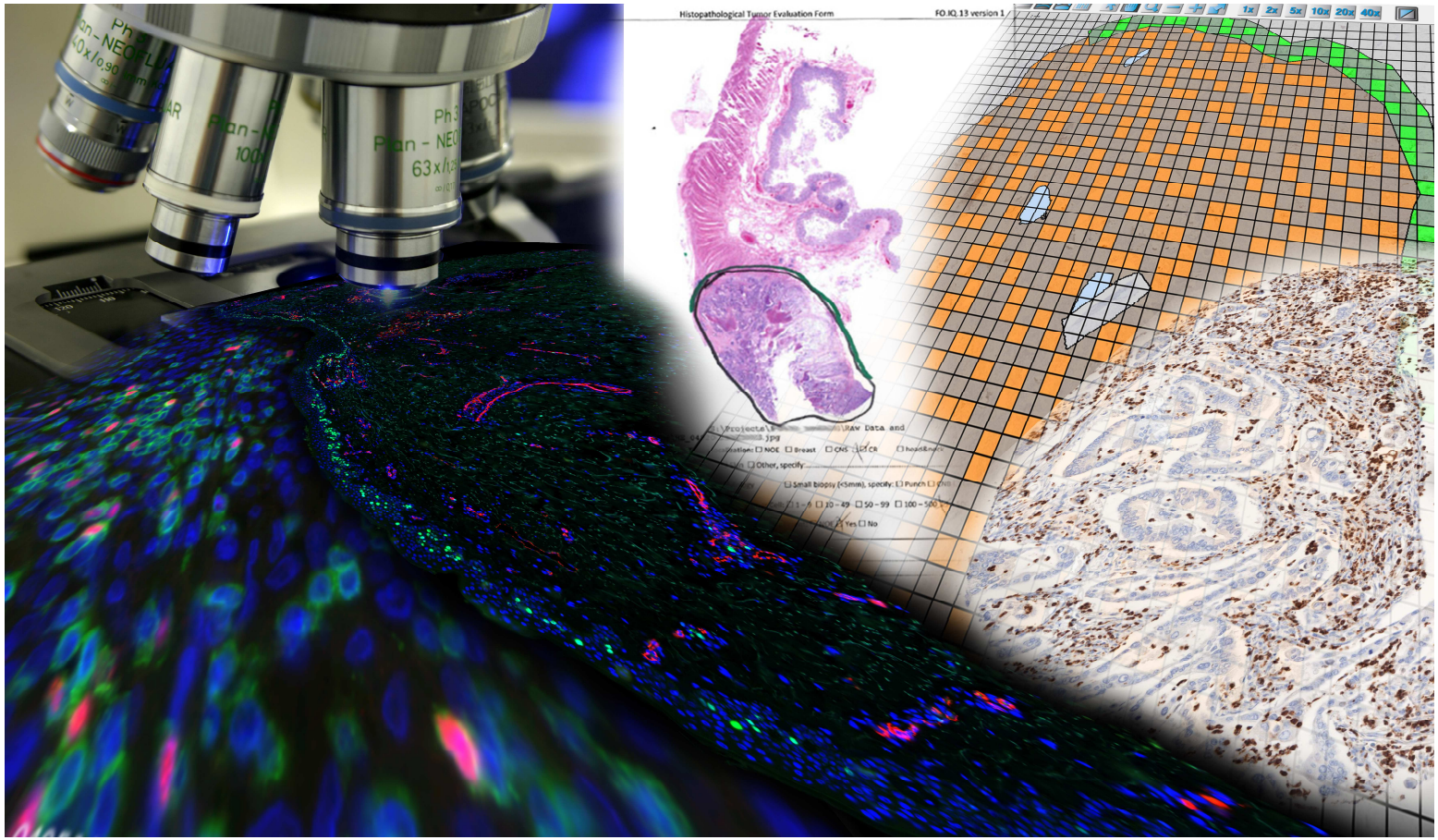
