Comparison of PD-L1 expression in primary and metastatic lung cancer biopsies

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Background

- Programmed death-1/programmed death ligand 1 (PD-1/PD-L1) inhibitors have been approved for use in lung cancer (**Table 1**)[1-4]
- PD-L1 expression, as assessed using an approved PD-L1 immunohistochemistry (IHC) assay, may be associated with greater clinical benefit from PD-L1 inhibitors in lung cancer[5,6]
- Multiple studies have demonstrated heterogeneous PD-L1 expression within the primary tumor and between the primary tumor and metastatic sites in lung cancer[7-9]
  - Most of these studies were retrospective analyses performed at a single institution[7-9]
- Characterizing the variation of PD-L1 expression between paired primary lung tumor and metastatic site biopsies from a real-world cohort of patients may contribute to an improved understanding of the biology of PD-L1 expression

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

Antibody clone	28-8[1,10]	22C3[2,11]	SP142[3,12]
Assay	Dako PD-L1 IHC 28-8 pharmDx	Dako PD-L1 IHC 22C3 pharmDx	Ventana PD-L1 (SP142)
For use with (drug)	Nivolumab ± ipilimumab	Pembrolizumab	Atezolizumab
Approval status in NSCLC	Companion[a]/ complementary[b]	Companion	Companion[c]/ complementary[d]
Approved cutoffs (scoring algorithm)	≥ 1%,[a,b] ≥ 5%,[b] ≥ 10%[b] (TC)	≥ 1% (TPS)	≥ 50% (TC) or ≥ 10% (IC)

[a]Companion diagnostic to nivolumab + ipilimumab in patients with 1L metastatic NSCLC expressing PD-L1 on ≥ 1% of TCs, with no EGFR or ALK genomic tumor aberrations; [b]Complementary diagnostic to nivolumab in patients with NS NSCLC that has progressed after platinum-based chemotherapy; [c]In patients with 1L metastatic NSCLC, with no EGFR or ALK genomic tumor aberrations; [d]In patients with metastatic NSCLC who had disease progression during or following platinum-containing chemotherapy.
1L, first-line; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; IC, immune cell; NS NSCLC, non-squamous non-small cell lung cancer; TC, tumor cell; TPS, tumor proportion score.

Study objective

- Compare PD-L1 expression between paired biopsies from primary tumor and metastatic sites in a real-world cohort of patients with lung cancer

Methods

Patient samples

- NeoGenomics Laboratories (Fort Myers, FL), a US national reference laboratory, reported PD-L1 test results between October 2015 and January 2020
- PD-L1 assays were performed per manufacturer's protocols for each respective assay at the time of analysis
- Patients were included if they met the following inclusion criteria:
  - Biopsies tested with the Dako PD-L1 IHC 28-8 or 22C3 pharmDx assays
  - Only 2 biopsy sites: 1 from the primary lung tumor and 1 from a metastasis
  - Both biopsies tested with the same PD-L1 IHC assay
  - Biopsies collected ≤ 3 months apart
  - Test ordered dates ≤ 3 months apart
- Patients were excluded if both assays were used on the same biopsy to eliminate bias towards either test
- Test results were linked to clinical characteristics provided by Symphony Healthcare Solutions (Phoenix, AZ) using unique identifiers, and analyzed by Bristol Myers Squibb

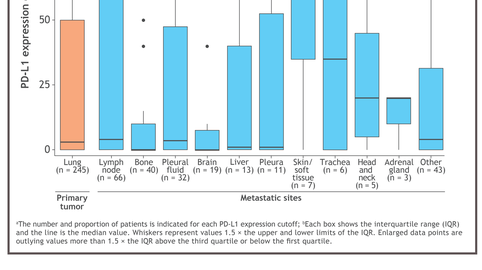
Analyses

- The percentage of TCs expressing PD-L1 was determined by trained pathologists from NeoGenomics Laboratories
- Positive, negative, and overall percentage agreement (PPA, NPA, and OPA) for PD-L1 expression between the primary tumor and metastatic sites was assessed at the 1%, 5%, 10%, 25%, and 50% cutoffs
- The difference in PD-L1 expression between the primary tumor and metastatic sites was assessed in the overall population and by metastatic site
- The agreement for PD-L1 expression between primary tumor and metastatic sites was assessed with Passing-Bablok regression
- The difference in PD-L1 expression was analyzed in patients who had their second biopsies collected after the result of the first biopsy was reported

