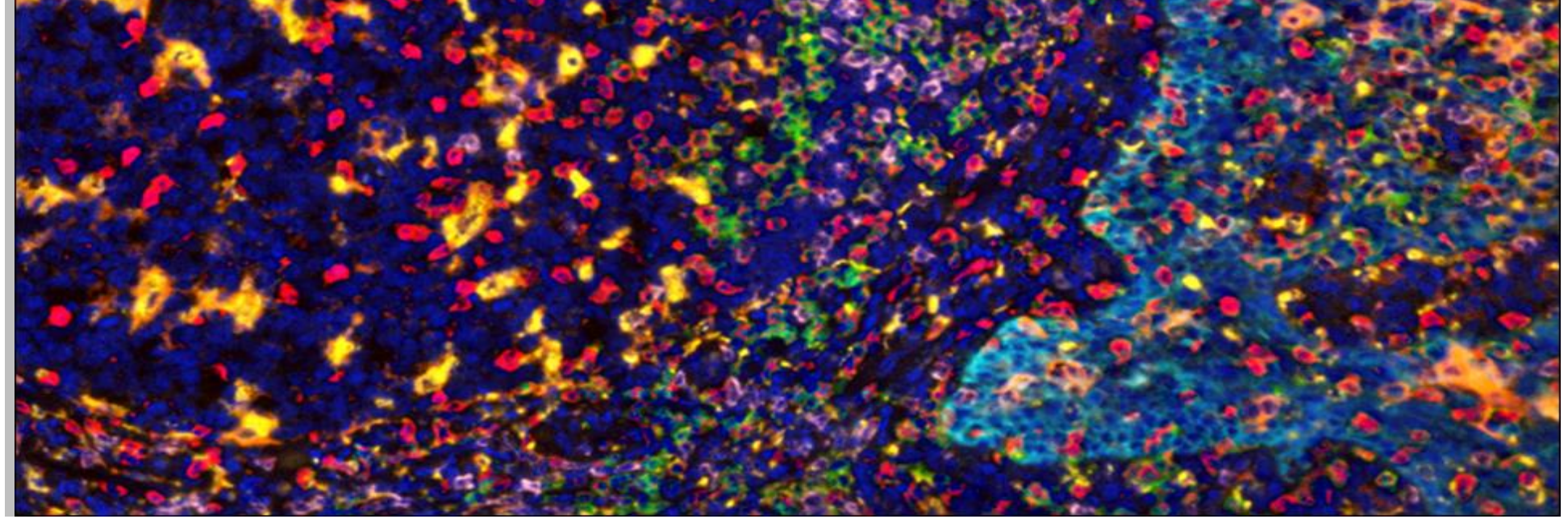


Papel del Patólogo en la Identificación de Marcadores Predictivos en Immunoterapia en Cancer de Pulmón

XXIV Congress Sociedad Chilena de Anatomia Patológica (SCHAP) November 11-13, 2020, Virtual Meeting, Santiago, Chile

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Ignacio I. Wistuba, M.D

Division Head ad interim, Division of Pathology and Laboratory Medicine.
Professor and Chair, Department of Translational Molecular Pathology
Co-Director, Khalifa Institute for Personalized Cancer Therapy (IPCT)
The University of Texas MD Anderson Cancer Center, Houston, TX.

Disclosures

- **Advisory Board:** Genentech/Roche, Bayer, Bristol-Myers Squibb, Astra Zeneca/Medimmune, Pfizer, HTG Molecular, Asuragen, Merck, GlaxoSmithKline, Guardant Health, Oncocyte, Flame, and MSD.
- **Speaker**: Medscape, MSD, Genentech/Roche, Platform Health, Pfizer, AstraZeneca, Merck
- **Research support**: Genentech, Oncoplex, HTG Molecular, DepArray, Merck, Bristol-Myers Squibb, Medimmune, Adaptive, Adaptimmune, EMD Serono, Pfizer, Takeda, Amgen, Karus, Johnson & Johnson, Bayer, Iovance, 4D, Novartis, and Akoya.

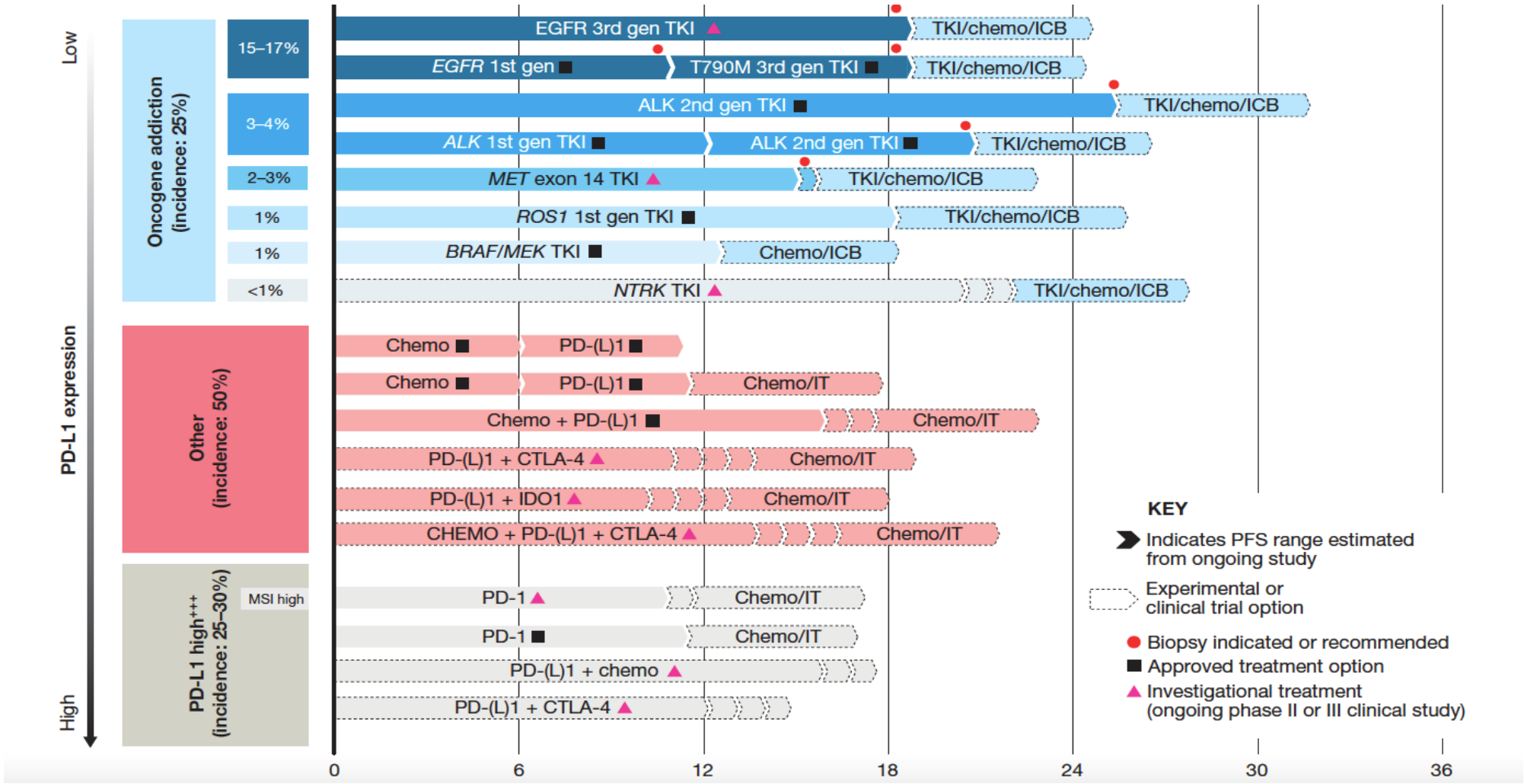
Paradigms in Lung Cancer Molecular Pathology - 2020

- Histology subtyping of lung cancer is clinically important
- Multiple clinically relevant molecular abnormalities ("driver alterations") have been detected and can be used to direct targeted therapy and improve patients' outcomes
- Liquid biopsy represents an alternative option for molecular testing, and potentially, early diagnosis
- Immunotherapy-related biomarkers are part of diagnosis: *PD-L1 IHC, microsatellite instability (MSI), and Tumor Mutational Burden (TMB)*
  - However, additional biomarkers are needed.

Pathology and Immunotherapy in Lung Cancer

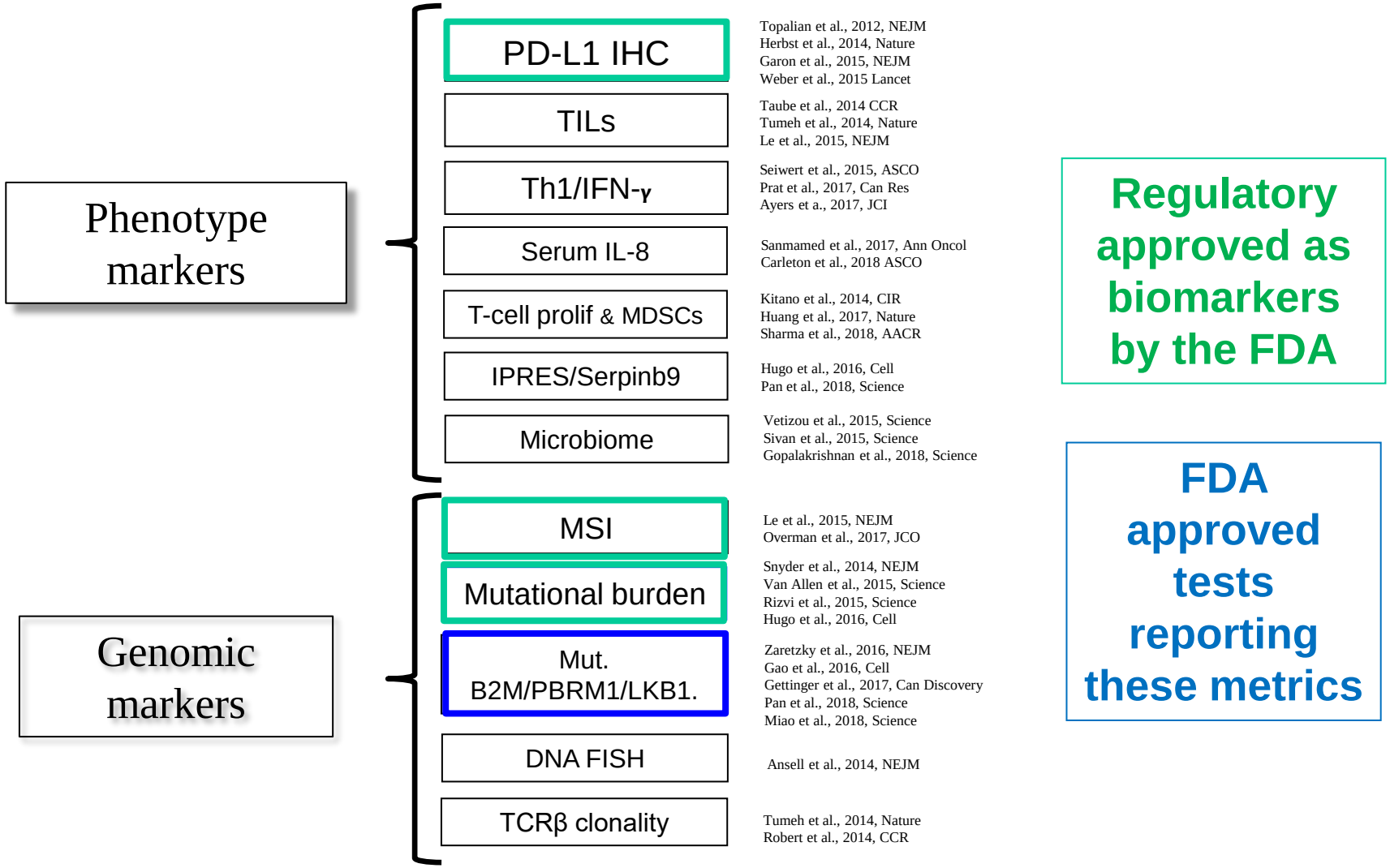
- PD-L1 immunohistochemical expression
- New immune-check points assessment and immune infiltration signatures
- Major pathological response assessment in immunotherapy neoadjuvant trials in lung cancer

Lung Cancer Targeted Therapy Landscape



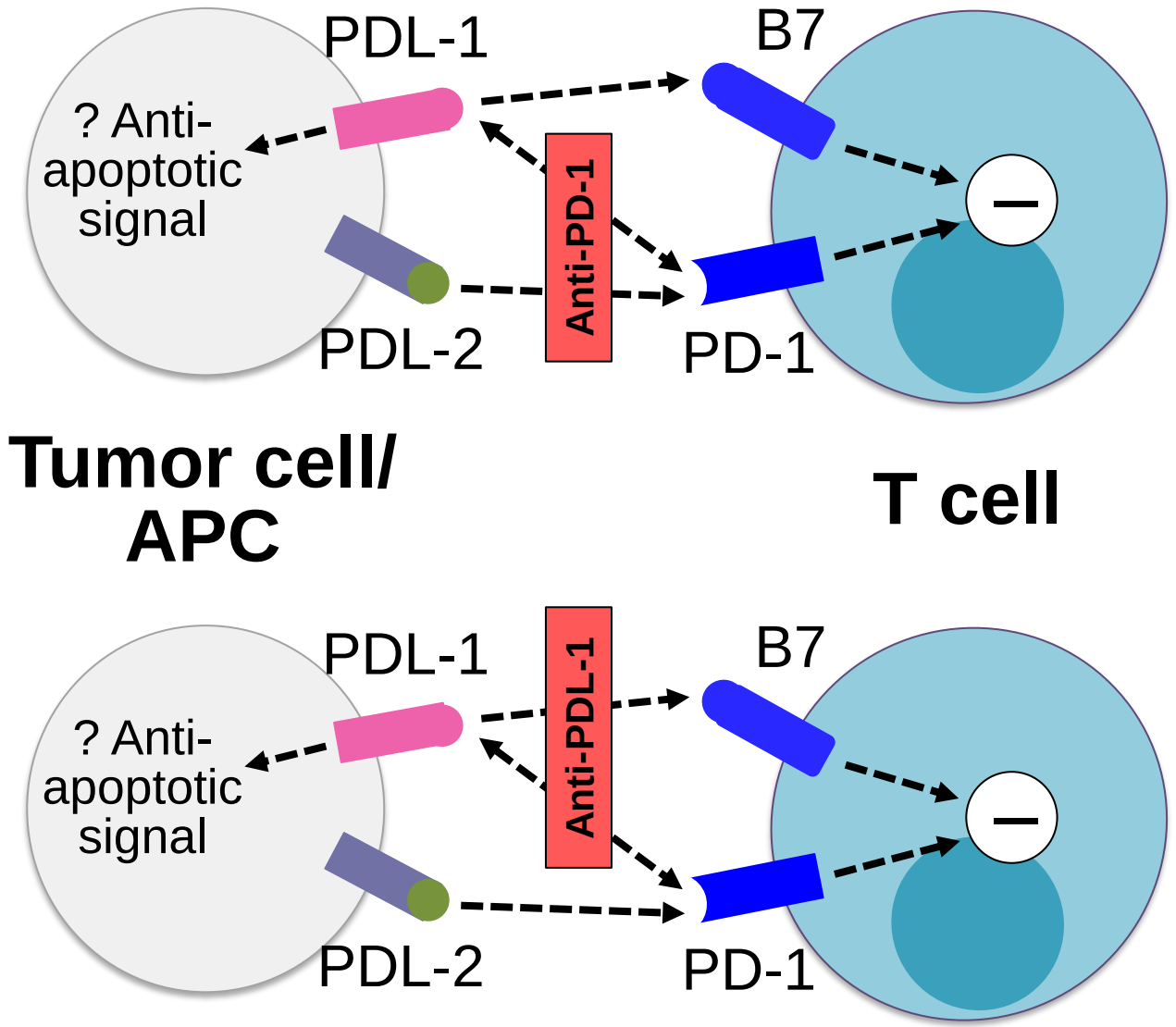
R S Herbst et al. Nature 553, 446–454 (2018)

Developing Markers for Immunotherapy



Slide Courtesy of Dr. Kurt Schalper, Yale Cancer Center

PD-1 vs. PDL-1 Blockade



PD-L1 IHC Testing

- Four separate PD-L1 assays have been developed and approved, and 5th is in development, in association with the clinical development of the different FDA-approved anti PD-(L) therapies in cancer.
- Challenges:
  - Different antibodies, detection systems, and staining instrument.
  - Scoring measure (malignant cells, immune cells) and threshold for positivity vary:
    - CPS: cell proportion score; TPS: tumor proportion score; IC: immune cells; etc.
  - Non-approved antibodies have been developed as laboratory-derived tests (LDTs).
  - The use of assays as companion and complementary.
- Several studies have assessed the analytic and technical concordance between the on-marker, multi-institutional and inter-clone assays/antibodies.
  - However, the analysis did not include patients treated with anti-PD-(L)1 therapies.