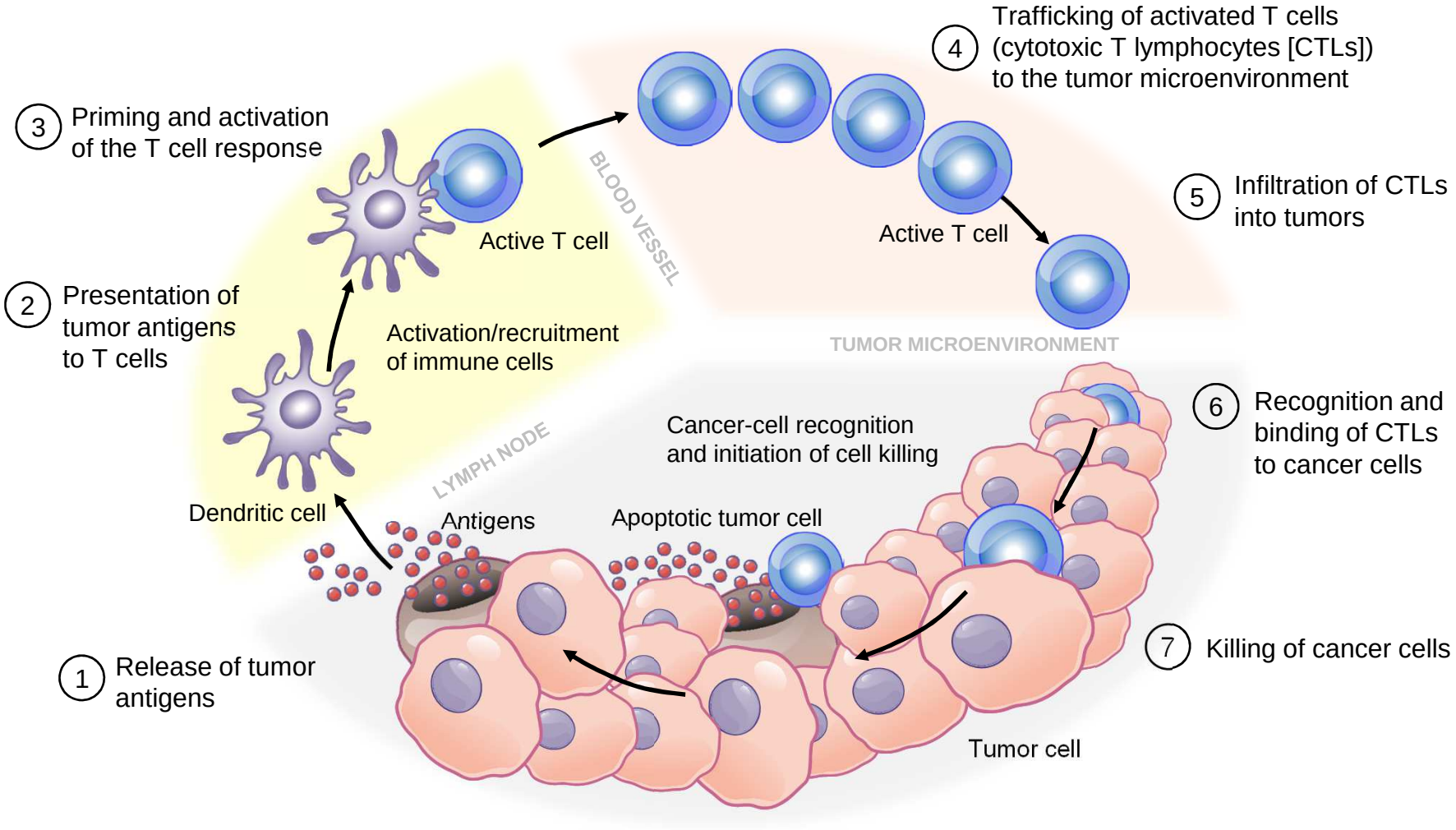
PD-L1 testing and the Pathologist