Results

- NeoGenomics Laboratories reported PD-L1 test results for biopsies from 126,180 patients between October 2015 and January 2020
- In total, 44,407 patients had a confirmed diagnosis of lung cancer, of whom 245 had paired biopsies from primary tumor and metastatic sites that met the inclusion criteria (**Figure 1**)
  - A subset of 144 patients had their second biopsy collected after the PD-L1 test result for the first biopsy was reported

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



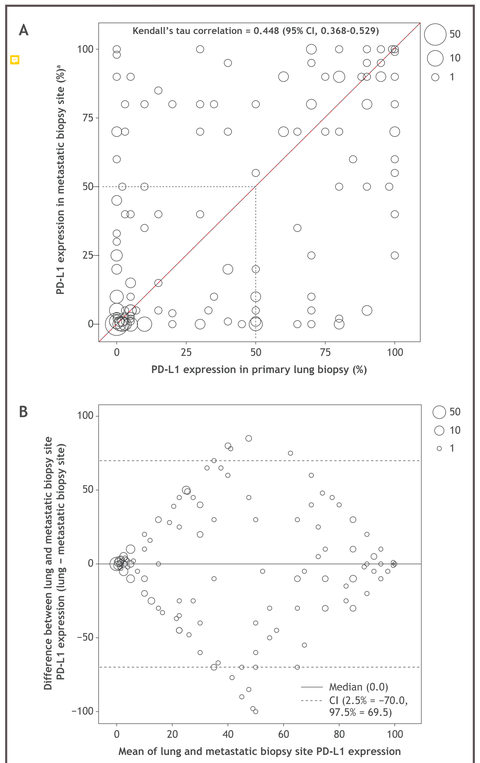
- PD-L1 expression levels were variable in primary tumor and metastatic sites (**Figure 2A**)
  - The proportion of patients in each PD-L1 expression category was similar in the primary tumor and metastatic sites
- PD-L1 expression levels were variable within and across biopsy sites (**Figure 2B**)

Figure 2. PD-L1 expression in (A) primary lung tumor and metastatic sites, and (B) biopsy site subgroups

[a]The number and proportion of patients is indicated for each PD-L1 expression cutoff; [b]Each box shows the interquartile range (IQR) and the line is the median value. Whiskers represent values 1.5 × the upper and lower limits of the IQR. Enlarged data points are outlying values more than 1.5 × the IQR above the third quartile or below the first quartile.

- Overall, the expression of PD-L1 on TCs was poorly correlated between primary tumor and metastatic sites (Kendall's tau 0.448 [95% CI, 0.368-0.529]) (**Figure 3**)

Figure 3. (A) Correlation of and (B) difference in PD-L1 expression between primary lung tumor and metastatic sites



[a]Passing-Bablok regression with lung biopsy 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 = 0.562 (95% CI, 0.470-0.642). Intraclass correlation = 0.628 (95% CI, 0.545-0.698). N = 245.

- The magnitude and direction of differences in PD-L1 expression between primary tumor and metastatic sites were heterogeneous (**Table 2**)
  - There was no consistent trend for higher or lower PD-L1 expression in biopsies from metastatic sites relative to the primary tumor

Table 2. Difference in PD-L1 expression between primary lung tumor and metastatic sites

PD-L1 expression difference (n = 245)	n (%)
0%-5%	133 (54)
6%-10%	28 (11)
11%-20%	9 (4)
> 20%	75 (31)
Lung = metastatic site	76 (31)
Lung < metastatic site	81 (33)
Lung > metastatic site	88 (36)

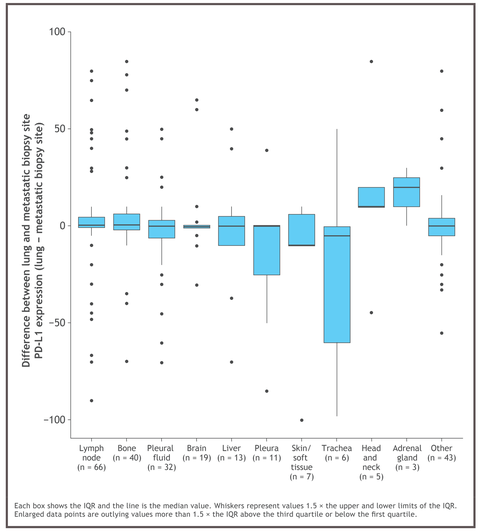
- OPA was 68%-82% (Cohen's kappa 0.35-0.53) across all cutoffs evaluated and appeared to increase with higher PD-L1 expression cutoffs (**Table 3**)

