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Inter-assay reliability of programmed cell death-ligand 1 in head and neck squamous cell carcinoma

Hempenius, Maaike Anna; Bisheshar, Sangeeta Kareshma; Slagter-Menkema, Lorian; van der Kamp, Martine Froukje; Halmos, Gyorgy Bela; Doff, Jan Johannes; Willems, Stefan Martin; van der Vegt, Bert

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ABSTRACT

Objectives: The programmed cell death-ligand 1 (PD-L1) 22C3 pharmDx assay is used as a companion diagnostic test to select head and neck squamous cell carcinoma (HNSCC) patients that may benefit from treatment with the checkpoint inhibitor pembrolizumab. Because the Dako platform is not universally available, we studied the performance of a 22C3 laboratory developed test (LDT) performed on a Ventana BenchMark Ultra compared to the 22C3 pharmDx assay.

Materials and methods: Serial sections from tissue micro arrays (TMAs) containing tumour tissue from 97 HNSCC patients were stained with the 22C3 pharmDx assay and 22C3 LDT. All TMA cores were scored by three dedicated head and neck pathologists for PD-L1 expression.

Results: Substantial interobserver agreement was reported for both the standardized 22C3 pharmDx assay and the 22C3 LDT (respectively Fleiss' κ 0.62, 95% CI 0.57–0.67 and 0.63, 95% CI 0.58–0.68). Concordance between the assays was almost perfect on core and patient level (respectively Weighted κ 0.84, 95% CI 0.79–0.89 and 0.84, 95% CI 0.75–0.92). Intratumor heterogeneity between the cores per patient case was similar in both assays.

Conclusion: After validation a 22C3 LDT is non-inferior to the standardized 22C3 pharmDx assay and can be safely used to select HNSCC patients for pembrolizumab treatment.

Introduction

Most head and neck squamous cell carcinoma (HNSCC) patients are diagnosed with locally advanced disease at presentation (T3-T4 primary or ≥N1 nodal staging) [1–3]. These patients have a high risk for local recurrence (15–40%) or/and distant metastasis (3–52%) [1,2,4–6]. Pembrolizumab, a PD-1 inhibitor, was approved by the US Food and Drug Administration (FDA) for first-line palliative treatment of metastatic or unresectable recurrent HNSCC [7]. Only few HNSCC patients benefits from PD-1 inhibitors, with reported overall response rates of 13–18% [8–10]. Eligibility for pembrolizumab is based on programmed cell death-ligand 1 (PD-L1) immunohistochemistry (IHC) [11]. The FDA approved the 22C3 pharmDx assay on the Dako Autostainer Link 48 platform as a companion diagnostic to select patients for pembrolizumab treatment [7]. Because the Dako platform is not universally available, there is a need for laboratory developed tests (LDTs) on alternative platforms to prevent diagnostic delays and keep costs reasonable [12,13]. This study assessed the performance of a 22C3 LDT performed on the BenchMark Ultra compared to the 22C3 pharmDx assay on the Dako platform.

Material and methods

100 stage I-IV HNSCC patients gave informed consent for inclusion in the OncoLifeS data-biobank. This data-biobank has been approved by the local medical ethics committee (no.2010/109) and is registered in the Dutch Trial Register (NL7839) and UMCG research register (201900297) [14].

Formalin-fixed paraffin-embedded primary tumour tissue, obtained from biopsies or resections, was included in two tissue micro arrays (TMAs) using a Manual Tissue Arrayer I (Beecher Instruments). Per patient, three 0.6 mm tumour cores were included. Three patients were excluded after TMA construction, due to missing cores, resulting in a final study population of 97 patients.

Two 5 μm sections were cut from each TMA for IHC and were stained for PD-L1 on two different automated staining platforms: (1) Autostainer Link 48, 22C3 pharmDx assay (Dako/Agilent;) according to manufacturer's protocol at VU University Medical Center Amsterdam. (2) BenchMark Ultra (Ventana), PD-L1 monoclonal mouse antibody (Clone 22C3, Dako/Agilent); antigen retrieval time 64 min (100 °C, Cell Conditioner #1, pH 9; Ventana); primary antibody dilution 1:50; incubation time 32 min; visualization OptiView diaminobenzidine detection kit (Ventana); counterstaining Mayer's hematoxylin (Klinipath). The 22C3 LDT was stained at the UMCG.

Three pathologists independently scored all cores for PD-L1 using the clinically relevant combined positive score (CPS) cut-offs <1, ≥1-20 and ≥20. CPS was determined as the number of PD-L1 positive tumour cells, lymphocytes, and macrophages divided by the total number of viable tumour cells, multiplied by 100 [4].

