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700 Ellicott Street | Buffalo NY, 14203

PD-L1 expression by RNA-sequencing and survival from pembrolizumab in non-small cell lung cancer (NSCLC)

PRESENTER: **MARY K. NESLINE**

Mary K. Nesline[1], Sarabjot Pabla[1], Yong Hee Lee[1], Paul DePietro[1], Amy Early[2] Roger Klein[1], Shengle Zhang[1], Jeffrey M. Conroy[1,2]

[1] OmniSeq, Inc., 700 Ellicott St., Buffalo, NY 14203, US *now part of* labcorp | Oncology
[2] Roswell Park Comprehensive Cancer Center, Elm and Carlton Streets, Buffalo, NY 14263, US

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Background

The PD-L1 immunohistochemistry companion diagnostic assay for pembrolizumab monotherapy (IHC 22C3 pharmDx) lacks clinical sensitivity, challenging immunotherapy selection for NSCLC patients with lower levels of expression.[1,2] Unlike IHC 22C3 testing, which restricts assessment of PD-L1 expression to viable tumor cells as a tumor proportion score (% TPS), mRNA next generation sequencing (RNA-seq) measures PD-L1 expression in the tumor microenvironment for both tumor and inflammatory background cells. RNA-seq previously demonstrated concordance with IHC and may be a sensitive and robust alternative testing method for multiple tumor types.[3] Here, we sought to optimize RNA-seq cutoff values for PD-L1 by RNA-seq in NSCLC and compare the clinical sensitivity to IHC.

Methods

All NSCLC patients included in the study (n=3,283) were previously tested for PD-L1 expression by both IHC 22C3 and clinically validated RNA-seq, measured as % rank (0-100) relative to a reference population based on normalized reads per million (nRPM). Patients were divided into an RNA-seq cut-off discovery cohort (n=3,168), and a test cohort (n=115) who received pembrolizumab monotherapy. In the discovery cohort, principal components analysis (PCA) was used to classify patients based on test results and explore PD-L1 cut-off values. In the test cohort, Kaplan Meier curves and a Cox proportional hazards regression models assessed overall survival (OS) hazard ratios (HR) for RNA-seq versus standard of care PD-L1 IHC cut-offs in the test cohort.

References

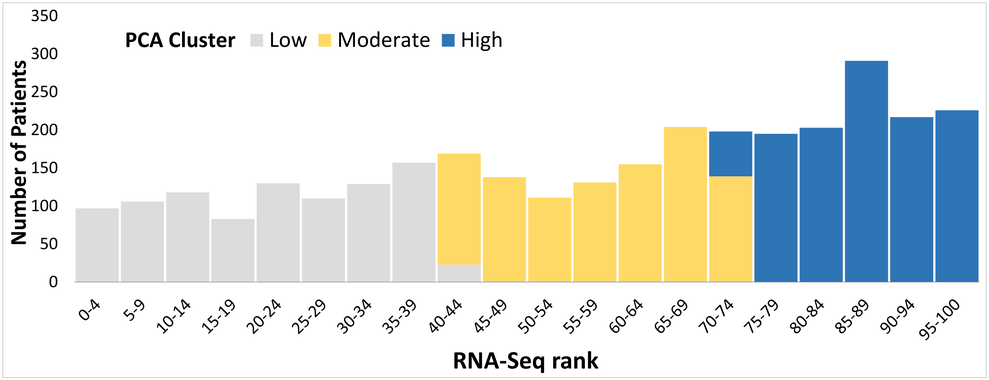
1. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project. J Thorac Oncol. 2018;13(9):1302-1311. doi:10.1016/j.jtho.2018.05.013
2. Wang M, Wang S, Trapani JA, Neeson PJ. Challenges of PD-L1 testing in non-small cell lung cancer and beyond. J Thorac Dis. 2020;12(8):4541-4548. doi:10.21037/jtd-2019-itm-010
3. Conroy JM, Pabla S, Nesline MK, et al. Next generation sequencing of PD-L1 for predicting response to immune checkpoint inhibitors. J Immunother Cancer. 2019;7(1):18. doi:10.1186/s40425-018-0489-5

Figure 1. Patient Identification

Table 1. Discovery cohort (n=3,168) principal component analysis (PCA) unsupervised patient clusters used to determine PD-L1 cut offs by RNA-seq

PCA Cluster	Over-represented	Under-represented	PD-L1 RNA-seq cut-off
High (n=966)	PD-L1 IHC (>=50% TPS) PD-L1 IHC (mean %TPS=74) PD-L1 RNA (>=75 rank) PD-L1 RNA (mean rank =84) histology=non-squamous gender=female	PD-L1 RNA (<75 rank) PD-L1 IHC (1-49% TPS) PD-L1 IHC (<1% TPS) histology=squamous gender=male	Mean rank =83.6 Median rank =87 SD=13.5 Range=24-100 **≥74 (Median rank -1 SD)**
Moderate (n=1,037)	PD-L1 IHC (1-49% TPS) PD-L1 RNA (mean rank =70) gender=female PD-L1 IHC (<1% TPS) age =40-49	PD-L1 IHC (>=50% TPS) PD-L1 IHC (mean %TPS=6.7) gender=Male age =60-69	Mean rank =69.8 Median rank =69 SD=12 Range=48-99 **41-73**
Low (n=1,165)	PD-L1 RNA (<75 rank) PD-L1 IHC (<1% TPS) gender=male PD-L1 IHC (1-49% TPS) histology=squamous	PD-L1 RNA (mean rank =26) PD-L1 RNA (>=75 rank) PD-L1 IHC (>=50% TPS) PD-L1 IHC (mean %TPS=4.6) gender=female histology=non-squamous	Mean rank = 25.6 Median rank =26 SD=14 Range=0=47 **≤40 (Median rank +1 SD)**