PD-L1 IHC Tests Available

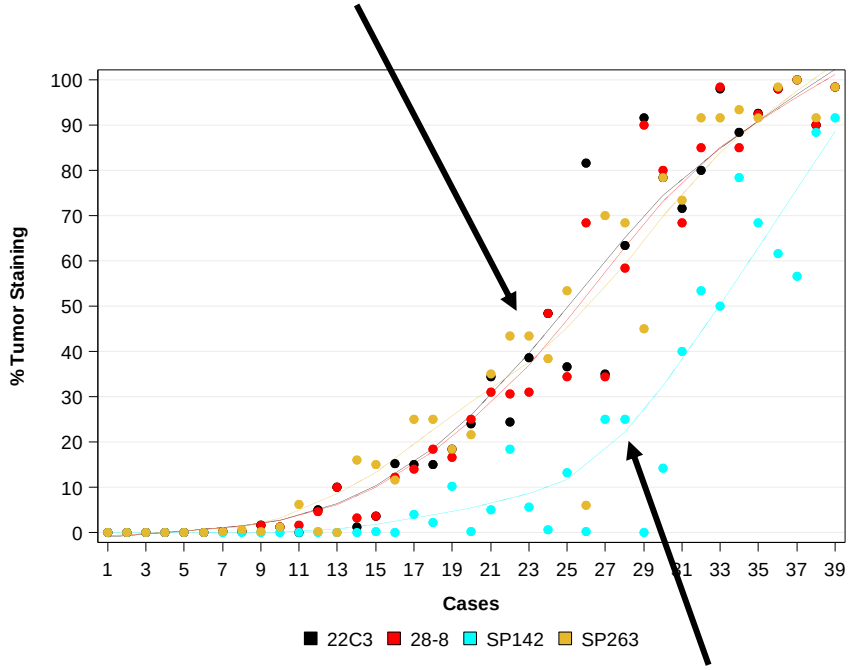
Commercial Assays	Autostainer	Detection system	Epitope (origin)
22C3 DAKO PharmDX	DAKO 48Link	Envision FLEX	Extracellular (mouse)
28.8 DAKO PharmDX	DAKO 48Link	Envision FLEX	Extracellular (rabbit)
PD-L1 (SP142) Ventana	Benchmark Ventana	Optiview + amplification	Intracytoplasmic (rabbit)
PD-L1 (SP263) Ventana	Benchmark Ventana	Optiview	Intracytoplasmic (rabbit)
73.10 DAKO PharmDX	DAKO 48Link	Envision FLEX	Intracytoplasmic (rabbit)
Concentrated Abs			
E1L3N Cell Signaling Technology (Rabbit monclonal) 22C3 DAKO (Mouse monclonal) 22C3 Abcam (Rabbit monoclonal QR1 Quartett (Rabbit monoclonal)			

Courtesy Dr. Sylvie Lantuejoul, Centre Léon Bérard, Lyon

Two PD-L1 IHC Expression Studies in Lung Cancer

Blue-Print Study (n=40)

CDx Dako: 22c3 and 28-8
CDx Ventana: SP263

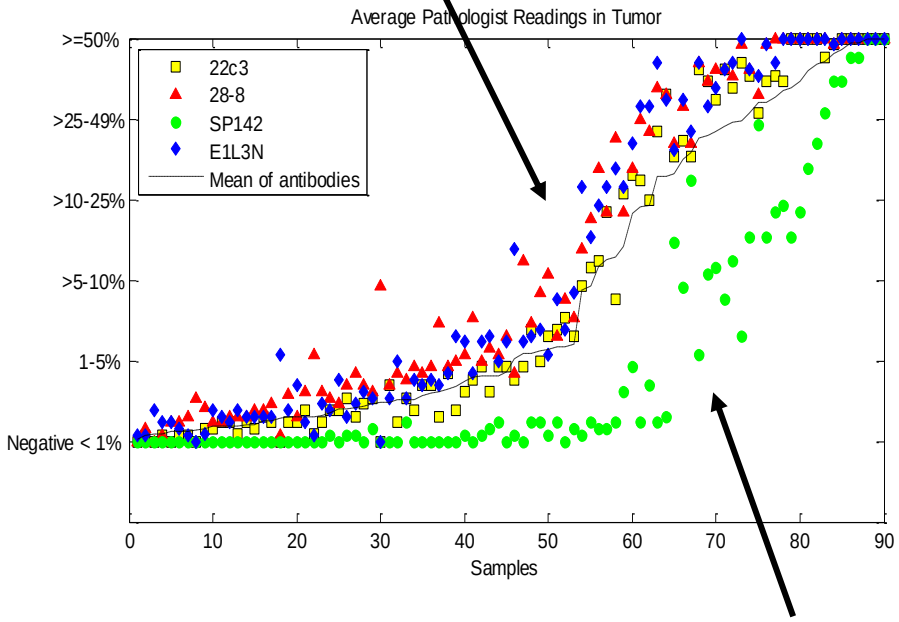


CDx Ventana: SP142

F. Hirsch et al, J Thoracic Oncol, 2016

NCCN Study (n=90)

CDx Dako: 22c3 and 28-8
LDT Cell Signaling: E1LN3



LDT Ventana: SP142

D. Rimm, JAMA Oncol, 2015

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Approved PD-L1 IHC Assays

Companion **Complementary**

Table 3. Approved Programmed Death Ligand-1 (PD-L1) Immunohistochemistry (IHC) Assays

Assay Name	Indication	Drug	PD-L1 Scoring Measure	Indication-Specific Cutoff	FDA Classification	EMA Classification
PD-L1 IHC 22C3 PharmDx	NSCLC, stage III, first line	Pembrolizumab	TPS	TPS ≥ 1%	PMA: companion	CE mark
	NSCLC, metastatic, second line		TPS	TPS ≥ 1%		
	Gastric or GEJ adenocarcinoma, recurrent locally advanced or metastatic		CPS	CPS ≥ 1		
	Cervical cancer, recurrent or metastatic			CPS ≥ 1		
	UC, locally advanced or metastatic			CPS ≥ 10		
	Esophageal squamous cell carcinoma, recurrent, locally advanced or metastatic			CPS ≥ 10		
PD-L1 IHC 28-8 PharmDx	Nonsquamous NSCLC	Nivolumab	% evaluable tumor cells exhibiting partial or complete membrane staining at any intensity	≥1%, ≥5%, ≥10%	PMA: complementary	CE mark
	Melanoma			≥1%		
	SCCHN			≥1%		
	UC			≥1%		
Ventana PD-L1(SP142) assay	UC—first line, cisplatin ineligible	Atezolizumab	IC	≥5% IC	PMA: companion	CE mark
	NSCLC		TC, IC	≥50% TC or ≥10% IC	PMA: complementary	
	TNBC	Atezolizumab	IC	≥1% IC	PMA: companion	
Ventana PD-L1 (SP263) assay	UC	Durvalumab	% tumor cells with any membrane staining above background, IC+	≥25% of tumor cells exhibit membrane staining; or ICP > 1% and IC+ ≥ 25%; or ICP = 1% and IC+ = 100%	PMA: complementary	CE mark
	NSCLC—first line	Pembrolizumab	TC	≥50%	NA	CE mark
	NSCLC—second line			≥1%		
	NSCLC—second line	Nivolumab		>1%, >5%, >10%		

Abbreviations: CPS, combined positive score, the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100; CE, European conformity; EMA, European Medicines Agency; FDA, US Food & Drug Administration; GEJ, gastroesophageal junction; IC, the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells of any intensity; IC+, the percentage of tumor-associated immune cells with staining at any intensity above background; ICP, immune cells present, the percentage of tumor area occupied by any tumor-associated immune cells; NA, Not applicable; NSCLC, non–small cell lung carcinoma; PMA, premarket approval; SCCHN, squamous cell carcinoma of the head and neck; TC, percentage of tumor cells with PD-L1 membrane staining at any intensity above background staining as noted on the corresponding negative control; TNBC, triple-negative breast cancer; TPS, tumor proportion score, the percentage of viable tumor cells showing partial or complete membrane staining at any intensity; UC, urothelial carcinoma.

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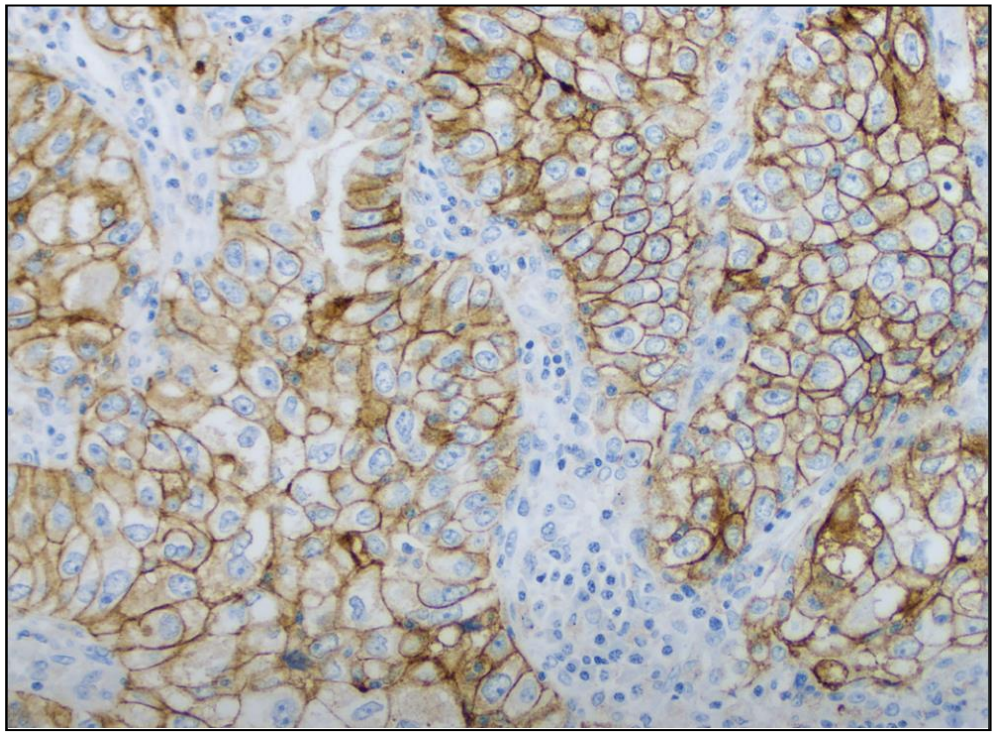
Eric. E Walk, Arch Pathol Lab Med, DOI 10.5858/arpa.2018-0584CP

PD-L1 IHC Interpretation in Lung Cancer



www.agilent.com
22C3 PharmDx interpretation manual

- Scoring of % tumor cells with membranous labeling, complete or partial ("TPS/tumor proportion score")
- Cut-points
  - **≥50%** → Pembrolizumab in **1st line**
  - **≥1%** → Pembrolizimab in **2nd line** (after chemo)
  - 0 → no Pembro
- A minimum of **100 viable tumor cells** must be present for the specimen to be considered adequate for PD-L1 evaluation.

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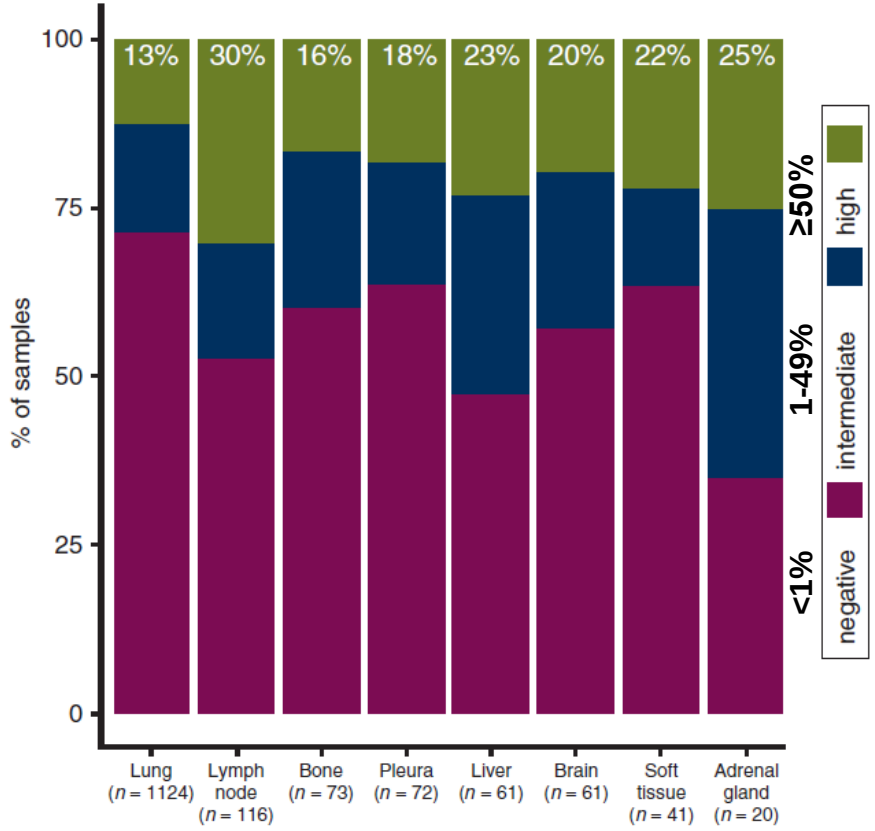
Slide Courtesy of Natasha Rekhtman, Memorial Sloan Kettering, NYC