Mark Kockx MD, PhD

HistoGeneX

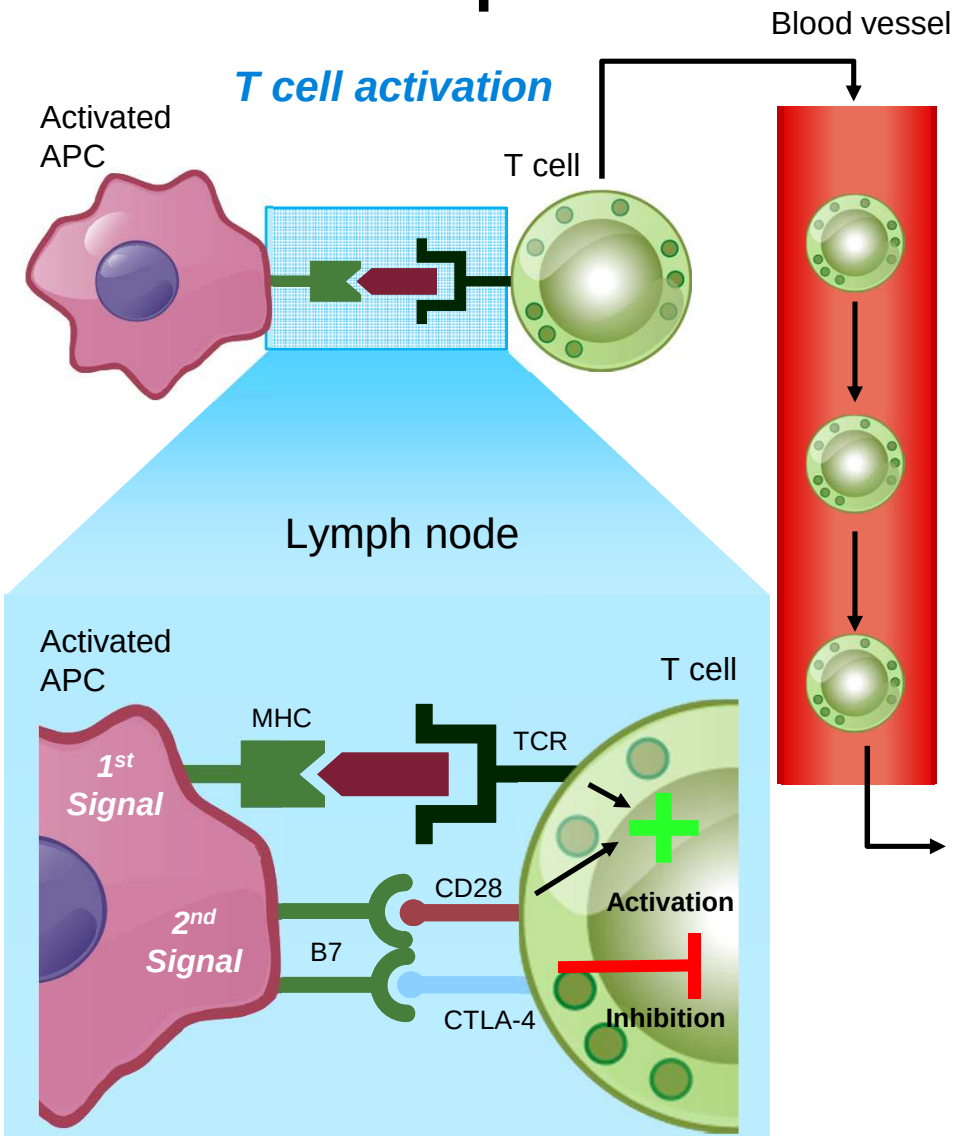


The Cancer-Immunity Cycle is Central toImmune Surveillance and Defence



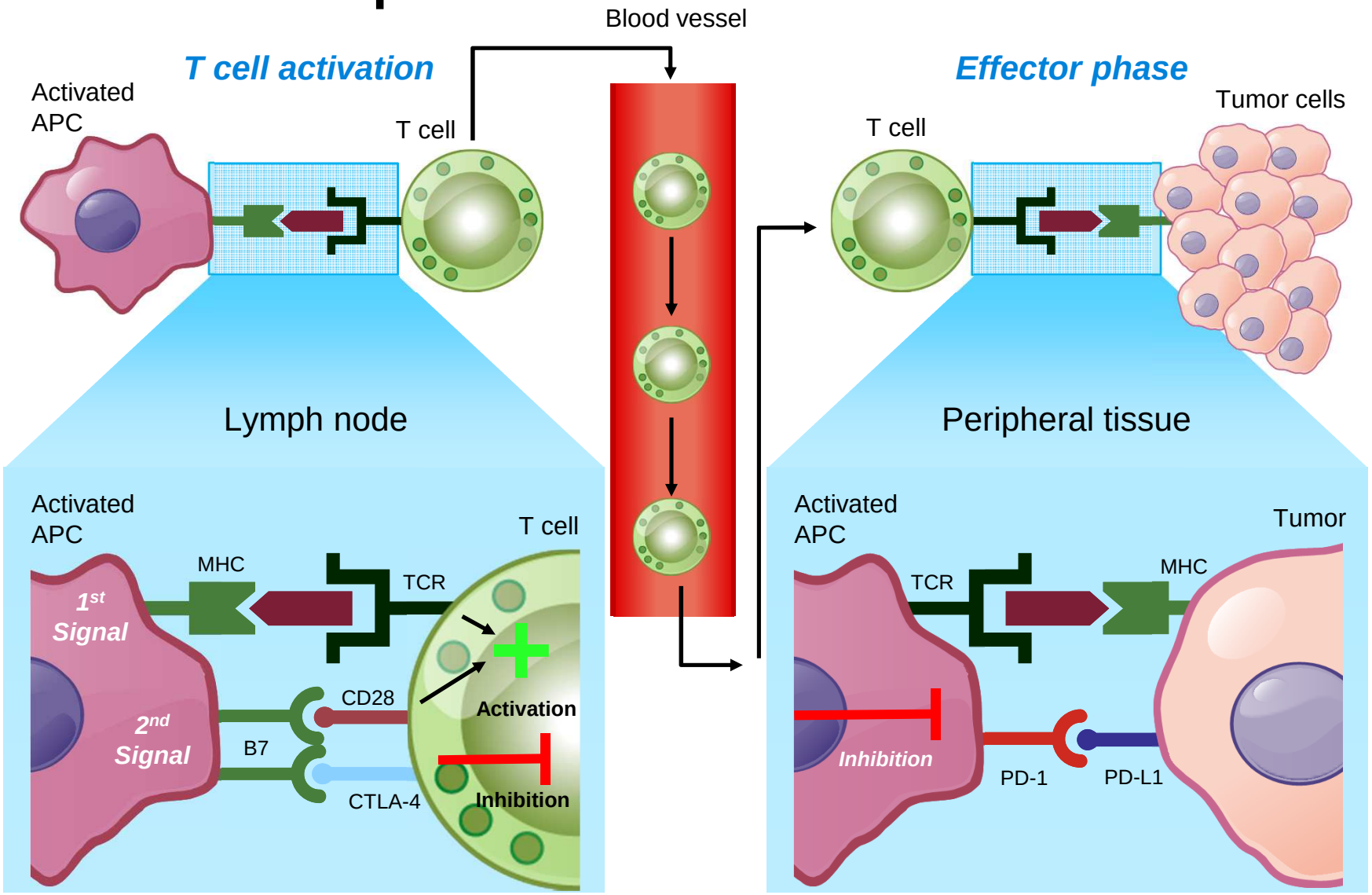
Chen DS, Mellman I. Immunity. 2013

Immune Checkpoints



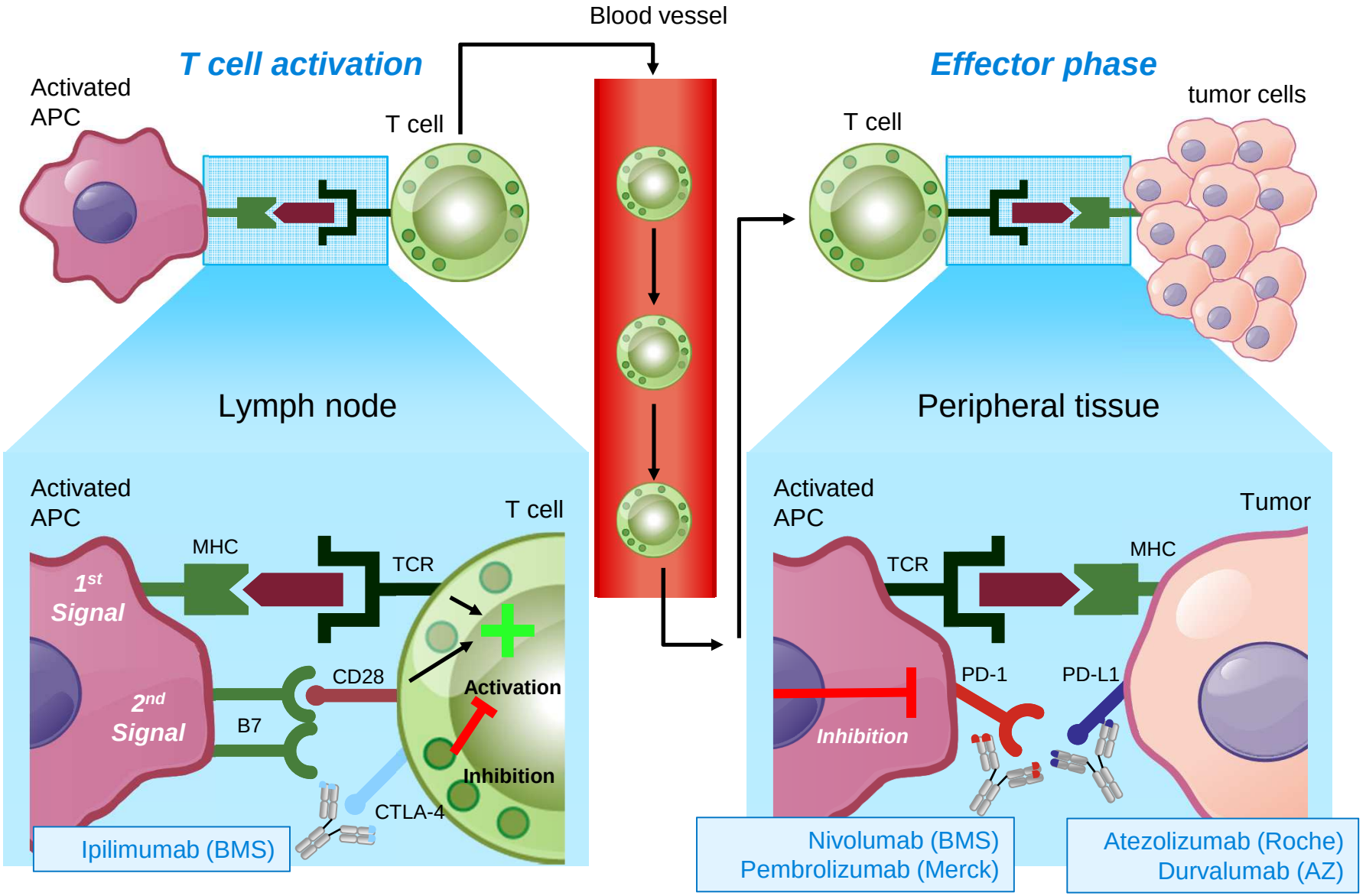
Adapted from Kyi C and Postow M. FEBS Letters 2014; 588:368–76

Immune Checkpoints



Adapted from Kyi C and Postow M. FEBS Letters 2014; 588:368–76
Ribas A. N Engl J Med. 2012;366:2517–19

Immune Checkpoints



Adapted from Kyi C and Postow M. FEBS Letters 2014; 588:368-376

PD-1/PD-L1

- Immuno-oncology

Anti-PD-1

- Nivolumab (Opdivo) (BMS)
  (melanoma, NSCLC, RCC)
- Pembrolizumab (Keytruda) (MSD)
  (melanoma, NSCLC)
- Pidilizumab (Curetech)
- AMP-224 (Amplimmune, GSK)
- AMP-514 (Amplimmune)
- PDR001 (Novartis)

Anti-PD-L1

- Atezolizumab (Tecentriq) (Roche)
  (urothelial cancer, NSCLC)
- BMS-936.559
- MEDI4736 (MedImmune, AZ)
- Avelumab (Merck, Pfizer)

