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Analysis of Real-World PD-L1 Testing and PD-L1 IHC 28-8 and 22C3 Assay Concordance in Patients With Breast Cancer

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Background

- Programmed death-1/programmed death ligand 1 (PD-1/PD-L1) inhibitors have been approved for use in a range of tumor types[1–5]
- PD-L1 expression, as determined by an approved PD-L1 diagnostic assay, may be associated with clinical benefit from PD-1/PD-L1 inhibitors in some tumor types, including breast cancer[6]
  - Atezolizumab in combination with nab-paclitaxel was approved by the US FDA in March 2019 for the treatment of patients with unresectable locally advanced or metastatic triple-negative breast cancer (TNBC) with PD-L1 immune cell (IC) staining of any intensity covering ≥ 1% of tumor area, as determined by an FDA-approved test[3]
  - Clinical trials of other PD-1/PD-L1 inhibitors in patients with breast cancer are ongoing, including studies exploring the utility of PD-L1 expression on TCs and ICs for predicting treatment outcome[7–10]
- Assay approval status varies across assays and drug indications, with some assays approved as companion diagnostics and others as complementary diagnostics[1–4] (**Table 1**)
  - The Ventana PD-L1 (SP142) assay is the only assay approved as a PD-L1 companion diagnostic in the treatment of patients with TNBC
- Concordance between PD-L1 assays has been shown across a range of tumor types, including lung cancer, melanoma, squamous cell carcinoma of the head and neck (SCCHN), and urothelial carcinoma (UC)[11–15]
  - Few studies have evaluated PD-L1 assay concordance in breast cancer samples

Table 1. Current FDA-approved PD-L1 IHC assays

Antibody clone	28-8[1,16]	22C3[2,17]	SP142[3,18]	SP263[4,19]
Assay (manufacturer)	PD-L1 IHC 28-8 pharmDx (Agilent/Dako)	PD-L1 IHC 22C3 pharmDx (Agilent/Dako)	PD-L1 (SP142) assay (Ventana)	PD-L1 (SP263) assay (Ventana)
For use with (drug)	Nivolumab	Pembrolizumab	Atezolizumab	Durvalumab
Approval status	Complementary (NSQ NSCLC, SCCHN, UC)	Companion (NSCLC, UC, gastric/ GEJ, CC, ESCC, SCCHN)	Companion (UC,[a] TNBC) Complementary (NSCLC, UC[b])	Complementary (UC)

[a]In cisplatin-ineligible patients with locally advanced or metastatic UC and PD-L1 expression on tumor-infiltrating ICs covering ≥ 5% of the tumor area; [b]in patients with locally advanced or metastatic UC who have disease progression during or following any platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant chemotherapy.
CC, cervical cancer; ESCC, esophageal squamous cell carcinoma; GEJ, gastroesophageal junction adenocarcinoma; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; NSQ, non-squamous.

Objectives

- Evaluate the real-world use and outcomes of testing with the Dako PD-L1 IHC 28-8 and 22C3 pharmDx assays and the Ventana PD-L1 (SP142) and PD-L1 (SP263) assays in patients with breast cancer
- Assess the analytical concordance between the 28-8 and 22C3 assays in matched samples from patients with breast cancer

Methods

Patient samples

- NeoGenomics Laboratories, Inc (Fort Myers, FL), a US national reference laboratory, provided results for PD-L1 tests performed between October 2015 and October 2019
  - PD-L1 expression was determined by trained pathologists
  - Results for the 28-8 assay for the entire study period, and for the 22C3 assay until December 2018, were reported as the percentage of tumor cells (TCs) with PD-L1 expression, as indicated in diagnostic labels at the time of testing
  - From January 2019 onwards, results for the 22C3 assay were reported as a combined positive score (CPS), defined as the number of PD-L1 staining cells (TCs, lymphocytes, and macrophages) divided by the total viable TCs, multiplied by 100
  - Results for the SP142 assay were reported as the percentage of ICs with PD-L1 expression, as indicated in diagnostic labels at the time of testing
- Clinical characteristics of patients who underwent PD-L1 testing were provided by Symphony Health Solutions (Phoenix, AZ) and were matched to PD-L1 test results using unique identifiers

