

## Background & Objective

Lung cancer is one of the leading causes of cancer-related mortalities globally and especially the United States.<sup>1</sup> The level of Program Death Ligand-1 (PD-L1) IHC expression is used to select patients with non-small cell lung carcinoma (NSCLC) for immunotherapy with anti-PD1 drugs such as pembrolizumab.<sup>2</sup> PD-L1 protein expression is determined by the tumor proportion score (TPS), where a TPS score  $\geq 1\%$  is eligible for treatment.<sup>3-4</sup> Since patient stratification for anti-PD1 drugs is based on IHC alone, our purpose is to determine the overall agreement between the widely applied and well-characterized PD-L1 IHC 22C3 pharmDx and a newly developed PD-L1 IHC Assay (clone RM320) in NSCLC.

## Methods

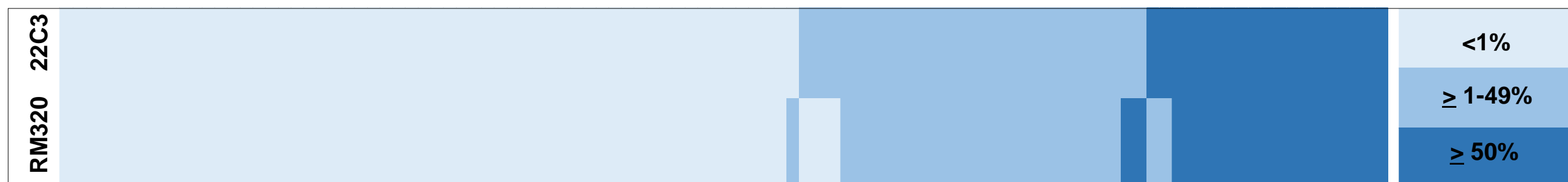
104 human formalin-fixed paraffin-embedded (FFPE) NSCLC cases in a tissue microarray were analyzed with two different IHC assays: (1) PD-L1 IHC 22C3 pharmDx, Agilent and (2) PD-L1 IHC Assay RM320, Sakura Finetek USA. Evaluation of PD-L1 IHC results was performed by a pathologist and a certified external reviewer using the Tumor Proportion score (TPS) based on the official recommended scoring guidelines and related cut-off levels at 1% and 50%.<sup>3-4</sup> Representative photomicrographs were taken with the Olympus VS200 SlideView.

## Results

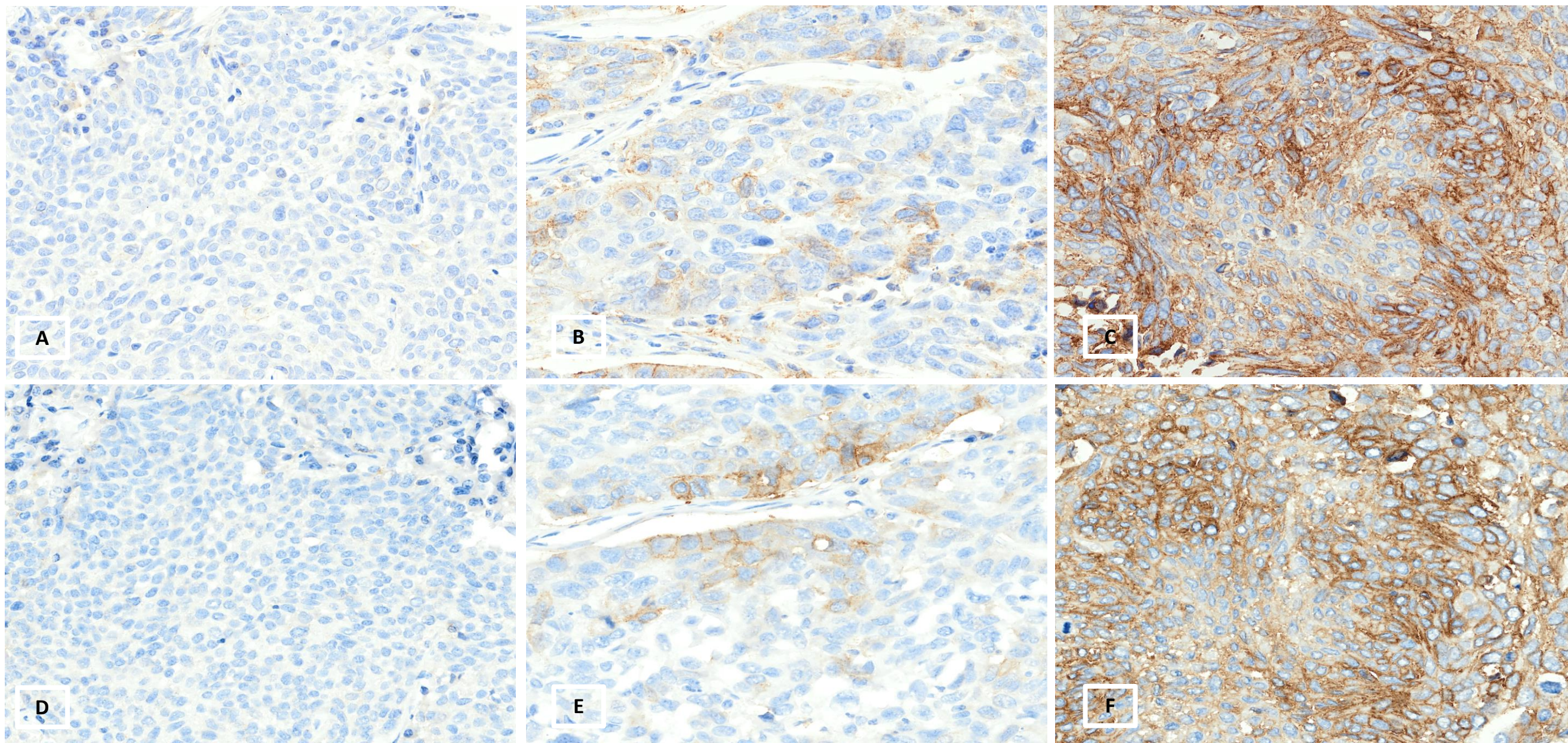
Using the PD-L1 IHC 22C3 pharmDx as a benchmark, 57.7% of the NSCLC cases (n=60) were classified as TPS-negative (<1%), 24.0% as TPS-low ( $\geq 1-49\%$ , n=25) and 18.3% as TPS-high ( $\geq 50\%$ , n=19). An overall agreement of 92% between the two methods was observed.