Table 3. Agreement for PD-L1 expression between primary lung tumor and metastatic sites across a range of cutoffs

PD-L1 expression cutoffs (% TC)	Primary lung tumor as reference		OPA (95% CI)	Cohen's kappa (95% CI)
	PPA (95% CI)	NPA (95% CI)		
1%	71 (63-77)	65 (55-73)	68 (62-74)	0.35 (0.23-0.47)
5%	71 (62-78)	75 (67-82)	73 (67-78)	0.46 (0.35-0.57)
10%	68 (59-77)	80 (73-86)	76 (70-80)	0.49 (0.38-0.60)
25%	65 (55-75)	85 (78-89)	78 (73-83)	0.51 (0.39-0.62)
50%	64 (52-74)	89 (83-93)	82 (77-86)	0.53 (0.41-0.66)

- No metastatic sites showed a trend for higher or lower PD-L1 expression on TCs than the primary tumor (**Figure 4**)

Figure 4. Difference in PD-L1 expression between lung tumor and metastatic sites



Each box shows the IQR and the line is the median value. Whiskers represent values 1.5 × the upper and lower limits of the IQR. Enlarged data points are outlying values more than 1.5 × the IQR above the third quartile or below the first quartile.

- Among the 144 patients who had their second biopsies collected after the result of the first biopsy was reported, 48% of patients with a negative first test (PD-L1 expression = 0%) had a positive result on the second test (PD-L1 expression ≥ 1%) (**Table 4**)
  - Of patients with high PD-L1 expression (50%-100%) on their first test, 22% had intermediate (1%-49%) or negative (0%) PD-L1 expression on their second test
  - Of patients with intermediate PD-L1 expression (1%-49%) on their first test, 57% had negative (0%) or high (50%-100%) PD-L1 expression on their second test

Table 4. Agreement for PD-L1 expression in patients whose second biopsy was collected after the first PD-L1 test was reported

		Second biopsy[a]			
		PD-L1 expression cutoffs (% TC)			
	First biopsy (reference)	0%, n (%)	1%-49%, n (%)	50%-100%, n (%)	Total, n (%)
PD-L1 expression cutoffs (% TC)	0%	38 (53)	27 (38)	7 (10)	72 (100)[b]
	1%-49%	13 (30)	19 (43)	12 (27)	44 (100)
	50%-100%	1 (4)	5 (18)	22 (79)	28 (100)[b]

[a]The 144 patients who had their second biopsy collected after the PD-L1 test result of the first biopsy was reported were included in this analysis; [b]The different categories do not add up to 100% due to rounding.

Conclusions

- PD-L1 expression on TCs was variable across biopsy sites in patients with lung cancer
- There was poor agreement between paired primary and metastatic biopsies regardless of the cutoff used for PD-L1 expression
  - The discrepancy in PD-L1 expression between primary tumor and metastases is consistent with the results of other studies[7-9]
  - Possible limitations of our study include the lack of controls for interobserver variability and heterogeneity of PD-L1 expression within the same biopsy
- This real-world study further highlights the heterogeneity of PD-L1 expression between paired biopsies from primary lung tumor and metastatic sites in lung cancer
- Further studies are required to uncover the mechanisms leading to differences in PD-L1 expression between the primary tumor and metastatic sites and the impact of these differences on clinical practice, including treatment selection and efficacy of PD-L1 inhibitors

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Acknowledgments

- Dako, an Agilent Technologies, Inc. company, for collaborative development of the PD-L1 IHC 28-8 pharmDx assay (Santa Clara, CA)
- Bristol-Myers Squibb Company (Princeton, NJ)
- The study was supported by Bristol-Myers Squibb Company
- Statistical analysis was conducted by Derrick Gallagher, PhD, and Timothy Ambrose, MS, of BioStat Solutions, Inc., funded by Bristol-Myers Squibb Company
- All authors contributed to and approved the presentation; writing and editorial assistance were provided by Thierry Deltheil, PhD, and Jay Rathi, MA, of Spark Medica Inc, funded by Bristol-Myers Squibb Company

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