Measures

- Test utilization over time was assessed using test volume for all 3 assays pooled and individually, and is presented by 3-month period (quarter)
- Test failure, defined as the absence of adequate sample with evaluable PD-L1 expression, is presented by quarter for all 3 assays pooled
- Test turnaround time (TAT), defined as the time from sample receipt by the laboratory to test-report availability, is presented by quarter for all 3 assays pooled

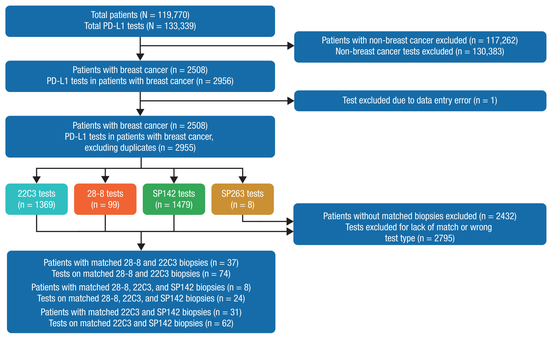
Analyses

- Analytical concordance between the 28-8 and 22C3 assays for samples tested between Q4 2015 and Q4 2018 was evaluated using Passing–Bablok regression, Kendall's tau correlation, and Spearman's correlation in patients with breast cancer who had matched samples
  - Patients were excluded from the concordance analysis if they had > 1 PD-L1 test using the same assay or if they had no matched biopsies
- Assay agreement (positive, negative, and overall percentage agreement [PPA, NPA, and OPA]) was assessed at the 1%, 10%, 25%, and 50% PD-L1 expression cutoffs
- To evaluate PD-L1 prevalence, test results were grouped by PD-L1 scoring algorithm and in PD-L1 expression categories of 0%, 1%–24%, 25%–49%, and 50%–100%
  - Patients with a single test result or ≥ 2 identical PD-L1 test results for the 28-8, 22C3, or SP142 assays were included
  - Patients with ≥ 2 discrepant PD-L1 test results were excluded to avoid potential misclassification

Results

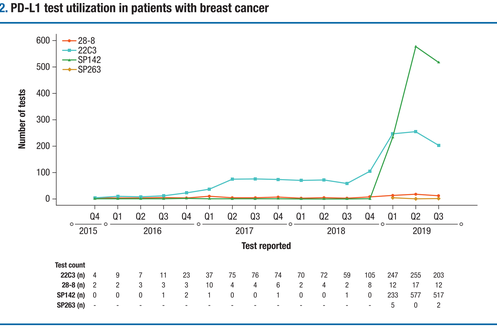
- A total of 133,339 PD-L1 tests on samples from 119,770 patients were included in the data set (**Figure 1**)
  - 2956 PD-L1 tests on samples from 2508 patients with a confirmed diagnosis of breast cancer were included in the analysis
  - 45 patients with breast cancer had matched samples tested with both the 28-8 and 22C3 assays between Q4 2015 and Q4 2018

Figure 1. Sample disposition for PD-L1 expression testing in patients with breast cancer



- The number of PD-L1 tests performed on samples from patients with breast cancer increased markedly over the study period (**Figure 2**)
  - The 22C3 and SP142 assays were each used for ~48% of tests on breast cancer samples
  - Increased use of the 22C3 and SP142 assays coincided with the FDA approval of atezolizumab + nab-paclitaxel for the treatment of unresectable or metastatic TNBC in March 2019

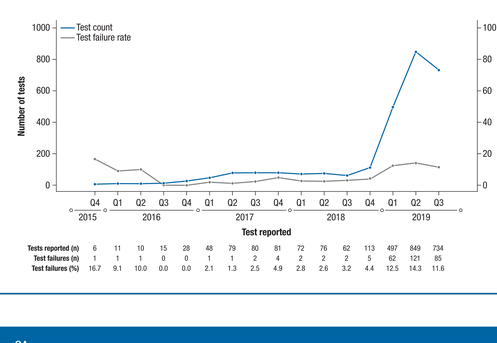
Figure 2. PD-L1 test utilization in patients with breast cancer