Cutoff	22C3		RM320	
	PPA	NPA	PPA	NPA
$\geq 1\%$	100% (44/44)	100% (60/60)	97.7% (43/44)	95.0% (57/60)
$\geq 50\%$	100% (19/19)	100% (85/85)	89.5% (17/19)	98% (83/85)

**Table 1. Agreement for PD-L1 Expression:** Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated when comparing PD-L1 Clone RM320 to PD-L1 IHC 22C3 pharmDx using a 2x2 table analysis. \*PPA and NPA values for RM320 were updated from the abstract.

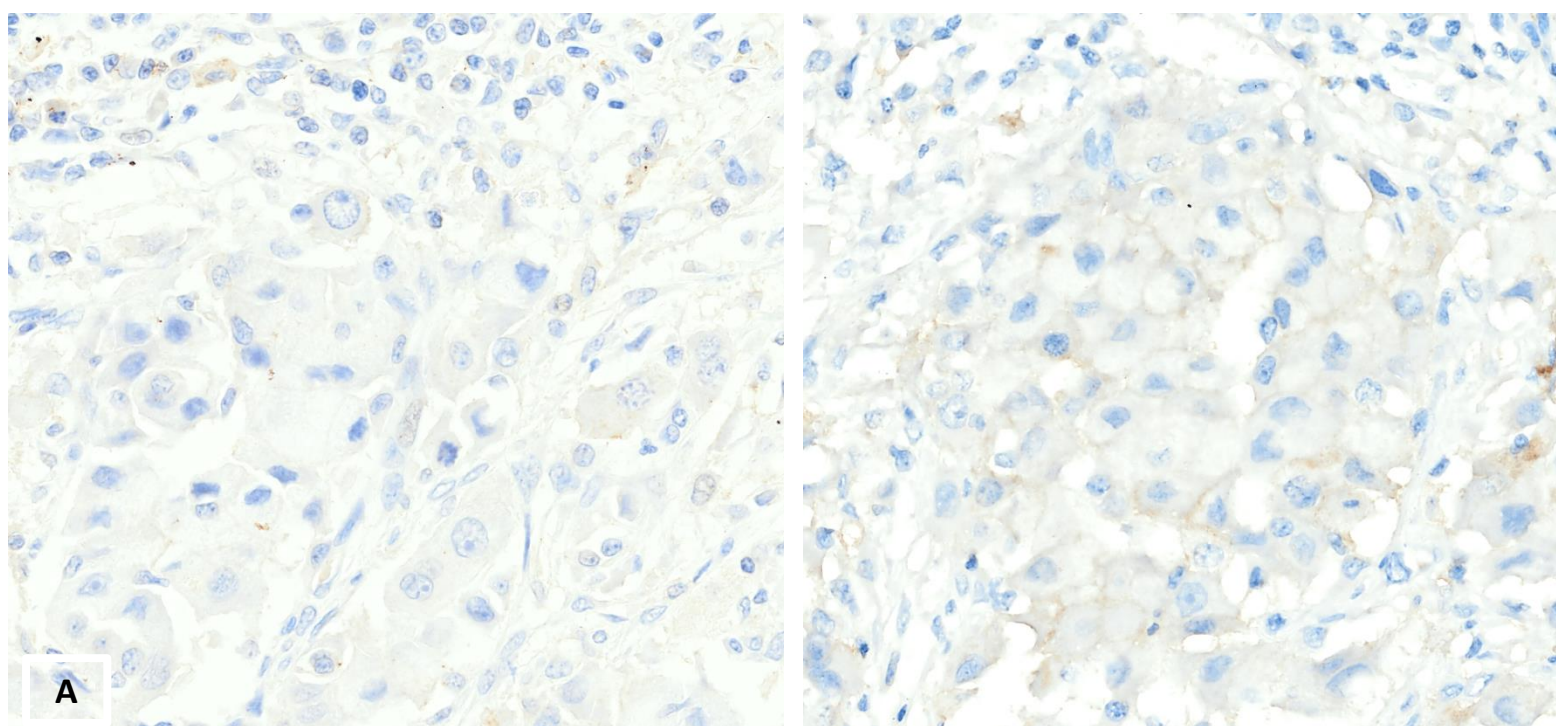


**Fig 1. Distribution of PD-L1 scores:** Map that depicts PD-L1 TPS scores for NSCLC (n=104)



**Fig 2. PD-L1 IHC Expression with TPS score**

Representative photomicrographs for PD-L1 IHC 22C3 pharmDx exhibiting a PD-L1 TPS-negative score (A, IHC 200x), TPS-low score (B, IHC 200x) and TPS-high score (C, IHC 400x) as well as PD-L1 IHC Assay using clone RM320 exhibiting a PD-L1 TPS-negative score (D, IHC 200x), TPS-low score (E, IHC 200x) and TPS-high score (F, IHC 400x).



**Fig 3. PD-L1 IHC Expression with TPS Negative and TPS Low score**

Representative photomicrographs for PD-L1 IHC 22C3 pharmDx exhibiting a PD-L1 TPS-negative score (A, IHC 200x) and PD-L1 IHC Assay using clone RM320 exhibiting a PD-L1 TPS-low score (B, IHC 200x)

## Conclusion

- A high overall agreement for PD-L1 expression was found with IHC PD-L1 assay with clone RM320 using the applied cut-off values and guidelines for TPS in NSCLC when compared to PD-L1 IHC 22C3 pharmDx.