Figure 2. Distribution of PD-L1 RNA-seq ranks



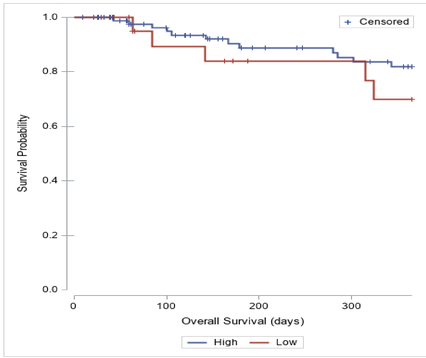
- Unsupervised PCA of the discovery cohort identified three distinct PD-L1 clusters separated by combinations of significant over- and under-representation of RNA-seq and IHC result measures and clinicopathologic characteristics from prior testing.
- Both the low and moderate clusters were significantly overrepresented by patients in the PD-L1 IHC low and negative groups.
- The moderate cluster was overrepresented by patients with moderately high PD-L1 RNA-seq ranks (median=70), while the low group was overrepresented by patients that were not PD-L1 high by RNA-seq.
- The high cluster was over-represented by patients high for PD-L1 by both IHC and RNA-seq.
- Over- and under-representation characteristics and RNA-seq rank distributions were used to derive an RNA-seq cut-off for each cluster as median rank (+/- 1SD for low and high).

Table 2. Pembrolizumab test cohort patient characteristics by PD-L1 RNA-seq cut-off

		PD-L1 RNA-Seq Cut Off			
		High (≥ 74) n=98	Moderate (41-73) n=22	Low (<40) n=6	Total n=115
PD-L1 IHC	≥50% TPS (High)	75 (0.77)	15 (0.68)	2 (0.33)	92 (0.80)
	1-49% TPS (Low)	13 (0.13)	6 (0.27)	4 (0.67)	23 (0.20)
Age (avg)		68.6	72.5	63.4	69.0
Female		46 (0.47)	13 (0.59)	4 (0.67)	63 (0.55)
Ever smoker (yes)		81 (0.83)	17 (0.77)	6 (1.00)	104 (0.90)
Histology	Non-Squamous	75 (0.77)	16 (0.73)	2 (0.33)	93 (0.81)
	Squamous	13 (0.13)	5 (0.23)	4 (0.67)	22 (0.19)
	<1	23 (0.23)	1 (0.05)	3 (0.50)	27 (0.23)
	1 to <2	55 (0.56)	16 (0.73)	3 (0.50)	74 (0.64)
	2 to <4	7 (0.07)	4 (0.18)	0 (0.00)	11 (0.10)
	Missing	3 (0.03)	0 (0.00)	0 (0.00)	3 (0.03)
	1	68 (0.69)	12 (0.55)	5 (0.83)	85 (0.74)
	2	13 (0.13)	5 (0.23)	1 (0.17)	19 (0.17)
	≥3	7 (0.07)	4 (0.18)	0 (0.00)	11 (0.10)
	Chemotherapy	15 (0.15)	8 (0.36)	1 (0.17)	24 (0.21)
	Targeted Therapy	4 (0.04)	3 (0.14)	0 (0.00)	7 (0.06)
	Immunotherapy	2 (0.02)	0 (0.00)	0 (0.00)	2 (0.02)

Figure 3. Pembrolizumab overall survival (1-year) unadjusted Kaplan Meier curves

a. PD-L1 IHC



b. PD-L1 RNA-Seq

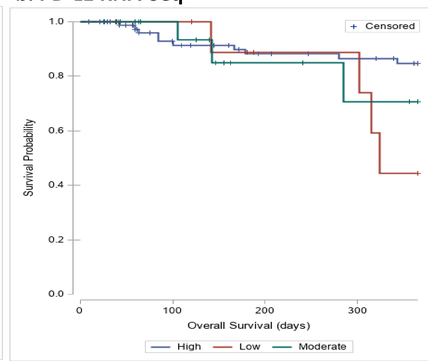


Table 3. Pembrolizumab overall survival (1-year) hazard ratios by PD-L1 marker status

PD-L1 Marker Status		Hazard Ratio (HR)	CI Low	CI High	P Value
IHC	High vs Low	0.21	0.04	1.07	0.06
RNA-Seq	High vs Moderate	0.05	0.00	0.63	0.02
	High vs Low	0.16	0.03	0.86	0.03
	Moderate vs Low	3.08	0.30	31.85	0.35

Cox regression analysis was performed for treatment HR prediction by PD-L1 IHC or PD-L1 RNA-seq status, adjusting for potential covariates: age, sex, smoking (ever/never), performance status, treatment line, prior treatments, tested tissue status (primary/metastatic) oncogenic driver mutation status, CD8 and tumor mutational burden (TMB) status.

- OS HRs were significantly improved for the RNA-seq high versus moderate (HR=0.05, CI 0.00-0.63, p=.02), and RNA-seq high versus low (HR=0.16, CI 0.03-0.86, p=.03) groups compared to IHC 22C3 high versus low groups, (HR=0.21, CI 0.04-1.07, p=.06).
- Findings were non-significant for the RNA-seq moderate versus low groups, which were limited in number. The moderate group also included a disproportionately high number of patients with poor performance status.

Conclusion

PD-L1 expression by RNA-seq demonstrated improved clinical sensitivity in predicting OS versus standard of care PD-L1 IHC in a pembrolizumab-treated NSCLC patient cohort. Additional studies with a greater number of patients are needed to further define a cutoff for patients who are “not high” by RNA-seq in the context of performance status.