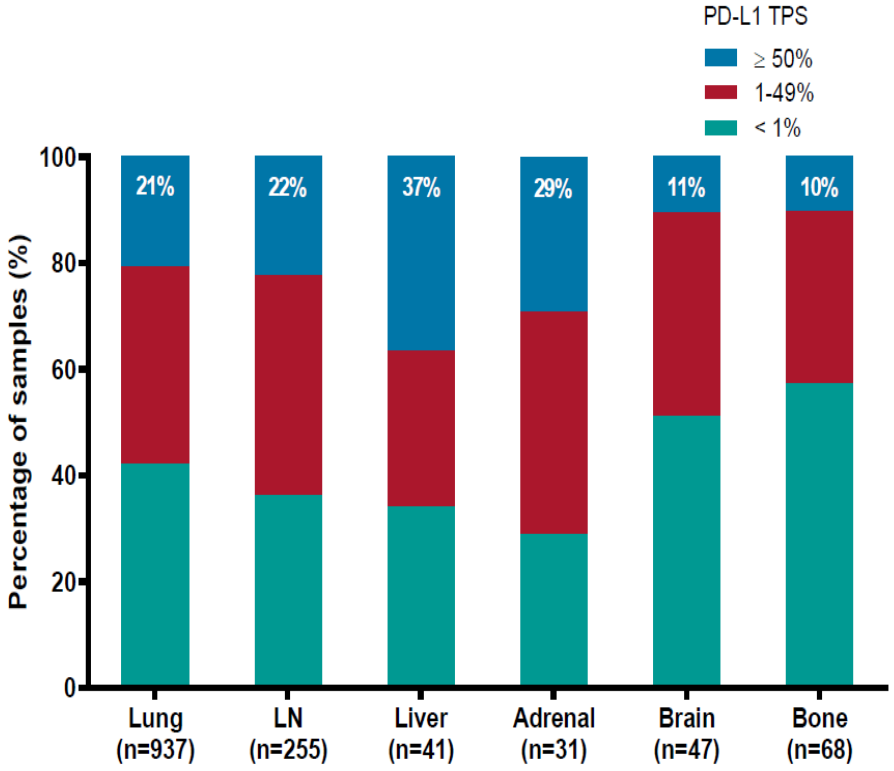
PD-L1 IHC Expression Intertumoral Heterogeneity and Patients Outcome

N=1,586 patients with lung adenocarcinomas treated with anti-PD-(L)1 blockade



A. J. Schoenfeld et al, Annals of Oncol, 2020

N=1,398 patients with NSCLC treated with anti-PD-(L)1 blockade

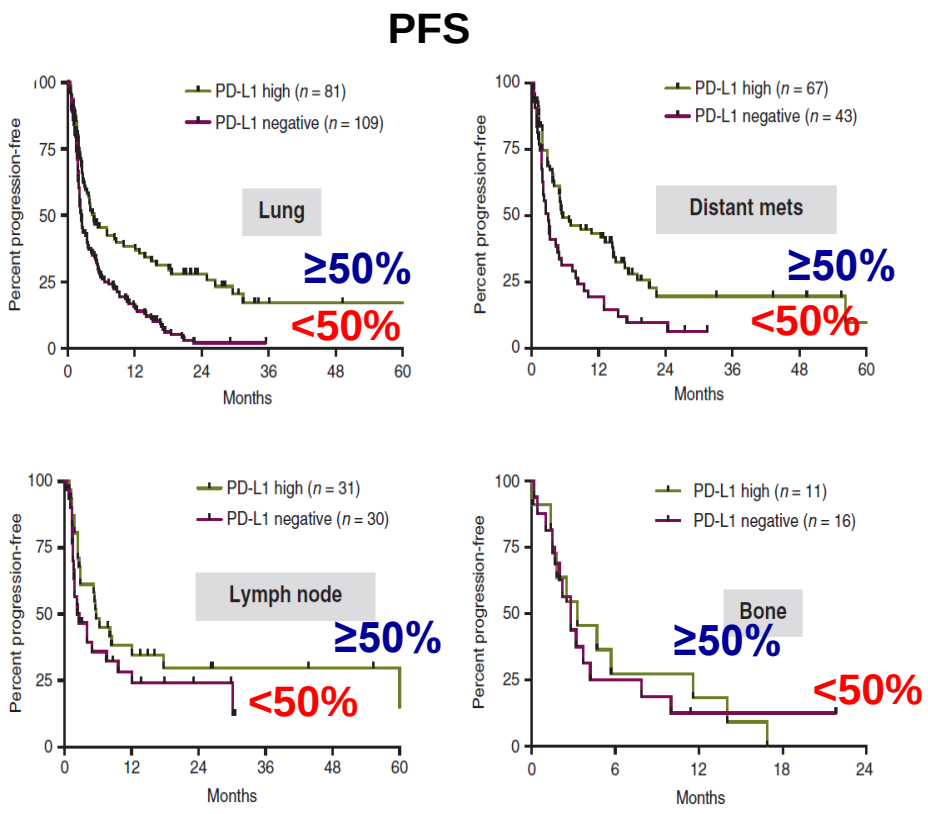


L. Hong, Journal of Thoracic Oncology, 2020

Cancer

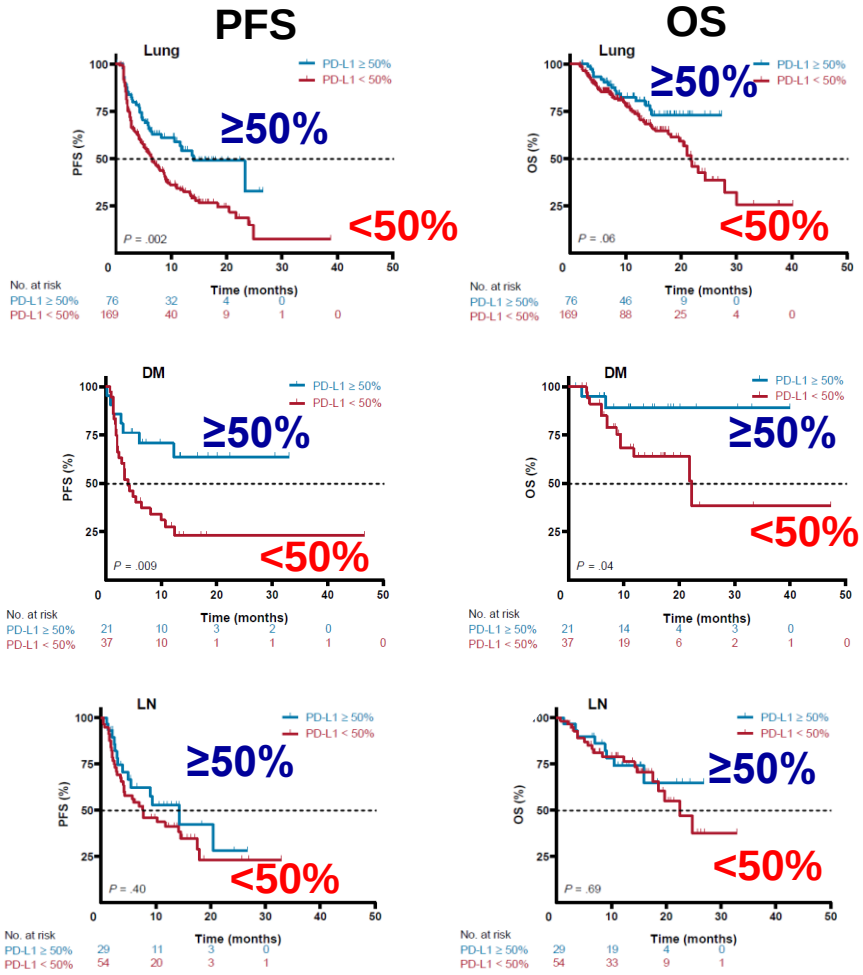
PD-L1 IHC Expression Inter-tumoral Heterogeneity and Patients Outcome

N=1,586 patients with lung adenocarcinomas treated with anti-PD-(L)1 blockade



A. J. Schoenfeld et al, Annals of Oncol, 2020

N=1,398 patients with NSCLC treated with anti-PD-(L)1 blockade



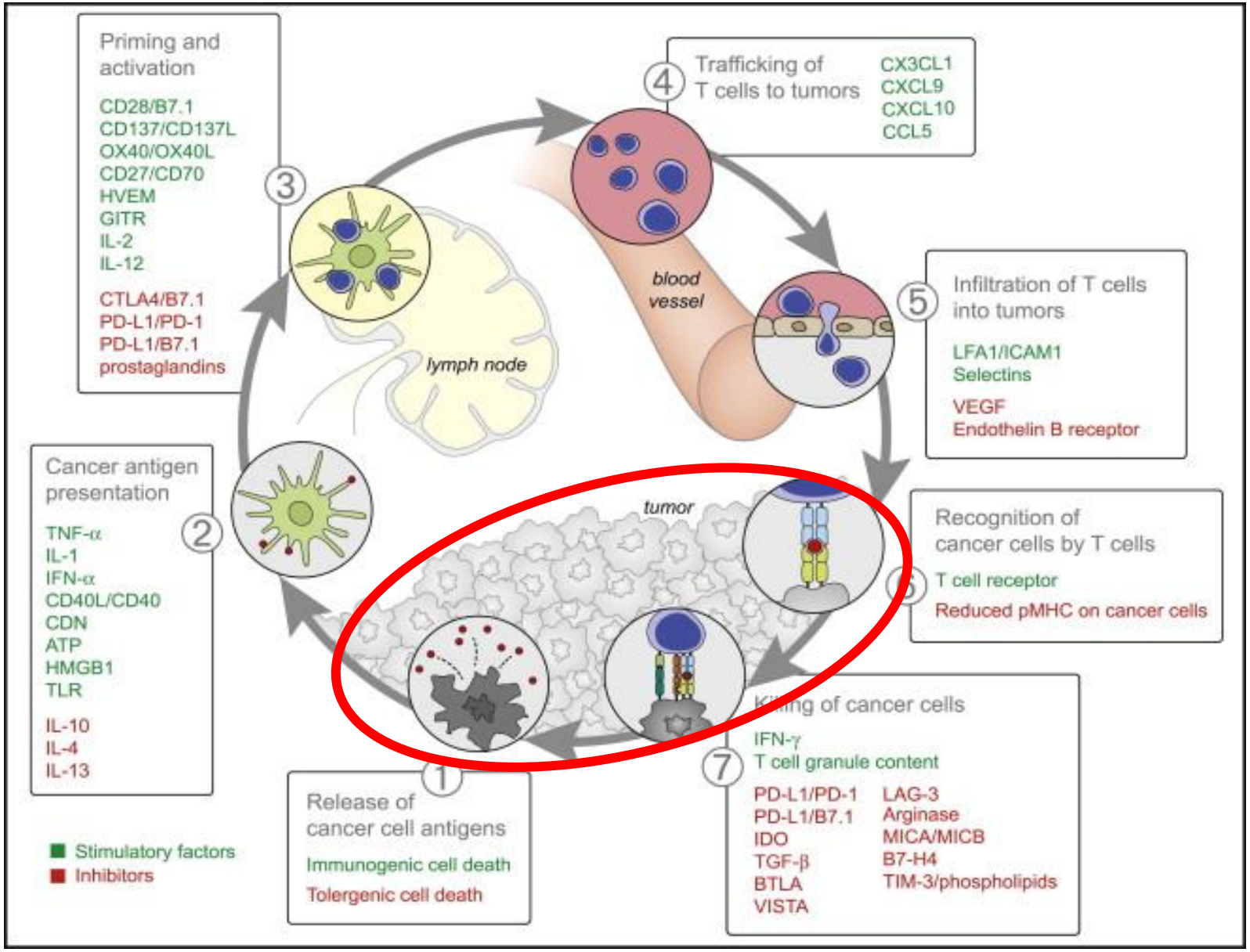
L. Hong, Journal of Thoracic Oncology, 2020

Moving Beyond PD-L1 as a Biomarker for Guiding Immunotherapy

- **Immune Response:**
  - Expression of new immune checkpoint targets
  - Immune cell infiltrates (IHC and multiplex approaches)
  - Gene expression signatures (mRNA assays)

- **Genomic:**
  - Microsatellite instability (MSI) High
  - Tumor Mutational Burden (TMB) for combination immune oncology therapies
  - Genomic predictors of response to therapy:
    - *STK1/LKB1* loss
    - Genes involved in inactivation of INF-γ pathway (mechanisms of resistance, *JAK* gene)

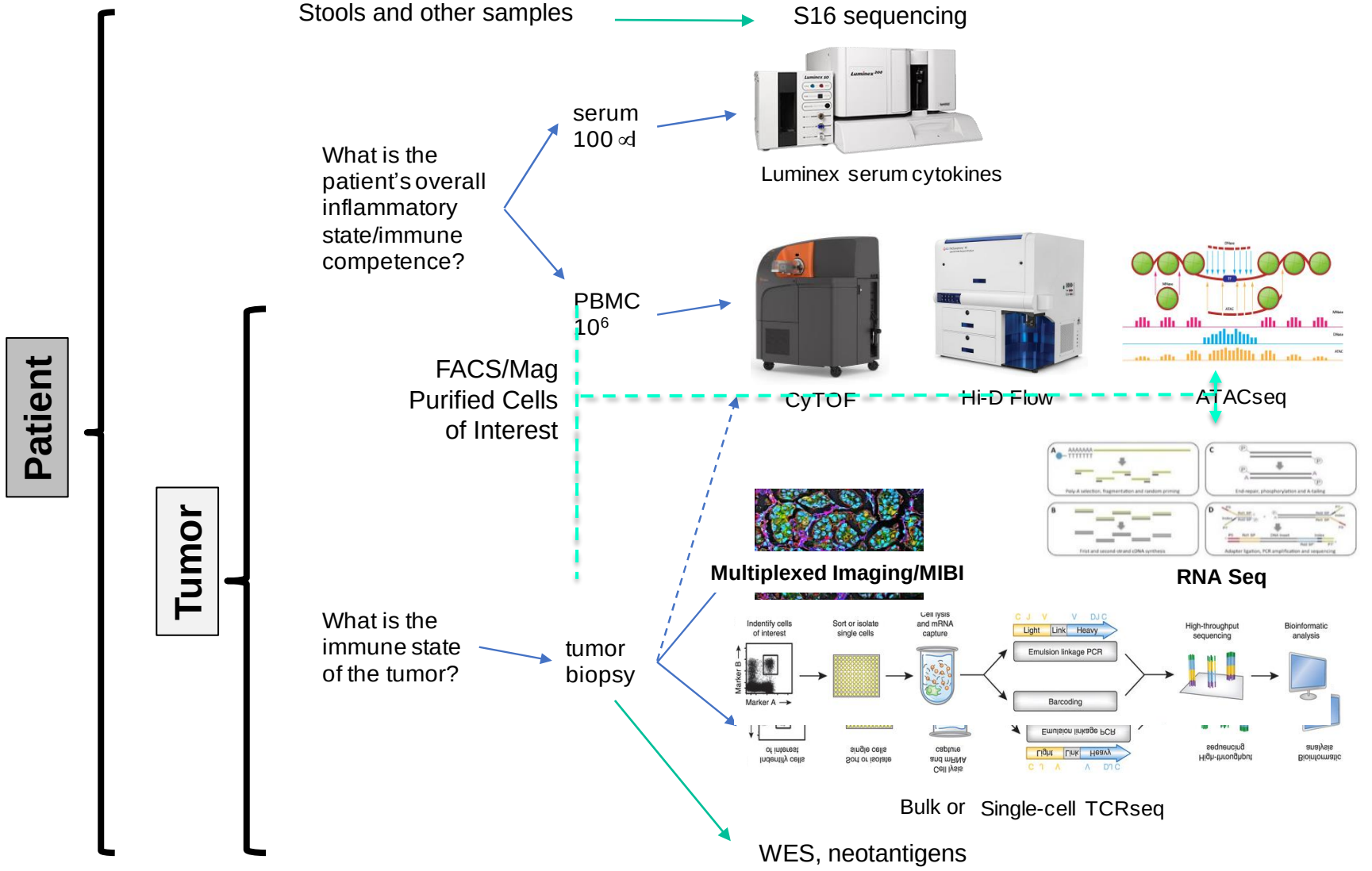
Cancer Immunity Cycle and Immune Checkpoints