Test count	Q4 2015	Q1 2016	Q2 2016	Q3 2016	Q4 2016	Q1 2017	Q2 2017	Q3 2017	Q4 2017	Q1 2018	Q2 2018	Q3 2018	Q4 2018	Q1 2019	Q2 2019	Q3 2019
22C3 (n)	4	9	7	11	23	37	75	76	74	70	72	59	105	247	255	203
28-8 (n)	2	2	3	3	3	10	4	4	6	2	4	2	8	12	17	12
SP142 (n)	0	0	0	1	2	1	0	0	1	0	0	1	0	233	577	517
SP263 (n)	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	2

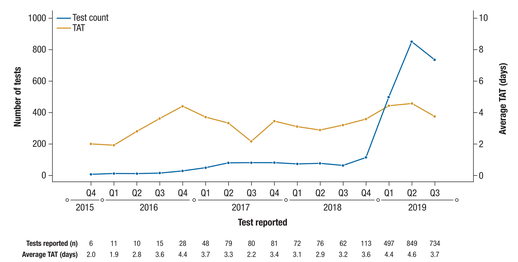
- Despite the large increase in test volume, test failure rates remained < 20% (**Figure 3**), and average TAT across all tests remained < 5 days (**Figure 4**)

Figure 3. PD-L1 test failure rate in patients with breast cancer



Tests reported (n)	Q4 2015	Q1 2016	Q2 2016	Q3 2016	Q4 2016	Q1 2017	Q2 2017	Q3 2017	Q4 2017	Q1 2018	Q2 2018	Q3 2018	Q4 2018	Q1 2019	Q2 2019	Q3 2019
Test failures (n)	1	1	1	0	0	1	1	2	4	2	2	2	5	62	121	85
Test failures (%)	16.7	9.1	10.0	0.0	0.0	2.1	1.3	2.5	4.9	2.8	2.6	3.2	4.4	12.5	14.3	11.6

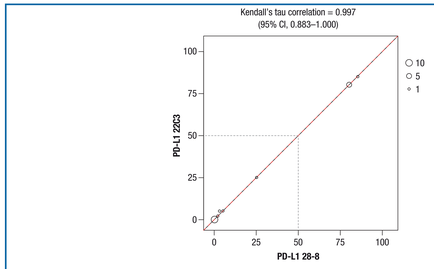
Figure 4. Impact of PD-L1 test volume on TAT for breast cancer samples



Tests reported (n)	Q4 2015	Q1 2016	Q2 2016	Q3 2016	Q4 2016	Q1 2017	Q2 2017	Q3 2017	Q4 2017	Q1 2018	Q2 2018	Q3 2018	Q4 2018	Q1 2019	Q2 2019	Q3 2019
Average TAT (days)	2.0	1.9	2.8	3.6	4.4	3.7	3.3	2.2	3.4	3.1	2.9	3.2	3.6	4.4	4.6	3.7

- Strong correlation was observed between the 28-8 and 22C3 assays when using % TC scoring in patients with breast cancer (**Figure 5**)
  - TC PD-L1 IHC scores with the 28-8 and 22C3 assays were identical for 96% of matched samples (26 of 27 patients) and the difference in score for the remaining patient was < 5%
  - OPA, PPA, and NPA between the 28-8 and 22C3 assays for PD-L1 expression on TCs was 100% (Cohen's kappa = 1.00) at the 1%, 10%, 25%, and 50% PD-L1 expression cutoffs

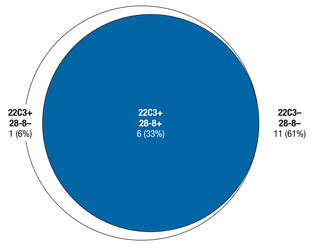
Figure 5. Correlation between the 28-8 and 22C3 assays for PD-L1 expression on TCs in matched samples from patients with breast cancer (N = 27)



Data are presented for matched 28-8 and 22C3 tests performed between Q4 2015 and Q4 2018. Passing–Bablok regression with the 28-8 assay as reference: slope = 1, intercept = 0. Identity line (dashed red) and the regression line (solid black) are coincident. Dotted gray line indicates PD-L1 expression of 50%. Symbol size scaled by sample size as indicated in the figure key. Spearman's correlation = 1.000 (95% CI, 0.999–1.000). CI, confidence interval.