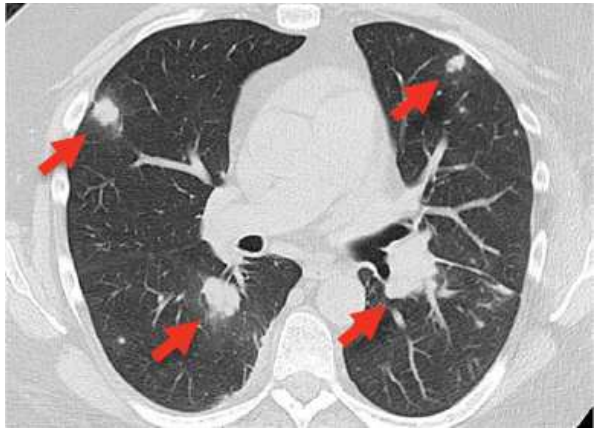
FDA approved

➔ PD-L1 IHC as a predictive biomarker ?

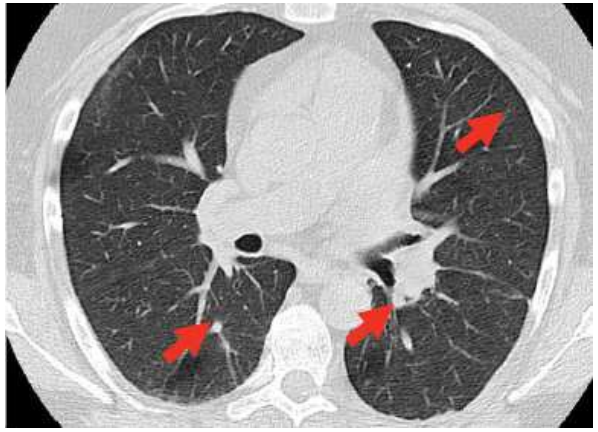
Mahoney K.M et al. *Clin.Ther.* 2015

Blocking PD-L1 Unleashes Immune Response

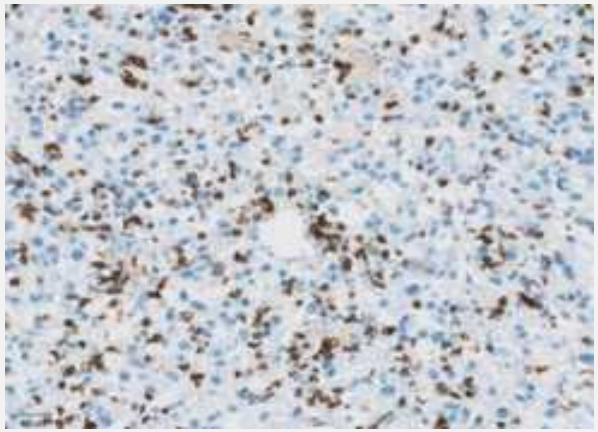
Atezolizumab in Patient with Renal Cell Carcinoma