Cancer

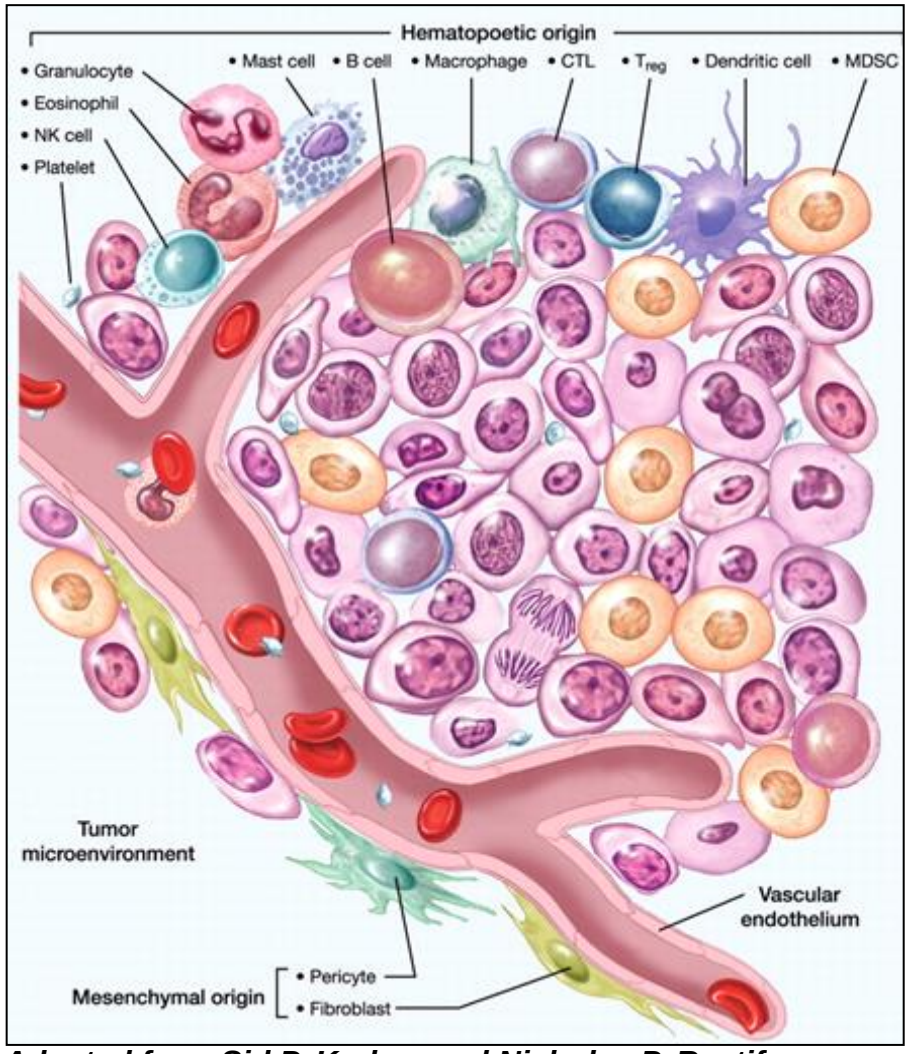
Adapted from Chen S, et al, Immunity, 2013

Immune-profiling Workflows



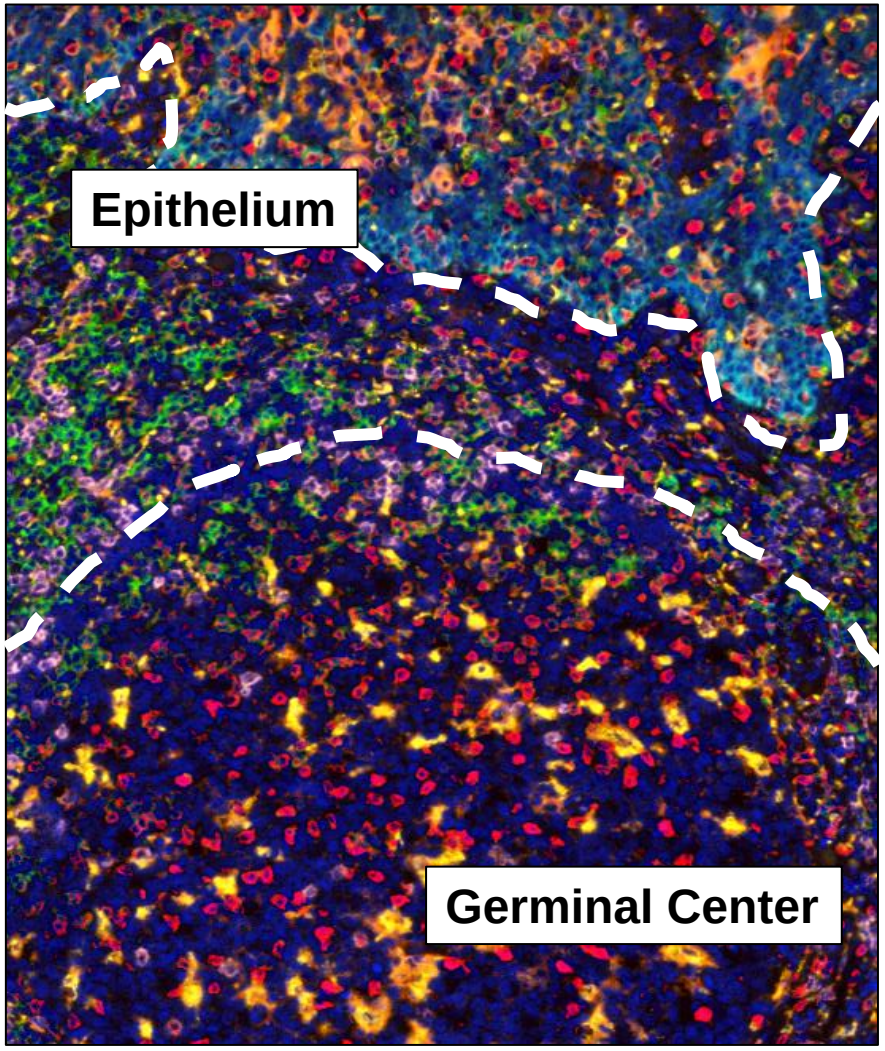
Modified from slide courtesy Holden Maecker, Stanford University CIMAC

Assessment of Immune Cells in Tumor Tissues



Adapted from Sid P. Kerkar, and Nicholas P. Restifo Cancer Res 2012;72:3125-3130

Tonsil



PD-L1 CD3 CD8 CD4 CD68 DAPI AE1/AE3

Immunopathology Laboratory

Translational Molecular Pathology – MD Anderson Cancer Center

Automated IHC/IF

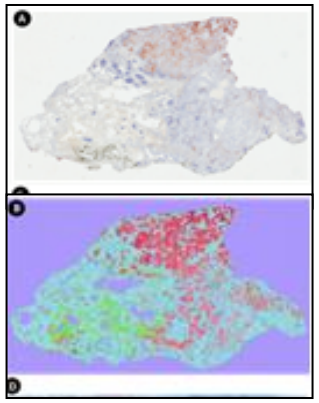
Leica™

Digital Pathology

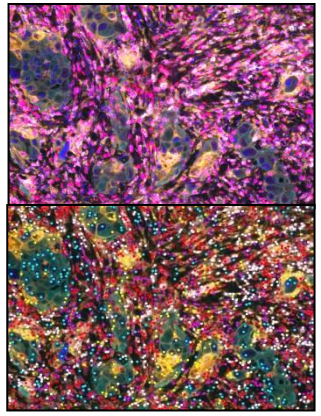
Aperio AT2™

Vectra/Polaris™

Image Analysis

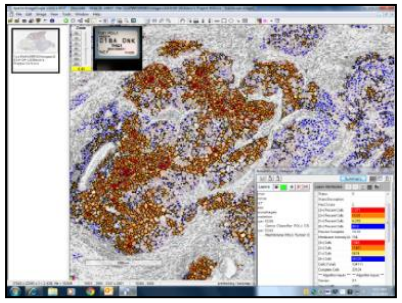


Aperio/Genie™

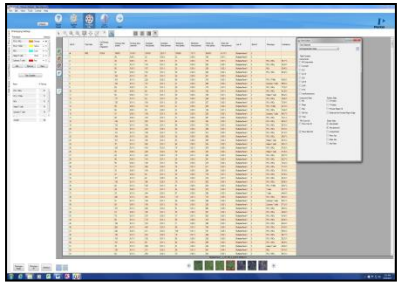


Vectra/Inform™

Data Analysis



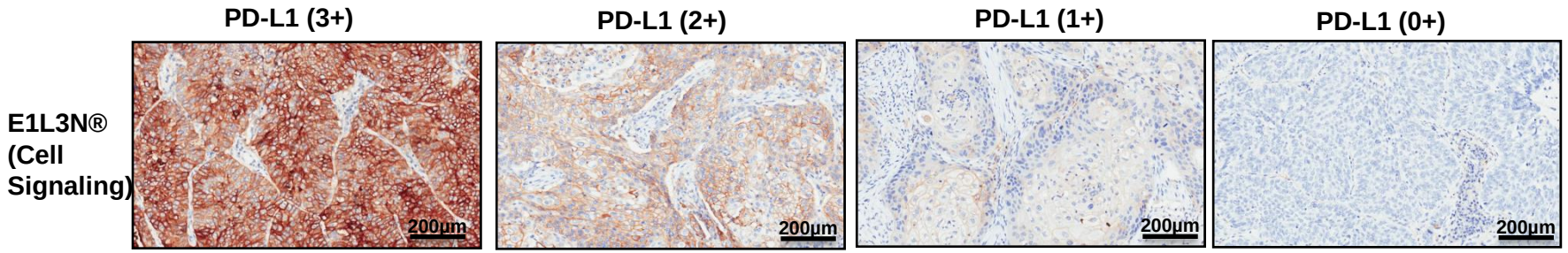
Genie™



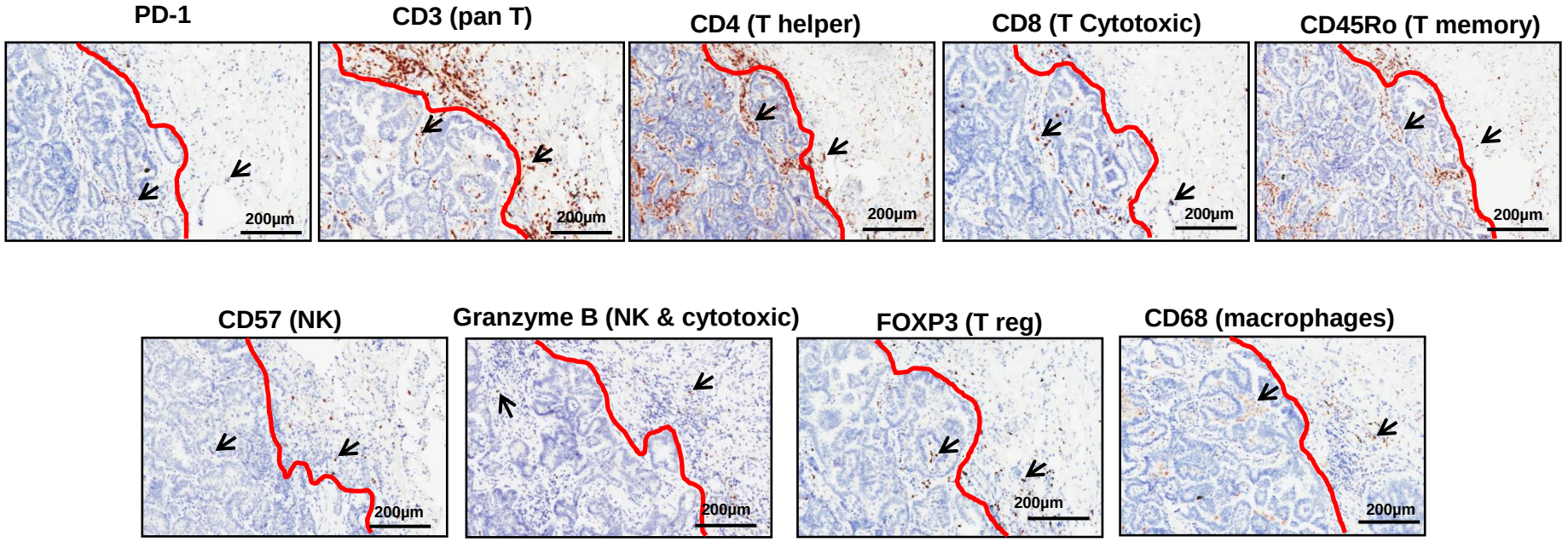
Inform™

E. Parra and Luisa Solis, MD Anderson Cancer Center, 2018

PD-L1 expression in NSCLC (H-score)



TAIC expression in NSCLC (Cell Density; 9 markers)



Cancer

Edwin Parra et al, Clinical Cancer Res, 2016

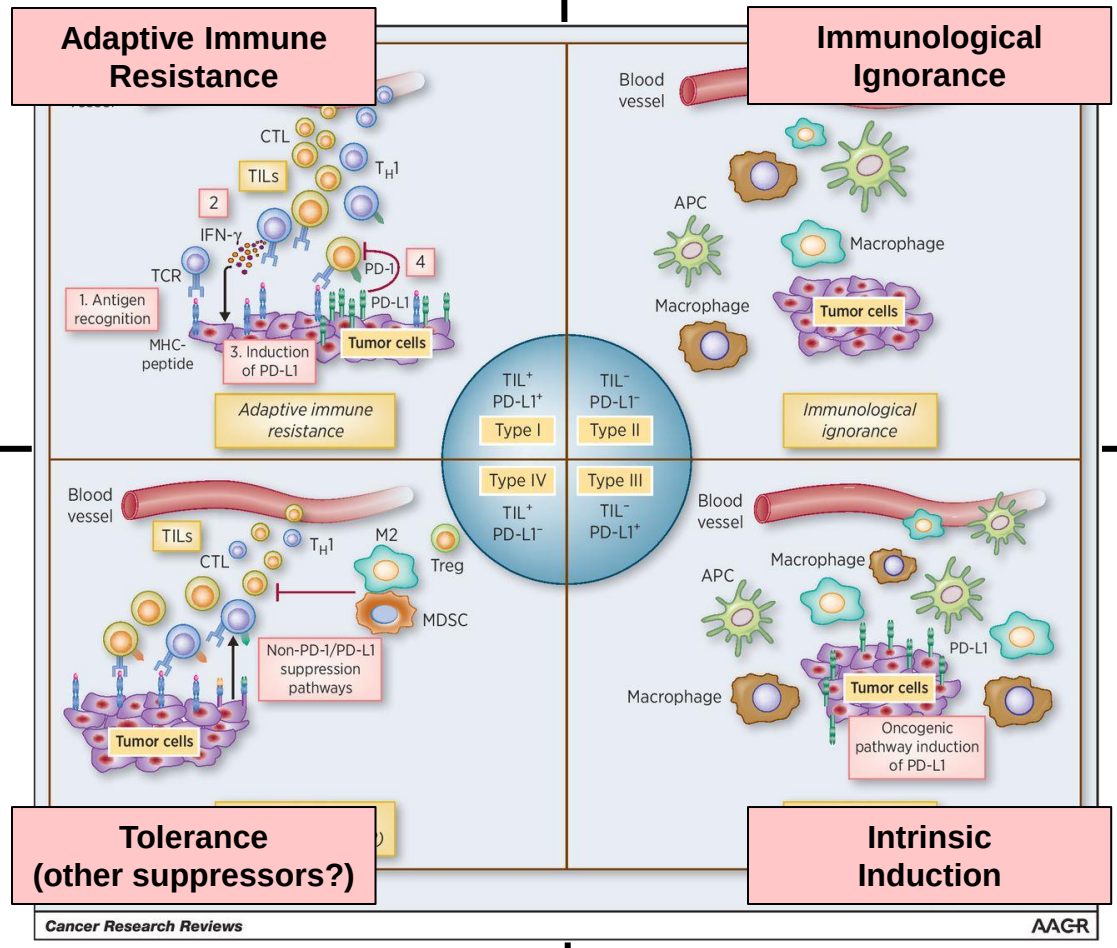
Types of Tumor Microenvironment in NSCLC