- More studies using NSCLC cases with positive PD-L1 expression levels are required to further evaluate the overall agreement of the PD-L1 IHC assay developed on the Tissue-Tek Genie<sup>®</sup> Advanced Staining System when compared to PD-L1 IHC 22C3 pharmDx using the TPS system.

**Disclaimer:** This study does not promote any *in-vitro* diagnostic use.

## References:

- Siegel RL et al. Cancer statistics, 2023. CA Cancer J Clin. 2023 Jan;73(1):17-48.
- Duma N et al. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc. 2019 Aug;94(8):1623-1640
- de Castro G Jr et al. Five-Year Outcomes With Pembrolizumab Versus Chemotherapy as First-Line Therapy in Patients With Non-Small-Cell Lung Cancer and Programmed Death Ligand-1 Tumor Proportion score  $\geq 1\%$  in the KEYNOTE-042 Study. J Clin Oncol. 2023 Apr 10;41(11):1986-1991.
- Reck M, Rodríguez-Abreu D, Robinson AG, et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. J Clin Oncol 2019;37:537-46.

## Contact information:

Aalborg University Hospital P.O.Box 561 DK-9100 Aalborg, Denmark | T: +45 9766 6567 | E: sn@rn.dk

Sakura Finetek USA, Inc. | 1750 West 214th Street, Torrance, CA 90501, USA | T: +1 310 972 7800/+1 800 725 8723 | F: +1 310 972 7888 | E: dwang@sakuraus.com