- Agreement between the 28-8 assay (% TC) and 22C3 assay (CPS) was high (**Figure 6**)

Figure 6. Agreement between the 28-8 (% TC) and 22C3 (CPS) assays in matched samples from patients with breast cancer (n = 18)

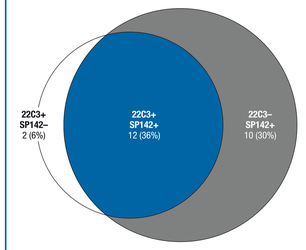


TC ≥ 1% / CPS ≥ 1[a]	28-8 as reference	22C3 as reference
PPA (n/N)	100 (6/6)	86 (6/7)
NPA (n/N)	92 (11/12)	100 (11/11)
OPA (n/N)	94 (17/18)	

Data are presented for 28-8 and 22C3 tests on matched samples with 22C3 tests performed between Q1 and Q4 2019. [a]PD-L1 expression cutoffs were TC ≥ 1% for the 28-8 assay and CPS ≥ 1 for the 22C3 assay.

- Agreement between the 22C3 assay (CPS) and SP142 assay (% IC) was moderate (**Figure 7**)
- Prevalence of PD-L1 expression ≥ 1% was 36% in patients tested with the 28-8 or 22C3 assays and scored with the % TC algorithm, 58% in patients tested with the 22C3 assay and scored with the CPS algorithm, and 66% in patients tested with the SP142 assay and scored with the % IC algorithm (**Figure 8**)

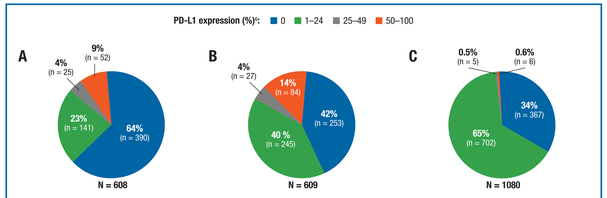
Figure 7. Agreement between the 22C3 (CPS) and SP142 (% IC) assays in matched samples from patients with breast cancer (n = 33)



CPS ≥ 1 / IC ≥ 1%[a]	22C3 as reference	SP142 as reference
PPA (n/N)	86 (12/14)	55 (12/22)
NPA (n/N)	47 (9/19)	82 (9/11)
OPA (n/N)	64 (21/33)	

Data are presented for 22C3 and SP142 tests on matched samples with 22C3 tests performed between Q1 and Q4 2019 . [a]PD-L1 expression cutoffs were CPS ≥ 1 for the 22C3 assay and IC ≥ 1% for the SP142 assay.

Figure 8. Prevalence of PD-L1 expression in patients with breast cancer using (A) the 28-8 and 22C3 assays (% TC),[a] (B) the 22C3 assay (CPS),[b] and (C) the SP142 assay (% IC)



All patients had a single test result or ≥ 2 identical test results. [a]Samples tested with the 22C3 assay between Q4 2015 and Q4 2018 were scored using the % TC algorithm; [b]Samples tested with the 22C3 assay between Q1 and Q4 2019 were scored using the CPS algorithm; [c]The CPS algorithm is reported on a scale of 0–100, not as a percentage.

Conclusions

- The number of PD-L1 tests performed on breast cancer samples at a single US reference laboratory increased markedly following the approval of the SP142 assay as a companion diagnostic assay to atezolizumab in March 2019
  - However, breast cancer samples made up only 2% of all samples tested over the study period
- Despite the increase in test volume, the proportion of test failures was < 20% and assay TAT remained < 5 days
- Concordance between the 28-8 and 22C3 assays for PD-L1 expression on TCs in matched samples from patients with breast cancer was high
  - Agreement between the 28-8 and 22C3 assays was 100% at all PD-L1 expression cutoffs evaluated
  - The high concordance and percentage agreement between the 28-8 and 22C3 assays were consistent with findings in other tumor types[11,14–16]
- Agreement between the 28-8 and 22C3 assays at the ≥ 1(%) cutoff remained strong despite the change to CPS scoring of the 22C3 assay in 2019
- Prevalence of PD-L1 expression ≥ 1% was higher with the CPS and % IC algorithms vs the % TC algorithm, while prevalence of PD-L1 expression ≥ 25% was higher with the % TC and CPS algorithms vs the % IC algorithm
- These findings provide context on the evolution of PD-L1 testing in patients with breast cancer; further studies with a focus on IC PD-L1 expression are needed

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