146 Adenocarcinomas (ADC) /108 Squamous Cell Carcinoma (SCC)

PD-L1 >5% / Intratumoral TAICs+ (CD3/CD68) moderate and severe density

PD-L1 +
TAICs +

ADC 20%
SCC 26%



Michele W.L. Teng et al. Cancer Res 2015;75:2139-2145

PD-L1 -
TAICs +

ADC 47%
SCC 40%

PD-L1 -
TAICs -

ADC 30%
SCC 28%

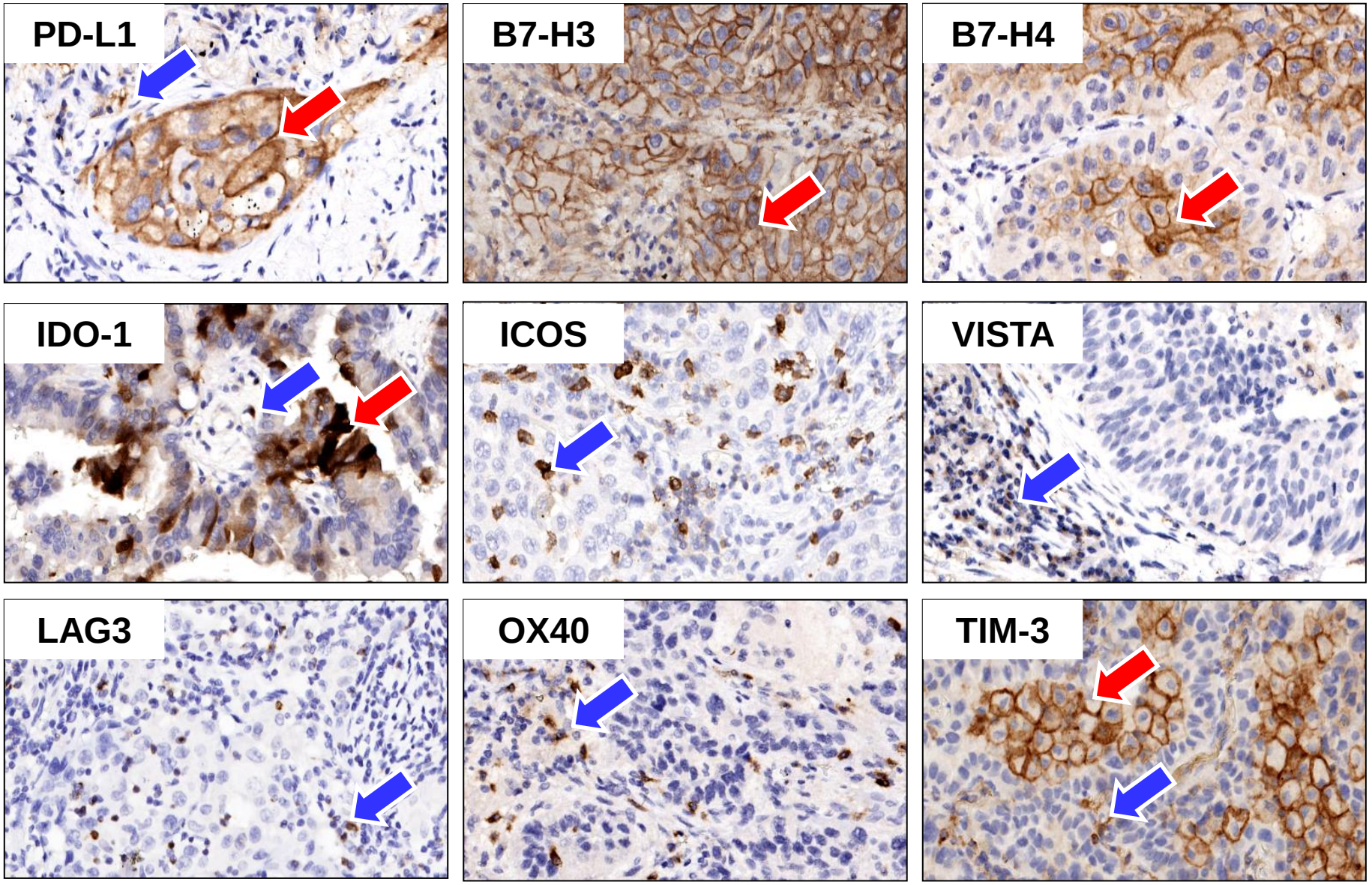
PD-L1 +
TAICs -

ADC 3%
SCC 6%

Cancer

Edwin Parra et al, Clinical Cancer Res, 2016

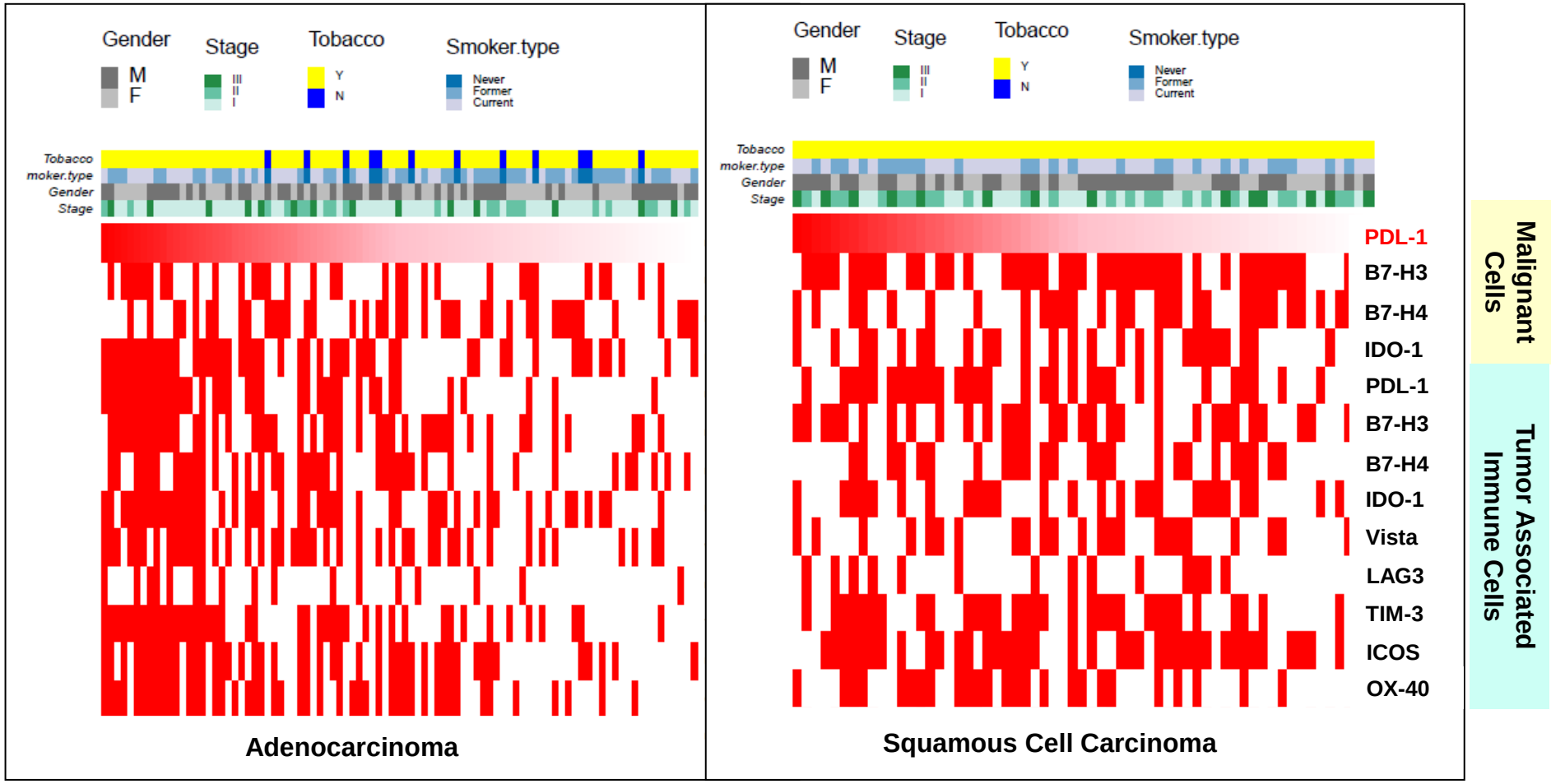
Immune Checkpoint Expression in NSCLC



Cancer

Edwin Parra and Pamela Villalobos et al, J Thoracic Oncol, 2018

Immune Checkpoints (N=9) IHC Expression in NSCLC (N=184 Cases)



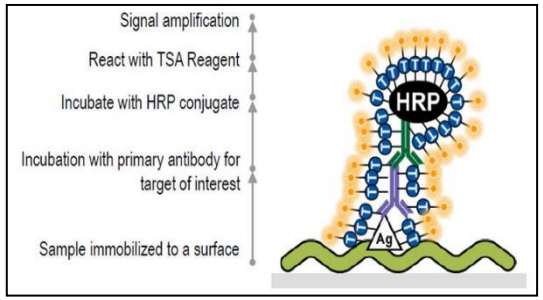
Cancer

Edwin Parra and Pamela Villalobos et al, J Thoracic Oncol, 2018

Multiplex Immunofluorescence (mIF) for Tissue Immune-profiling

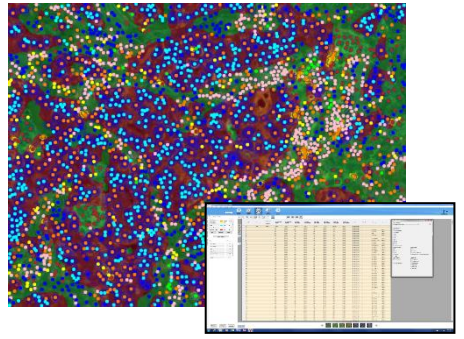
Edwin Parra-Cuentas, MD, PhD

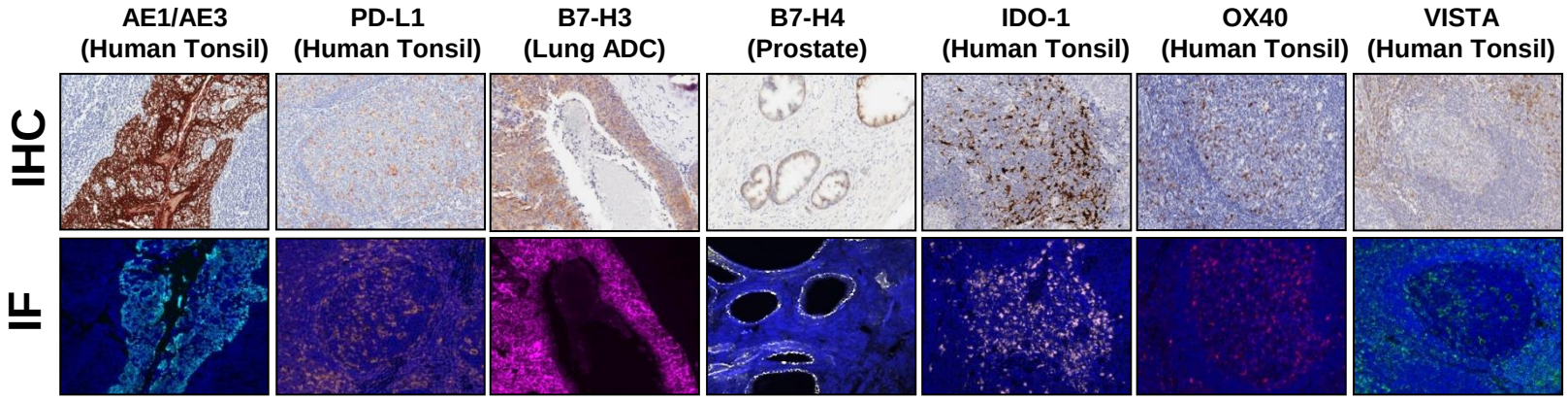
Opal f - TSA System for Multiplex Polaris IF



Polaris f (Perkin Elmer)

InForm f (Perkin Elmer)



