

Supplemental Material:

Table 1: Description of the cohort from which tissues* were selected

Characteristic	Number of Patients	Percentage of Patients
All Patients	90	100%
Age at Diagnosis		
<70	33	37%
≥70	57	63%
Sex		
Male	42	47%
Female	48	53%
Histotype**		
Adenocarcinoma	45	50%
Squamous cell	45	50%
Stage		
I	29	32%
II	31	34%
III-IV	13	14%
N/A	17	19%
Tumor size, cm		
<2	12	13%
2-5	62	69%
>5	5	6%
N/A	11	12%
Lymph node status		
Negative	59	66%
Positive	21	23%
N/A	10	11%

*All tissues used in the study were from resection specimens. No biopsy tissue or FNA material was used.

**No systematic differences were assessed between squamous and adenocarcinoma in this study

Table 2: Comparison of SP142 protocol done for this study (XT(Mayo)) vs the current FDA approved protocol (Ultra/Ventana).

Protocol Parameter	Ultra (Ventana)	XT (Mayo)
Deparaffinization	Selected	Selected
Cell Conditioning	CC1 Cell Conditioning 48 minutes	CC1 Cell Conditioning 64 minutes
Pre- primary antibody peroxidase	Selected	Selected
Primary Antibody	VENTANA PD L1 (SP142)	Spring PD-L1 Clone SP142
OptiView HQ Linker	8 minutes (default)	8 minutes (default)
OptiView Amplification	8 minutes (default)	8 minutes (default)
Amplifier and Amplification	Selected	Selected
Amplifier and Amplification H2O2	8 minutes	4 min
Amplification Multimer	8 minutes	4 min
Counterstain	Hematoxylin II, 4 minutes	Hematoxylin II, 8 minutes
Post Counterstain	Bluing Reagent, 4 minutes	Bluing Reagent, 4 minutes

Table 3: The protocol for the LDT assay for PD-L1 using E1L3N on the Leica Bond platform

Immunohistochemistry protocol for PD-L1 E1L3N staining:

Staining was performed on the Bond RX automated staining platform. Slides were processed in 4 batches, at each staining run the PD-L1 specific index tissue microarray (TMA) YTMA 337 was included for quality control and reproducibility purposes. Slides were baked at 60 degree Celsius for 20 minutes on the automated staining platform, then deparaffinized using the Leica Bond Dewax solution and rehydrated with Alcohol and Bond Wash Solution according to the Bond “Bake and Dewax” protocol. Antigen retrieval was performed using the Leica Bond Epitope Retrieval 2 (pH 9) for 20 minutes at 97 degree Celsius, followed by incubation with the Bond Peroxide Block for 5 minutes. Slides were then incubated with the primary PD-L1 rabbit monoclonal antibody from Cell Signaling Technology, clone E1L3N (CST #13684), at a concentration of 3.3 ug/ml, followed by signal amplification and visualization using the Leica Bond Polymer Refine Detection Kit (catalogue number: DS9800). Operating parameters for application of the detection system reagents were followed as suggested and incorporated into the software by Leica Biosystems. Finally slides were dehydrated and cover-slipped.

Table 4: The unified scoring system* for PD-L1

For Tumor Cells

Category	Range	Drugs Justifying Category Cut-point
A	Negative or 0%	All drugs
B	1% to 4%	Nivolumab and Pembrolizumab
C	5% to 9%	Nivolumab and Atezolizumab
D	10% to 24%	Nivolumab
E	25% to 49%	Durvalumab
F	50% or more	Pembrolizumab and Atezolizumab

For Immune Cells

Category	Range	Drugs Justifying Category Cut-point
A	Negative or 0%	All drugs
B	1% to 9%	Atezolizumab (IC1 and IC2)
C	10% or more	Avelumab and Atezolizumab

*Note that this system is designed to include all cut-points included in any current assay for the 4 drugs in trials at the time of the study design. The system was designed to categorize the percentages for optimal statistical comparison.

Table 5: Intraclass Correlation Coefficient (ICC) for the Antibodies and Platforms

ICC for Tumor (4 antibodies)	ICC for Tumor (3 antibodies)	ICC for Immune Cells (4 antibodies)	ICC for Immune Cells (3 antibodies)
0.813	0.971	0.277	0.804