Abbreviations: CPS, Combined Positive Score; FDA, US Food and Drug Administration; HNSCC, Head and Neck Squamous Cell Carcinoma; IHC, Immunohistochemistry; LDT, Laboratory Developed Test; PD-L1, Programmed Cell Death-Ligand 1; TMA, Tissue Micro Array; UMCG, University Medical Center Groningen.

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Table 1
Inter-assay concordance of PD-L1 CPS for 22C3 pharmDx and 22C3 LDT per pathologist (n = 254 cores).

Pathologist 1		22C3 pharmDx			
		<1	**≥1–20**	**≥20**	**Total**
22C3 LDT	**<1**	82	6	0	88
	≥1–20	16	62	11	89
	≥20	0	19	58	77
	Total	98	87	69	254
			κ = 0.77, 95 % CI 0.71–0.83		
Pathologist 2		**22C3 pharmDx**			
		<1	**≥1–20**	**≥20**	**Total**
22C3 LDT	**<1**	116	9	0	125
	≥1–20	22	17	9	48
	≥20	4	11	66	81
	Total	142	37	75	254
			κ = 0.75, 95% CI 0.69–0.81		
Pathologist 3		**22C3 pharmDx**			
		<1	**≥1–20**	**≥20**	**Total**
22C3 LDT	**<1**	95	3	0	98
	≥1–20	9	45	12	66
	≥20	0	13	77	90
	Total	104	61	89	254
			κ = 0.84, 95% CI 0.80–0.89		

CPS, combined positive score; LDT, laboratory developed test; κ = weighted kappa; CI, confidence interval.

Results

Ultimately, for both assays 254 TMA cores of 97 patients were scored by three pathologists (Table 1). The 22C3 LDT showed stronger and slightly more granular staining (Fig. 1).

Between three pathologists substantial interobserver agreement was found for both assays (22C3 LDT: Fleiss' κ 0.63, 95 %CI 0.58–0.68 and 22C3 pharmDx: κ 0.62, 95 %CI 0.57–0.67). For the LDT, all pathologists agreed on the CPS in 163 of 254 TMA cores, resulting in an overall percent agreement of 64.2%. Two of three pathologists agreed in 88 (34.6%) cores. All pathologists disagreed in three (1.2%) cores. The absolute values were identical for the 22C3 pharmDx. The CPS category that most pathologists attributed a core to was considered the consensus CPS. Cores without majority were discussed until agreement was reached. For the 22C3 LDT 99 (39.0%) cores received consensus CPS < 1, 72 (28.3%) CPS ≥ 1–20 and 83 (32.7%) CPS ≥ 20. For the 22C3 pharmDx 113 (44.5%) cores received a CPS < 1, 62 (24.4%) CPS ≥ 1–20 and 79 (31.1%) CPS ≥ 20.

Seventy-two patients had three TMA cores available. The highest consensus CPS of the cores was considered the consensus CPS per patient case. Both assays had 51 (70.8%) of 72 patients in which the three cores had the same consensus CPS. Concordance between the cores per case was substantial for both assays (22C3 LDT: Fleiss' κ 0.69, 95 %CI 0.60–0.79 and 22C3 pharmDx: κ 0.68, 95 %CI 0.58–0.77). In the 22C3 LDT 27 (31.8%) cases were negative (CPS < 1), 23 (27.1%) positive (CPS ≥ 1–20) and 35 (41.2%) strongly positive (CPS > 20). For the 22C3 pharmDx 30 cases (35.3%) were negative (CPS < 1), 22 (25.9%) positive (CPS ≥ 1–20) and 33 (38.8%) strongly positive (CPS > 20).

An almost perfect inter-assay agreement was found between consensus CPS per core and patient case for the 22C3 LDT and 22C3 pharmDx (respectively Weighted κ 0.84, 95 %CI 0.79–0.89 and κ 0.84, 95 %CI 0.75–0.92). When using the CPS ≥ 1 and ≥ 20 cut-off in the 22C3 pharmDx to determine PD-L1 positivity, respectively one (0.7%) and nine (11.4%) false negative cores in the 22C3 LDT were found. On patient level this translated to one (1.8%) and three (9.1%) false negative cases, respectively.