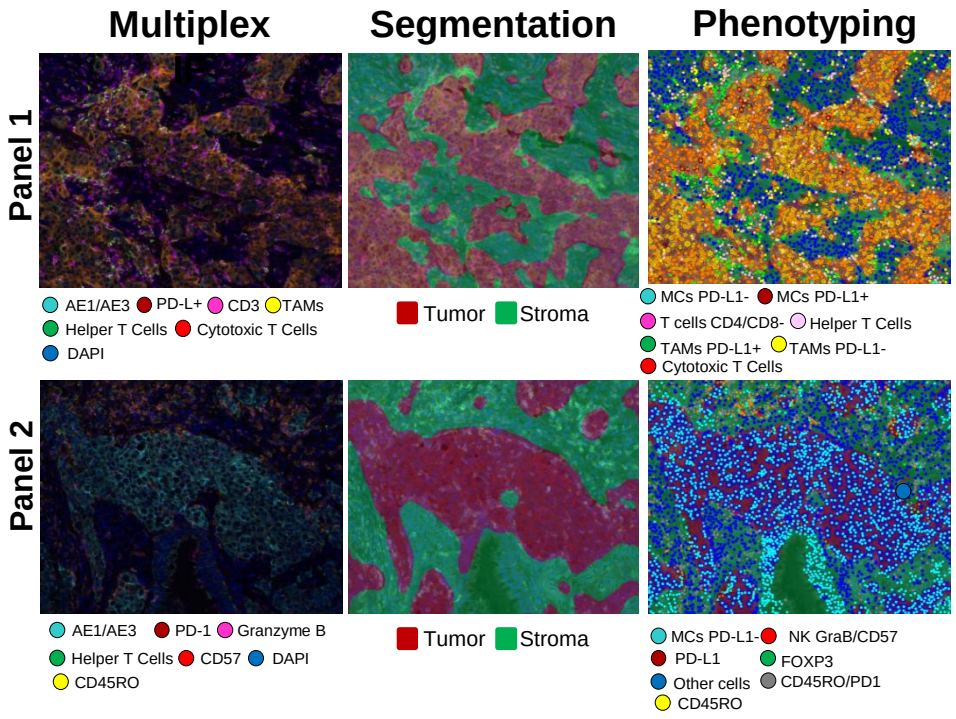


Cancer

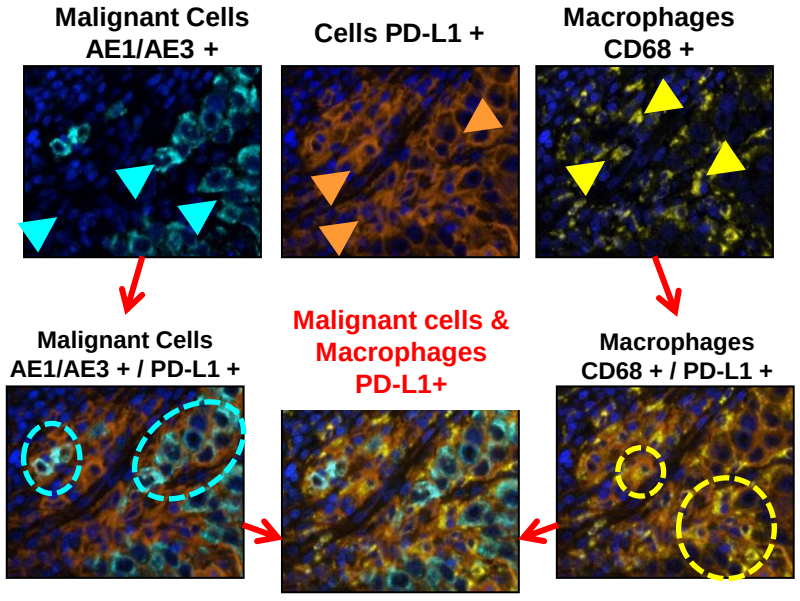
E. Parra, et al, MD Anderson Cancer Center, 2018

mIF Vectra/Polaris™ Segmentation and Co-localization Analysis

mIF Segmentation



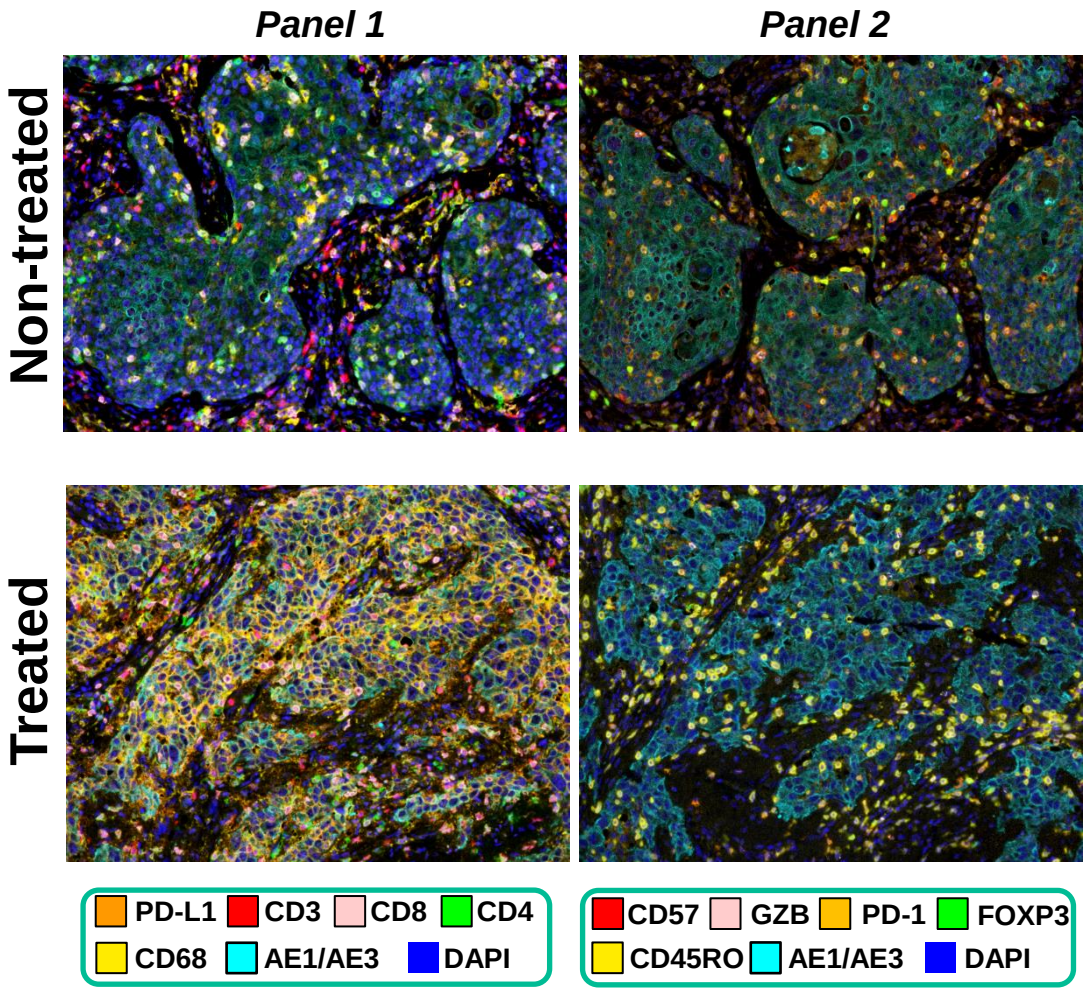
Panel 1 Co-localization: PD-L1



E. Parra et al, MD Anderson Cancer Center

Immune Cells Infiltration in Chemotherapy-treated Lung Cancer

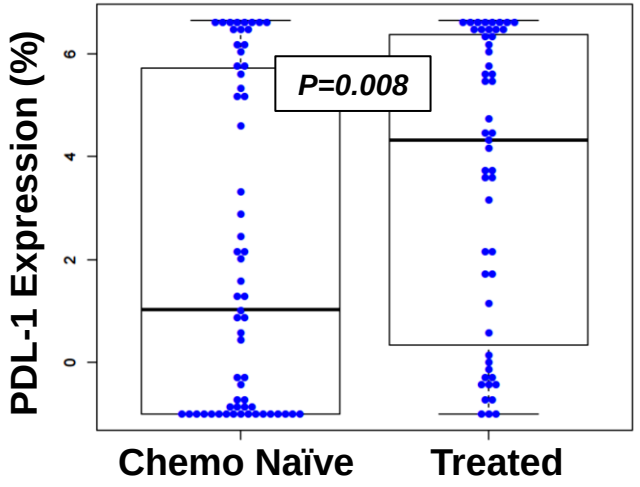
Multiplex Immunofluorescence (mIF)



Increase of Immune Cells Subpopulations

Markers (cells/mm^2)	Non-small Cell Lung Carcinoma (NSCLC) (N=112)		P-Value*
	Chemo Naïve (N=61)	Neo-adjuvant-treated (N=51)	
CD3+	903.21	↑1501.99	0.021
CD57+	206.87	↑ 527.35	<0.001
CD45RO+	668.75	↑1180.26	0.019
CD45RO/PD1+	153.73	↑ 443.04	<0.001
PD1+	336.02	↑ 795.21	<0.001

Increase of Malignant Cells PD-L1 IHC Expression



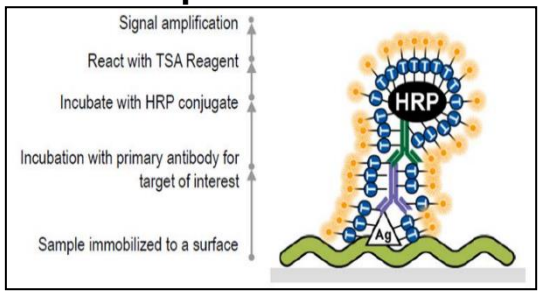
Cancer

E. Parra et al, JITC 2018

Tissue Multiplex Immune Profiling Methodologies

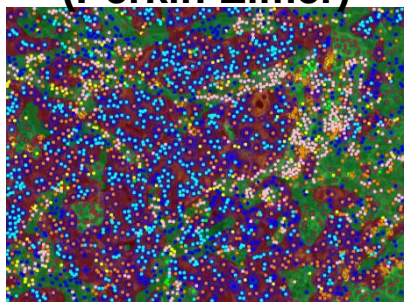
Multiplex Immunofluorescence - *9 markers/panel*

Opal™ - TSA System for Multiplex Polaris IF



Vectra/Polaris™ (Perkin Elmer)

InForm™ (Perkin Elmer)



E. Parra, et al, Sci Rep. 2017 Oct 17;7(1):13380.

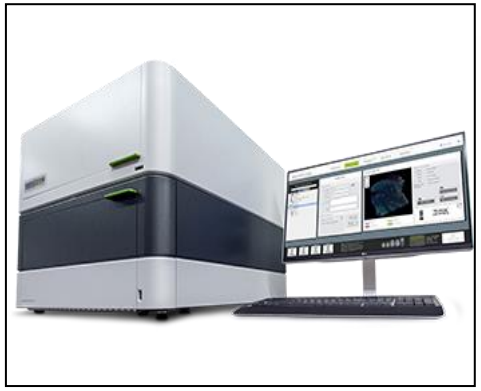
Imaging Mass Cytometry (*CyTOF, Helios, Fluidigm*)

Cosma A, Cytometry A. 2017 Jan;91(1):12-13.

Multiplexed Ion Beam Imaging (*MIBISCOPE, IonPath*)

Keren L, et al. Cell. 2018 174(6):1373-87.e19.

Nanostring GeoMx™ Digital Spatial Profiler

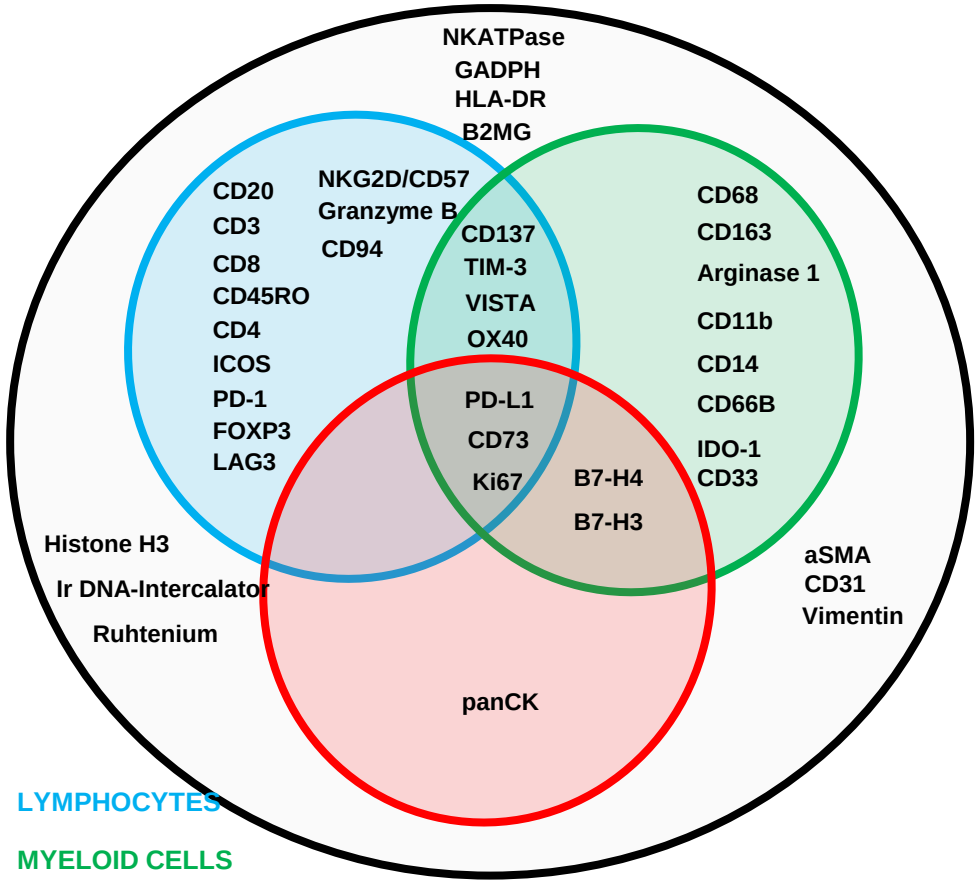


Toki MI et al, Clin Cancer Res. 2019

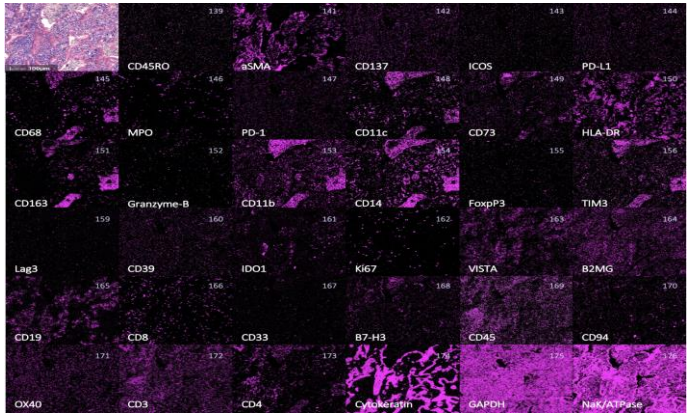
CODEX™ Digital Spatial Profiler

Imaging Mass Cytometry (IMC) by Hyperion (Fluidigm)

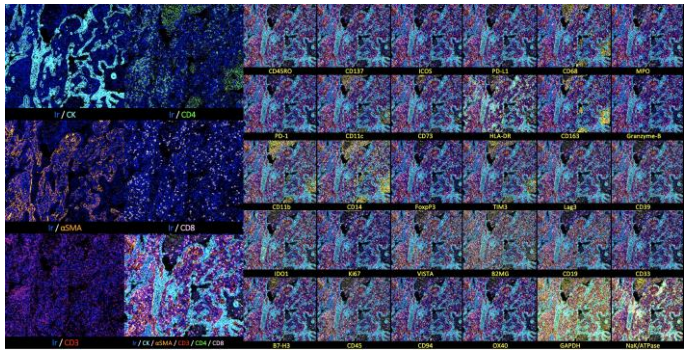
Development of a Immune Panel (N=35 markers)



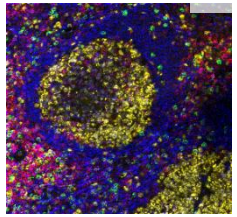
Single Markers



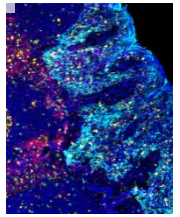
Combined Markers



Tonsil



Ir/CD3/CD8/Ki67

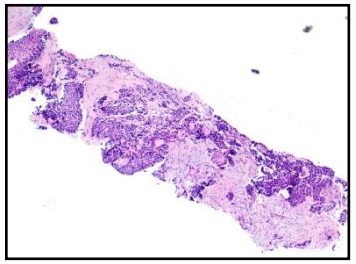


Ir/CD3/CD8/Ki67 CK

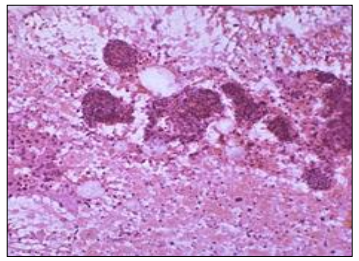
Pedro Rocha, Alexandro Francisco et al , MD Anderson Cancer Center, AACR 2020

