	0 (0.0)	1
Nodal/metastatic (primary unspecified)	20	16 (80.0)	10 (50.0)	12.5
Other (nasopharynx, parotid, unclassified)	7	6 (85.7)	4 (57.1)	30
Overall	98	89 (90.8)	58 (59.2)	20

Table 2
CPS positivity by specimen type (n = 98)

Specimen type	n	CPS ≥ 1 (%)	CPS ≥ 20 (%)	Median CPS
Biopsy	77	69 (89.6)	42 (54.5)	20
Resection	21	20 (95.2)	16 (76.2)	50
p-value (Fisher's exact)	—	0.68	0.085	—

Discussion

Local CPS ≥ 20 positivity was significantly higher than the KEYNOTE-048 reference population (59.2% vs ~ 43%, $p = 0.0015$). CPS ≥ 1 positivity was also higher (90.8% vs ~ 85%) but this difference was not statistically significant ($p = 0.12$) and should be interpreted as a trend given the sample size. The clearest local deviation from the reference population is therefore at the high-expression (CPS ≥ 20) threshold, which is also the threshold most strongly linked to pembrolizumab monotherapy benefit in KEYNOTE-048.

The audited series was weighted toward oral cavity primaries (39/98, 39.8%), which showed the highest CPS ≥ 20 rate of any subsite (74.4%) and may partly explain the elevated overall rate. Resections appeared to show a higher CPS ≥ 20 rate than biopsies (76.2% vs 54.5%, $p = 0.085$), which could potentially reflect intratumoural heterogeneity in PD-L1 expression, with larger resection specimens sampling a greater proportion of the primary tumour. Case selection also warrants discussion, as the series was retrospectively identified rather than within. Patients that had been tested for PD-L1 CPS had been pre-selected on a clinical basis rather than in a controlled trial setting. Assay variability would not be considered a major factor given the PharmDx 'locked down' of the Agilent assay. However, inter-observer scoring variability cannot be excluded, as three individual pathologists had scored the cases included in this audit separately.

These findings highlight that the local PD-L1-positive population may differ materially in composition from the trial population from which current CPS-based treatment thresholds were derived – a distinction relevant to clinicians interpreting CPS results in the context of expected treatment benefit. As immunotherapy indications expand into earlier-stage, potentially curable disease following KEYNOTE-689,[2] understanding local CPS distribution relative to trial-defined reference populations is likely to become increasingly important. Artificial intelligence (AI)-assisted scoring tools may be of benefit in the nature (with human supervision) in PD-L1 scoring to improve potential intra- and inter-observer variability [4].

Limitations

The series was retrospectively identified, and therefore, completeness as a fully consecutive sample of all CPS-tested cases in the period could not be confirmed, which may introduce selection bias. The specimen-type comparison is limited by a small resection group ($n = 21$). Although patients from several referral centres were included, the audit was conducted at a single centre, limiting generalisability.

Conclusion

PD-L1 CPS ≥ 20 positivity in this local HNSCC series was higher than the KEYNOTE-048 reference population, with a similar but non-significant trend at the CPS ≥ 1 threshold. Positivity varied by tumour subsite and by specimen type, with resections showing higher CPS ≥ 20 rates than biopsies. Regular, prospectively defined, fully consecutive re-audits are recommended to establish whether these findings are reproducible and to inform whether further standardisation of testing or scoring practice is warranted.

Declarations

Funding: None declared.

Competing interests: KM has received honoraria from MSD, the manufacturer of pembrolizumab and the 22C3 pharmDx PD-L1 assay discussed in this manuscript.

BT declares no competing interests.

Ethics/audit registration: Registered with the local clinical audit department, University Hospital Southampton NHS Foundation Trust (Audit reference SEV: 7182).

Author contributions: BT collected the data and drafted the manuscript. KM designed the audit, performed the statistical analysis, and takes responsibility for the accuracy of the data. Both authors reviewed and approved the final manuscript.

Data availability statement: Anonymised case-level data (CPS value, tumour subsite, and specimen type for each of the 98 cases) are available as a supplementary table accompanying this article.

Declaration of AI use: Generative AI (Claude, Anthropic) was used to assist with drafting and structuring this manuscript from data and analysis provided by the authors. All data, analysis, interpretation, and conclusions are those of the authors, who reviewed and take full responsibility for the accuracy and content of the manuscript.

Consent to Participate declaration: not applicable

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1PDL1CPSdata2.xlsx](#)