Discussion

We found similar results between a PD-L1 22C3 LDT and the PD-L1 22C3 pharmDx assay regarding interobserver agreement and intratumour CPS heterogeneity. Almost perfect agreement was found between the two assays at core and patient level. However, despite this, even few false negative results are problematic because they could result in withholding a potentially beneficial treatment from patients. A few studies have investigated the performance of a 22C3 LDT on the Ventana

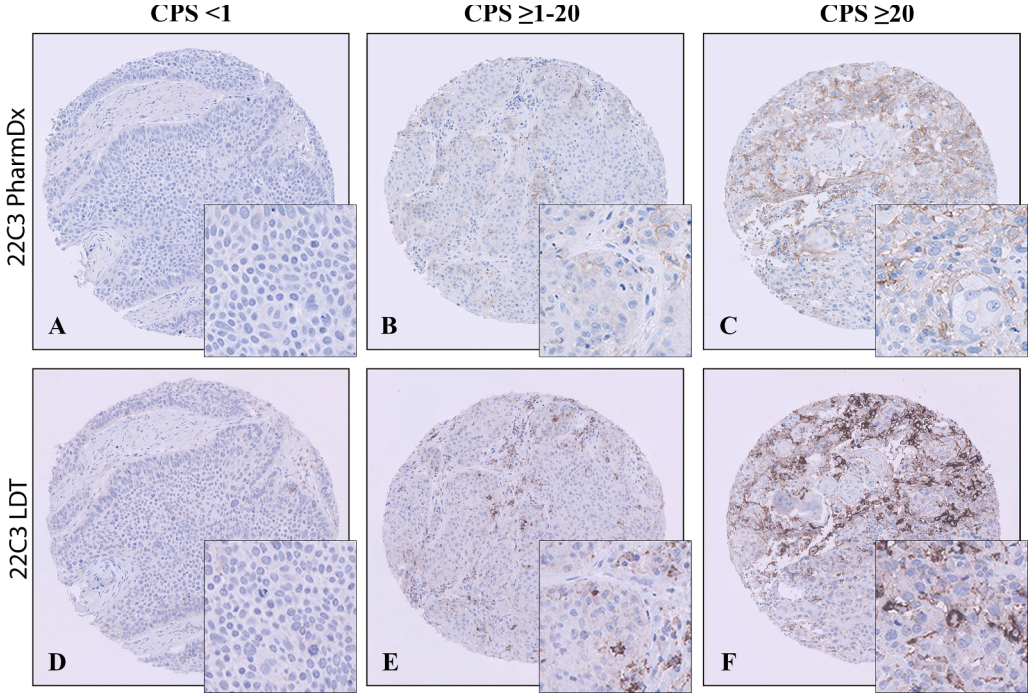

Fig. 1. TMA cores stained for PD-L1 using the pharmDx assay (upper row) and 22C3 LDT (lower row) with CPS < 1 (A,D), ≥1–20 (B,E) and ≥20 (C,F). All cores received a unanimous CPS from three pathologists. Images at 100x and 400x magnification.

BenchMark and the 22C3 pharmDx. One study reported more false negatives with a 22C3 LDT (12%), but analysed only a relatively small sample size of 30 TMA cores [13]. Another study conducted their analysis on case level and found a moderate to poor concordance between the two assays (ICC 0.68, 95 %CI 0.57–0.75) [12]. Our concordance was higher, possibly because our consensus CPS per patient case was determined based on the highest CPS of three cores, whereas the consensus of the prior was based on the mean. Another factor might be differences in staining protocol of the LDT. A limitation of our study is the use of TMA cores, instead of whole tissue slides, as PD-L1 is subject to intratumour heterogeneity. However, the use of multiple cores from one tumour should compensate for this.

Conclusion

A 22C3 LDT is non-inferior to the standardized 22C3 pharmDx assay and can safely be used to assess PD-L1 status for HNSCC in pathology departments that do not have access to the standardized assay.

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Declaration of Competing Interest

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Maaike Anna Hempenius[a,*,1], Sangeeta Kareshma Bisheshar[a,b,1], Lorian Slagter-Menkema[a,c], Martine Froukje van der Kamp[c], Gyorgy Bela Halmos[c], Jan Johannes Doff[a], Stefan Martin Willems[a], Bert van der Vegt[a]

[a] *Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*
[b] *Department of Pathology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands*
[c] *Department of Otolaryngology and Head and Neck Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*

[*] Corresponding author at: Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, PO Box 30001, 9700 RB Groningen, the Netherlands.
E-mail address: m.a.hempenius@umcg.nl (M.A. Hempenius).

[1] M.A.H. and S.K.B. contributed equally to this work and therefore share first authorship.