Cancer

Diagnostic Algorithm for Lung Cancer Diagnosis



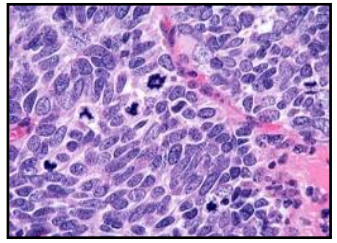
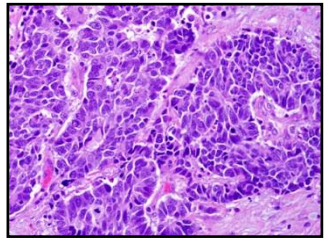
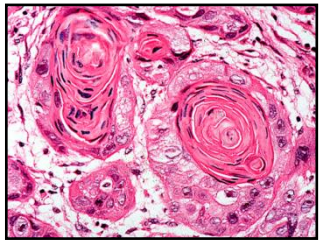
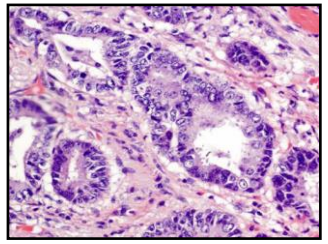
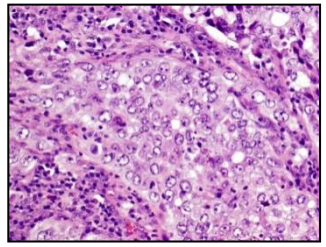
Biopsy



Cytology

Blood

cfDNA Testing
(Liquid Biopsy)
Genotyping + TMB

SCLC	LCNEC	Squamous	Adenoca	NSCLC-NOS
				
Morphology	Morphology IHC NE (+)	Morphology IHC p63/p40 (+)	Morphology IHC TTF1 (+)	Morphology IHC (-)

Multiplex Genotyping (NGS panels): ***EGFR & BRAF*** **mutations,** ***ALK*****,** ***ROS1 , RET*** **and** ***NTRK*** **fusions;** ***MET ex14*** **splicing:** ***Tumor Mutational Burden (TMB)***

Multiplex IHC/mIF Approaches*: PD-L1; TIL activation signatures? New IO targets?*

Cancer

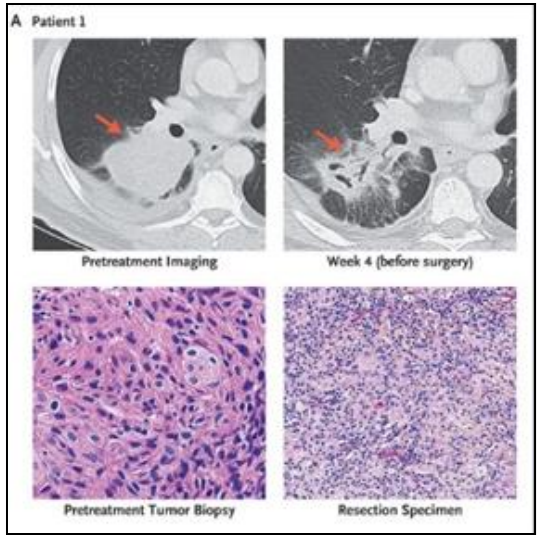
Neoadjuvant IO in Lung Cancer

- Based on the success of immune checkpoints inhibition on NSCLC, several neo-adjuvant phase II/III clinical trials of IO and IO + chemotherapy have been completed or are in progress.
- The clinical trials endpoint consider either ≤10% major pathological response (MPR) or recurrence/disease free survival.
- However, there is no standardized and validated pathology methodology to process surgically resected lung tumor specimens and to evaluate MPR in this setting.

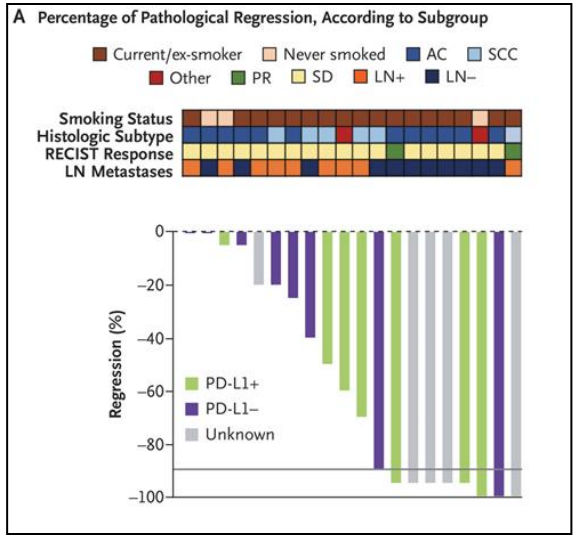
PM Forde et al. N Engl J Med 2018; TR Cottrell, Annals of Oncology, 2018; GM Blumenthal, J Thoracic Oncol, 2018

Neoadjuvant PD-1 Blockade in Resectable NSCLC (n=21 Cases)

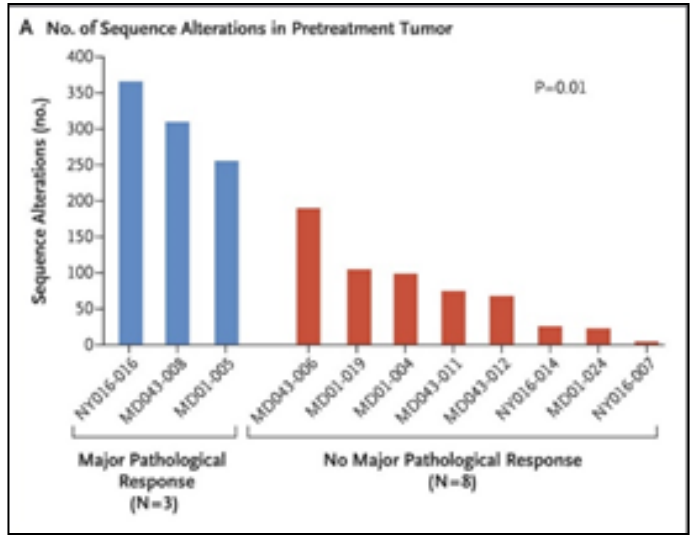
- MPR (≤10 malignant cells) in 9/20 (45%)
- No associated to PD-L1 IHC expression
- WES-based on 11 cases, MPR associated to tumor mutational burden
- TC receptor sequencing showed T-cell clones expansion in tumor and blood in 8/9 patients



MPR - Histology



PD-L1 IHC Expression



Tumor Mutational Burden

PM Forde et al. N Engl J Med 2018. DOI: 10.1056/NEJMoa1716078

Neoadjuvant I/O or I/O + CT
Summary (July 2019)

Therapies	N	MPR	pCR	ORR
Nivo	21	9 (43%)	NR	9.5%
Nivo or Nivo+Ipi	44	11/41 (25%)	8 (18%)	9 (20)
Atezo (LCMC3)	82	15 (18%)	4 (5%)	6 (7%)
Sintilimab	22	10 (45%)	3 (14%)	NR
Total I/O alone	169	45 (27%)	15/148 (10%)	24/147 (16%)
Nivo +CT	30	24 (80%)	18 (60%)	NR
Atezo +CT	14	7 (50%)	3 (21%)	8 (57%)
Total I/O + CT	44	31 (70%)	21 (48%)	8/14 (57%)

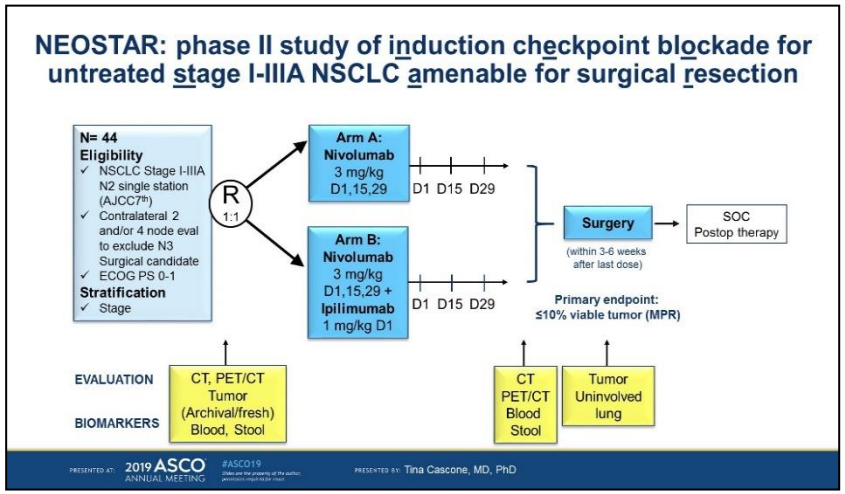
MPR= Major Pathological Response
pCR=pathological Complete Response
NR=not reported

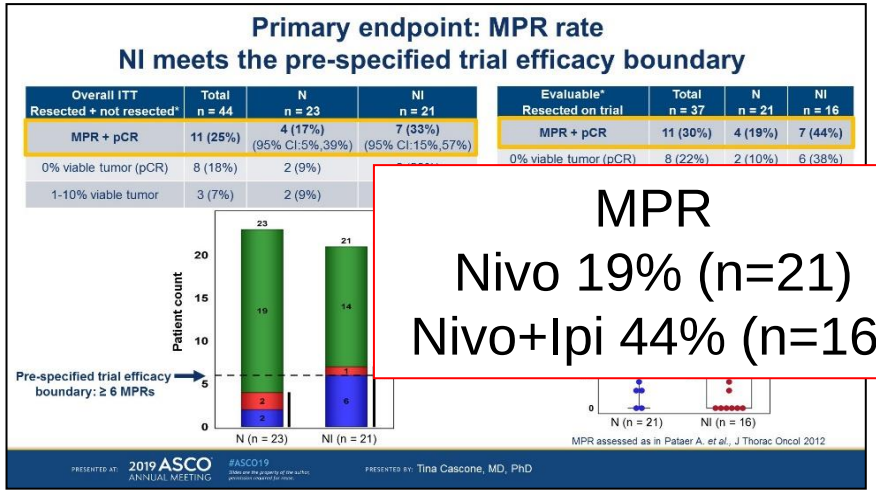
Slide courtesy of Dr. Paul Bunn, Colorado Cancer Center, USA.

Neoadjuvant Immunotherapy in Lung Cancer

Endpoints Major Pathological Response

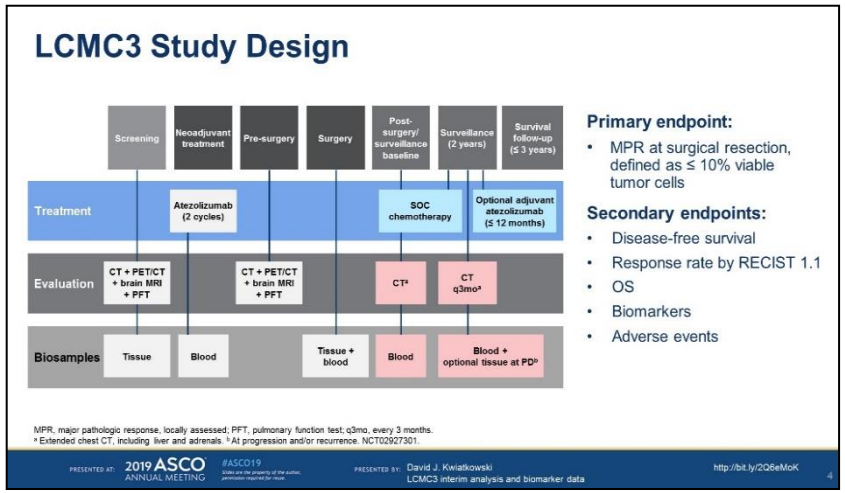
- ≤10% Malignant Viable Tumor -

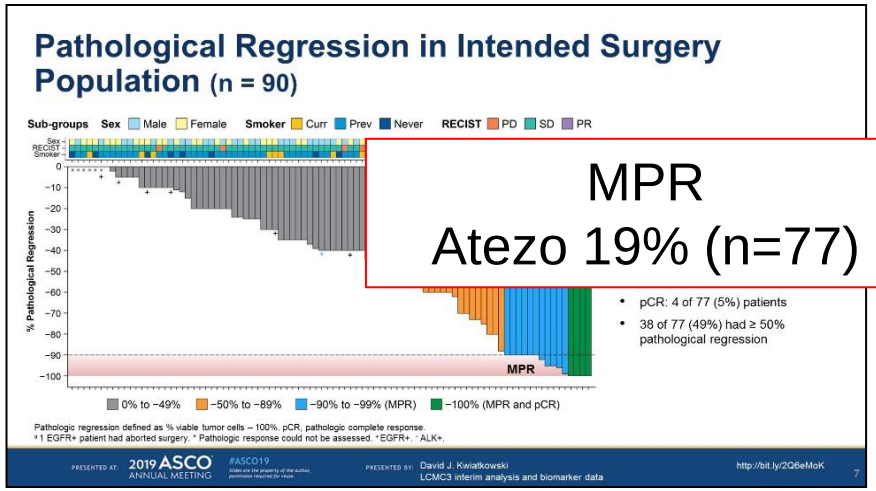




MPR
Nivo 19% (n=21)
Nivo+Ipi 44% (n=16)

T. Cascone et al, 2019 ASCO Annual Meeting





MPR
Atezo 19% (n=77)

D. Kwiatkowski at, 2019 ASCO Annual Meeting

Lung Cancer Neoadjuvant Chemotherapy and MPR

- Several clinical trials have evaluated pathological response in NSCLC chemotherapy and chemo-radiotherapy trials[1].
- MPR refers to primary tumor response, not to lymph nodes.
  - Pathological nodal response refers to down-staging and clearance.
  - It depend on accuracy of nodal assessment and only applicable to patients with confirmed nodal disease at diagnosis.
- Despite that the appropriate pathology methodology to assess MPR has not been established, data suggest that ≤10% residual tumor cells has prognostic value[2,3].

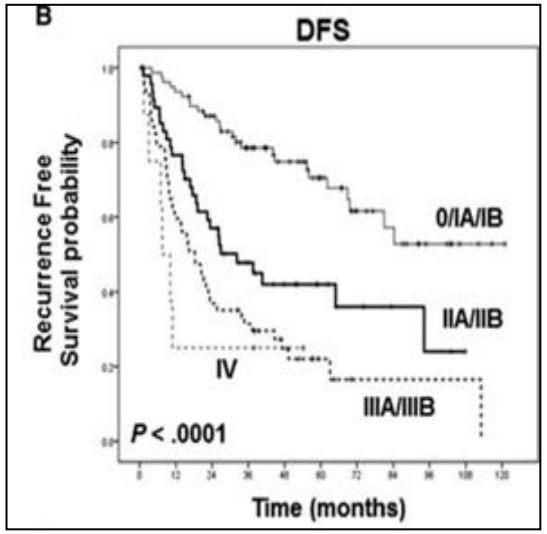
[1]Hellman et al, Lancet Oncol, 2014; [2]Junker et al, J Cancer Res Oncol, 1997; [3]Pataer et al, J Thorac Oncol, 2012;

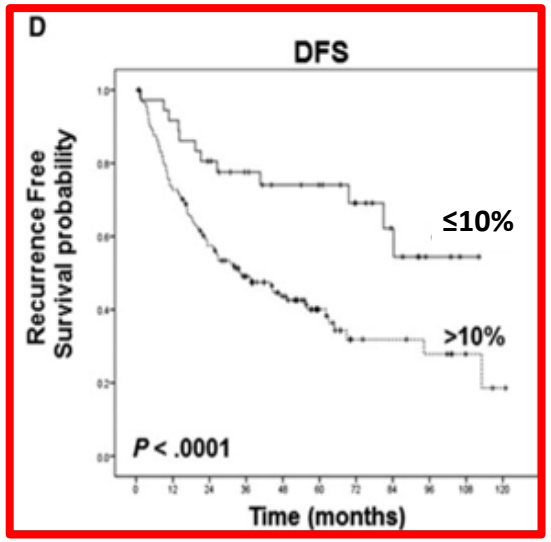
Neoadjuvant Immunotherapy in Lung Cancer

Major Pathological Response Assessment

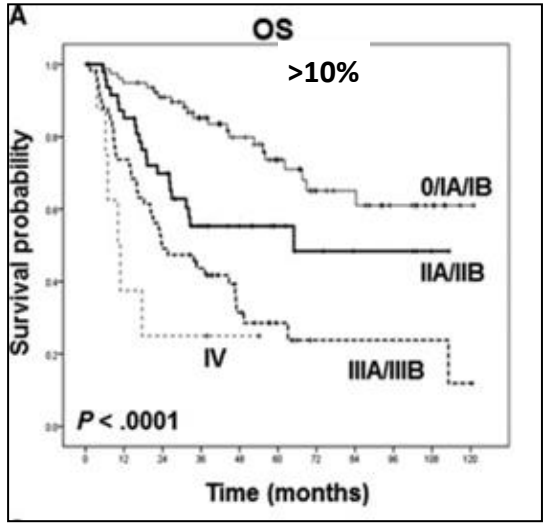
- A retrospective analysis: 192 patients treated and 166 controls NSCLCs.
- At least one H&E-stained section per/cm of tumor greatest diameter was examined.
- The number of slides examined per case ranged from 5-30.

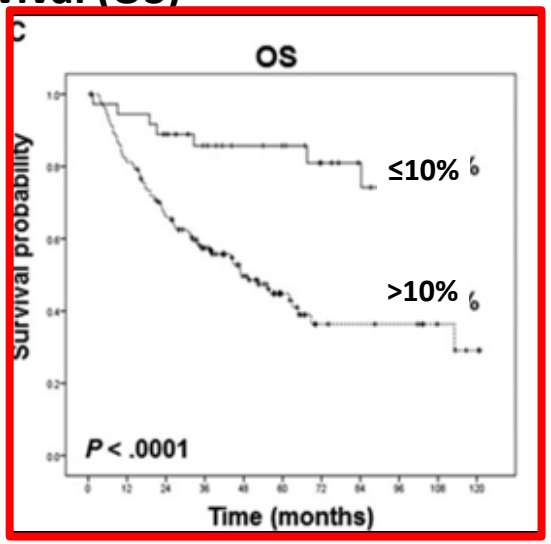
Disease Free Survival (DFS)



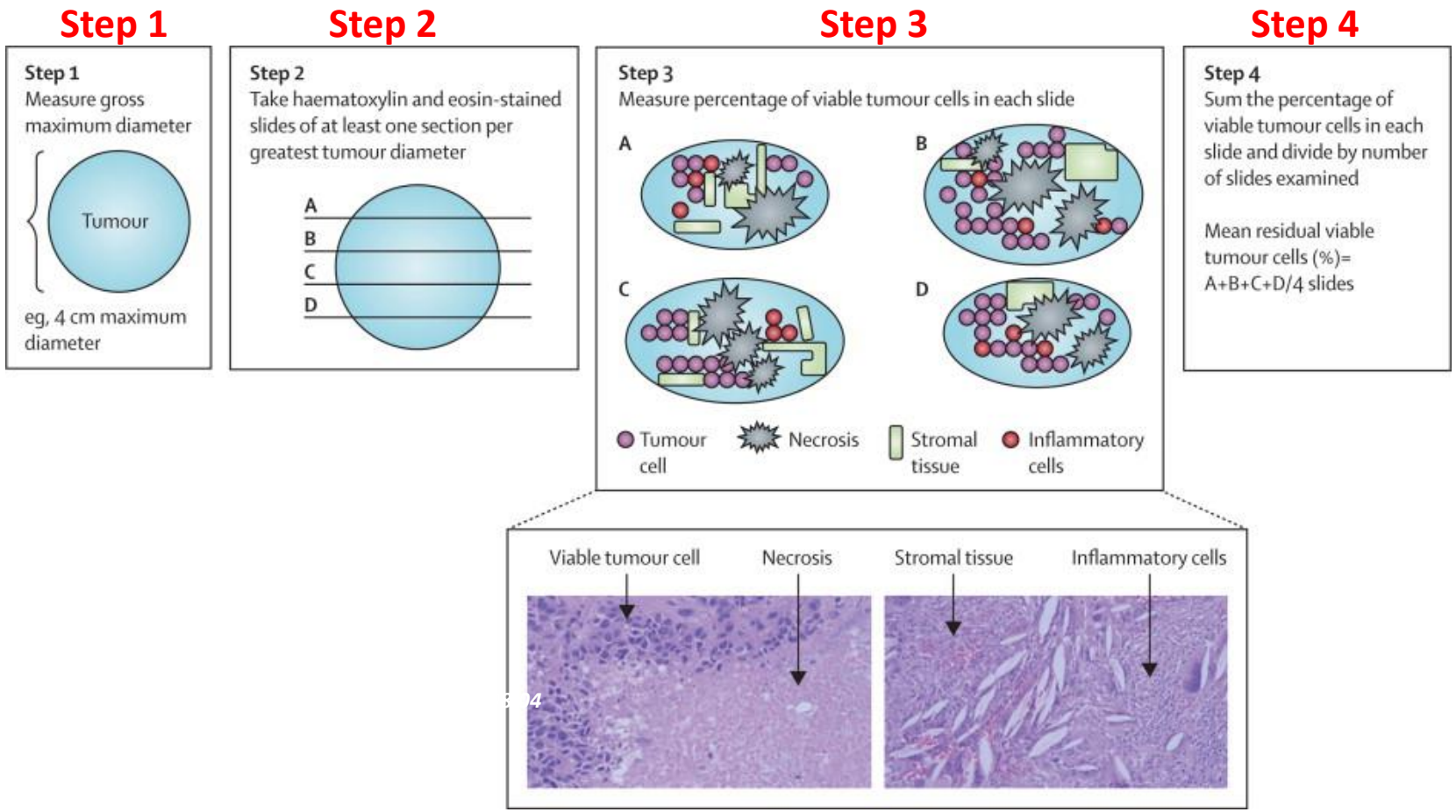


Overall Survival (OS)





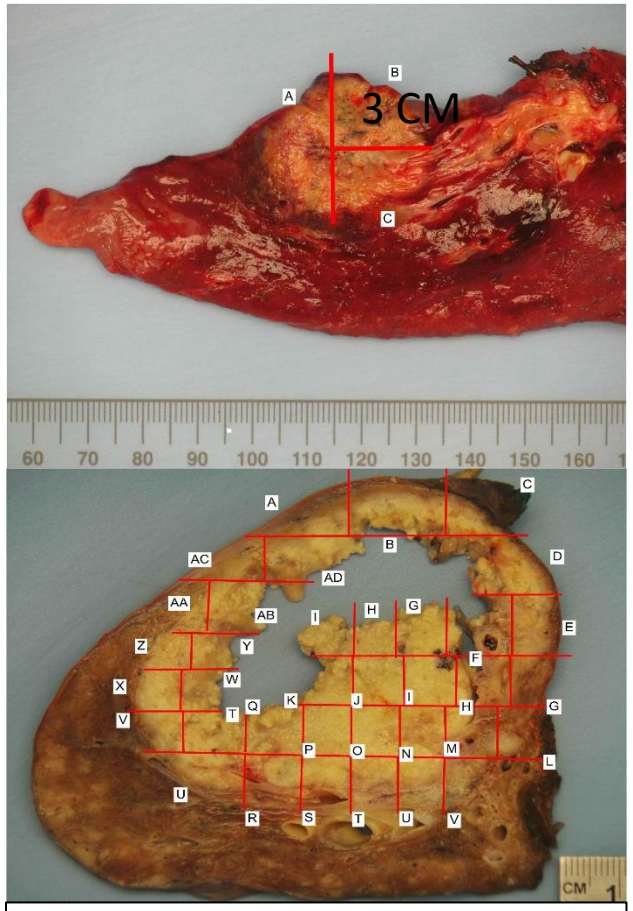
MPR Assessment in Neoadjuvant Chemotherapy-treated NSCLC Tumors – 4 Steps



Hellman et al, Lancet Oncol, 2014

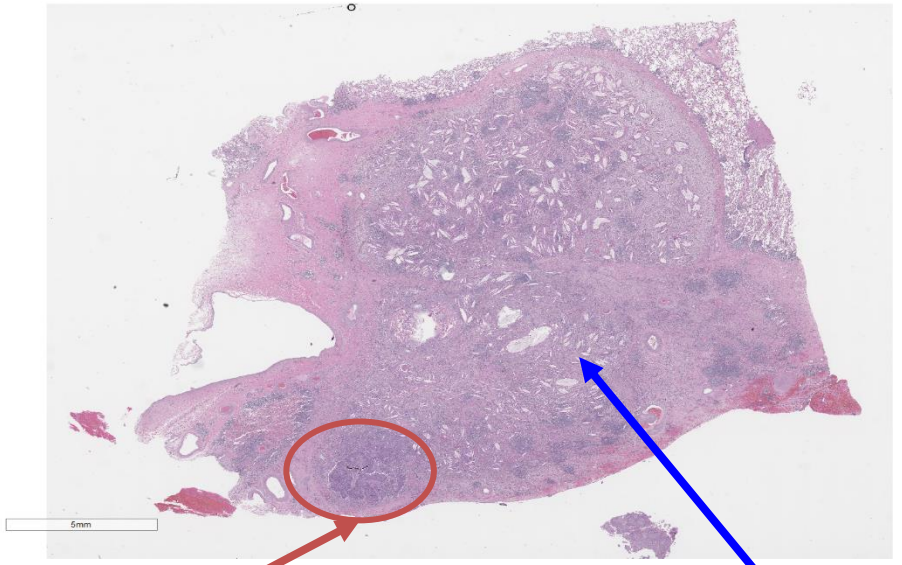
Neoadjuvant Immunotherapy in Lung Cancer

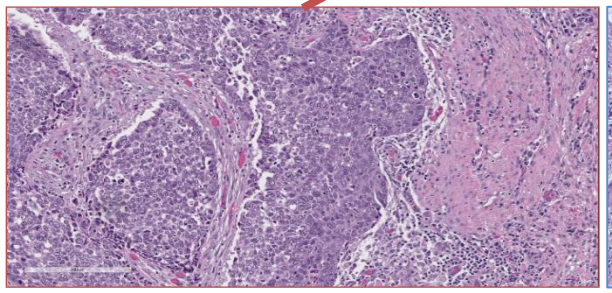
Major Pathological Response Assessment



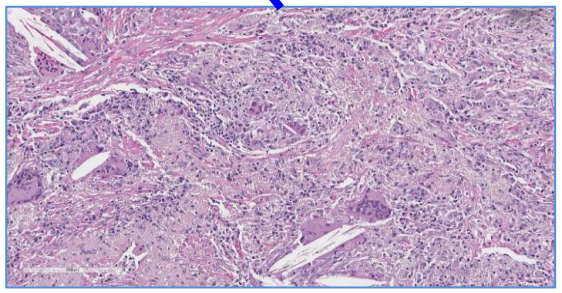
GROSS-HISTOLOGIC CORRELATION IS ESSENTIAL

Images Courtesy of Dr. William Travis, Memorial Sloan Kettering, NYC



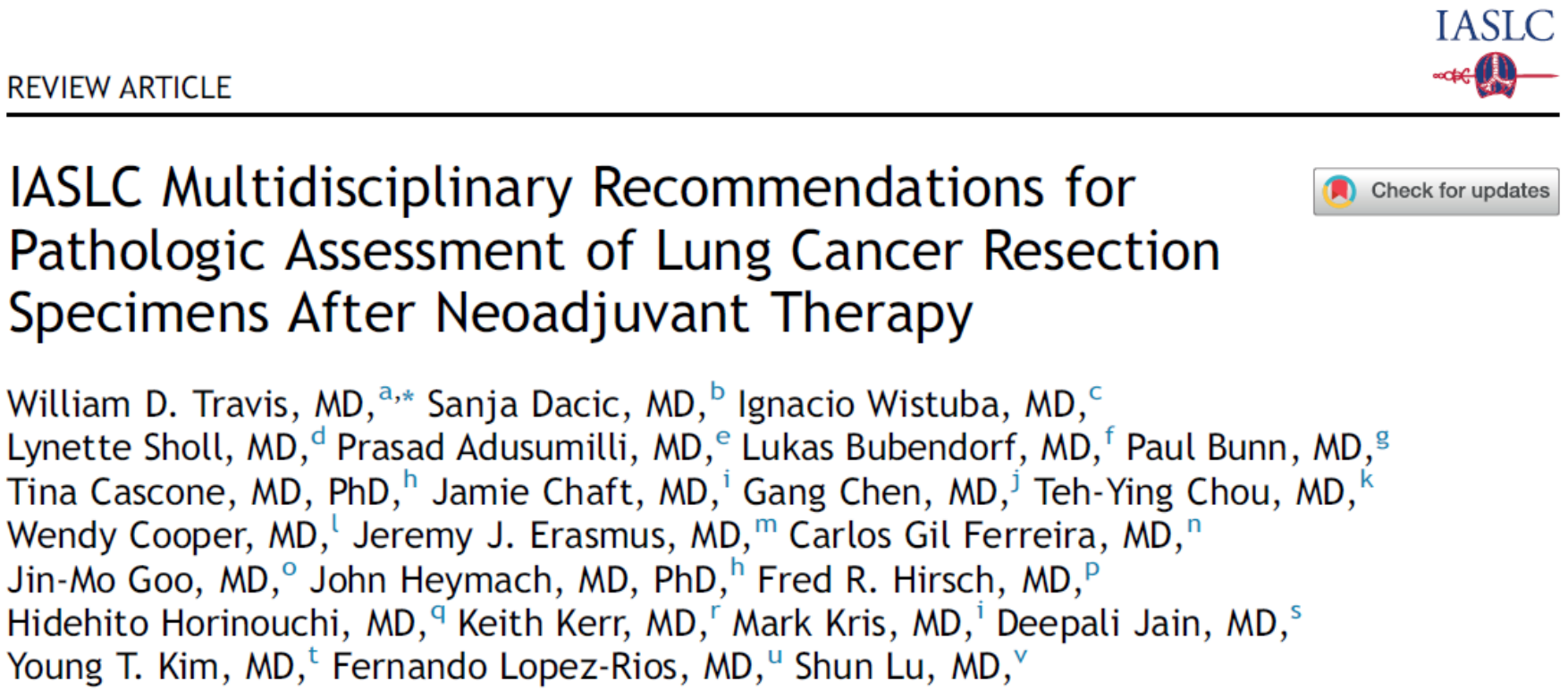


Malignant Cells



No malignant Cells
Tumor Bed

IASLC Multidisciplinary Recommendations for Assessment of Lung Cancer Resection Specimens Following Neoadjuvant Therapy



REVIEW ARTICLE

IASLC

IASLC Multidisciplinary Recommendations for Pathologic Assessment of Lung Cancer Resection Specimens After Neoadjuvant Therapy

Check for updates

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Travis et al (IASLC Pathology Committee), J Thorac Oncol 2020

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Thank You