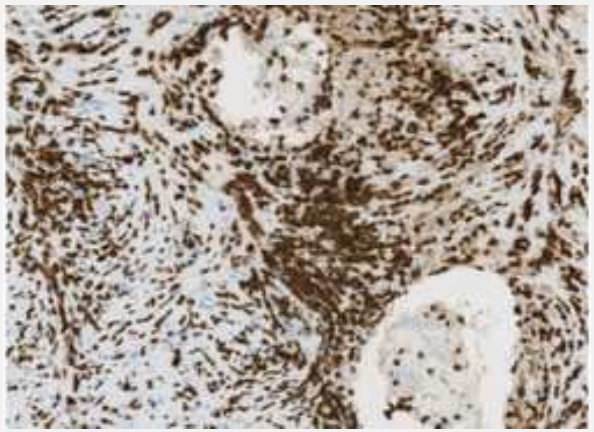
Pre-treatment



Post-cycle 2



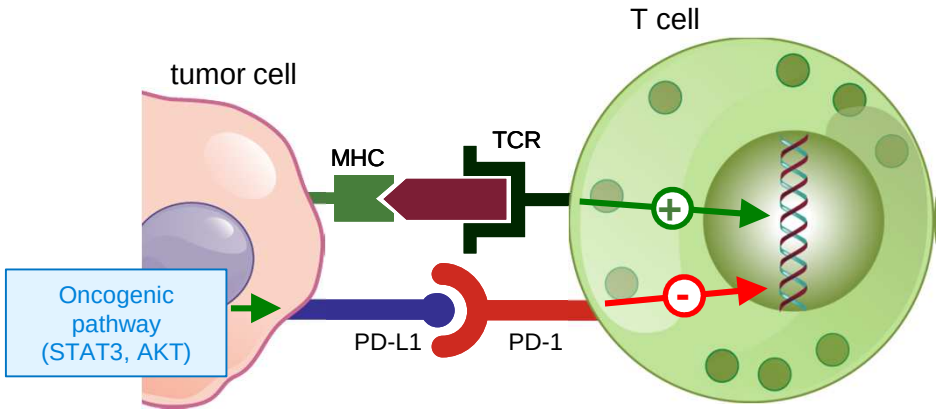
CD8 pre-treatment



CD8 on treatment ~4 weeks

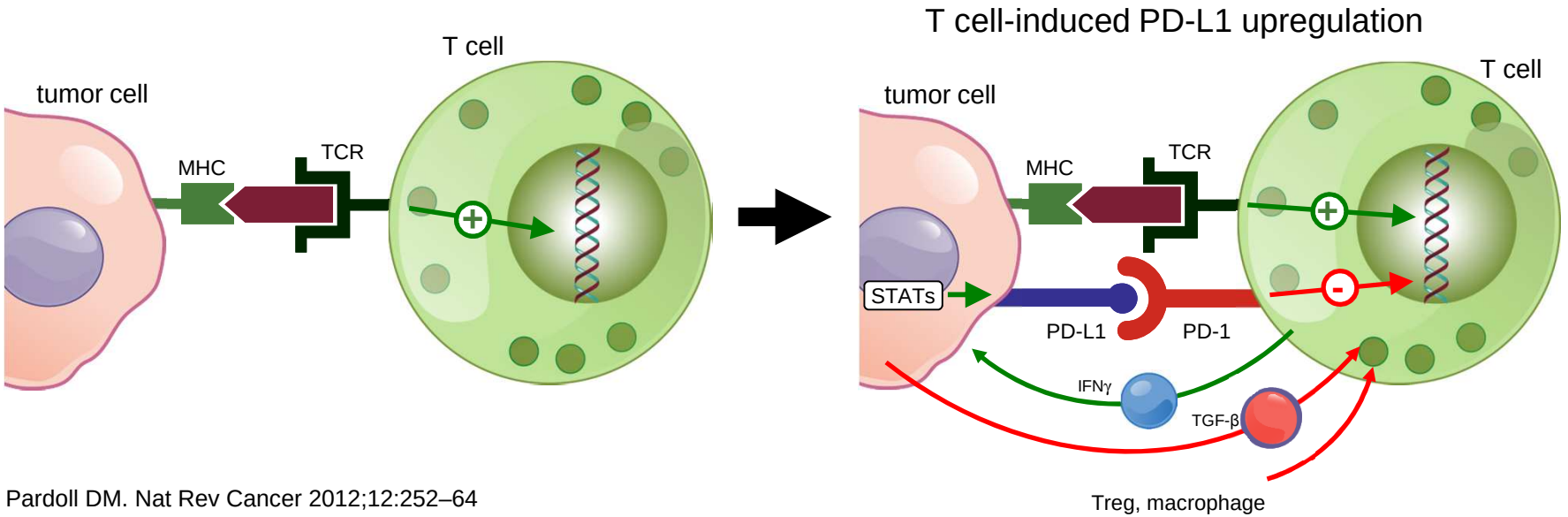
PD-L1 can be expressed in two Ways

Constitutive (tumor cell intrinsic) resistance



Constitutive oncogenic signalling induces PD-L1 expression on tumor cells

Adaptive or inducible resistance



Pardoll DM. Nat Rev Cancer 2012;12:252–64

PD-L1 IHC assays on the market

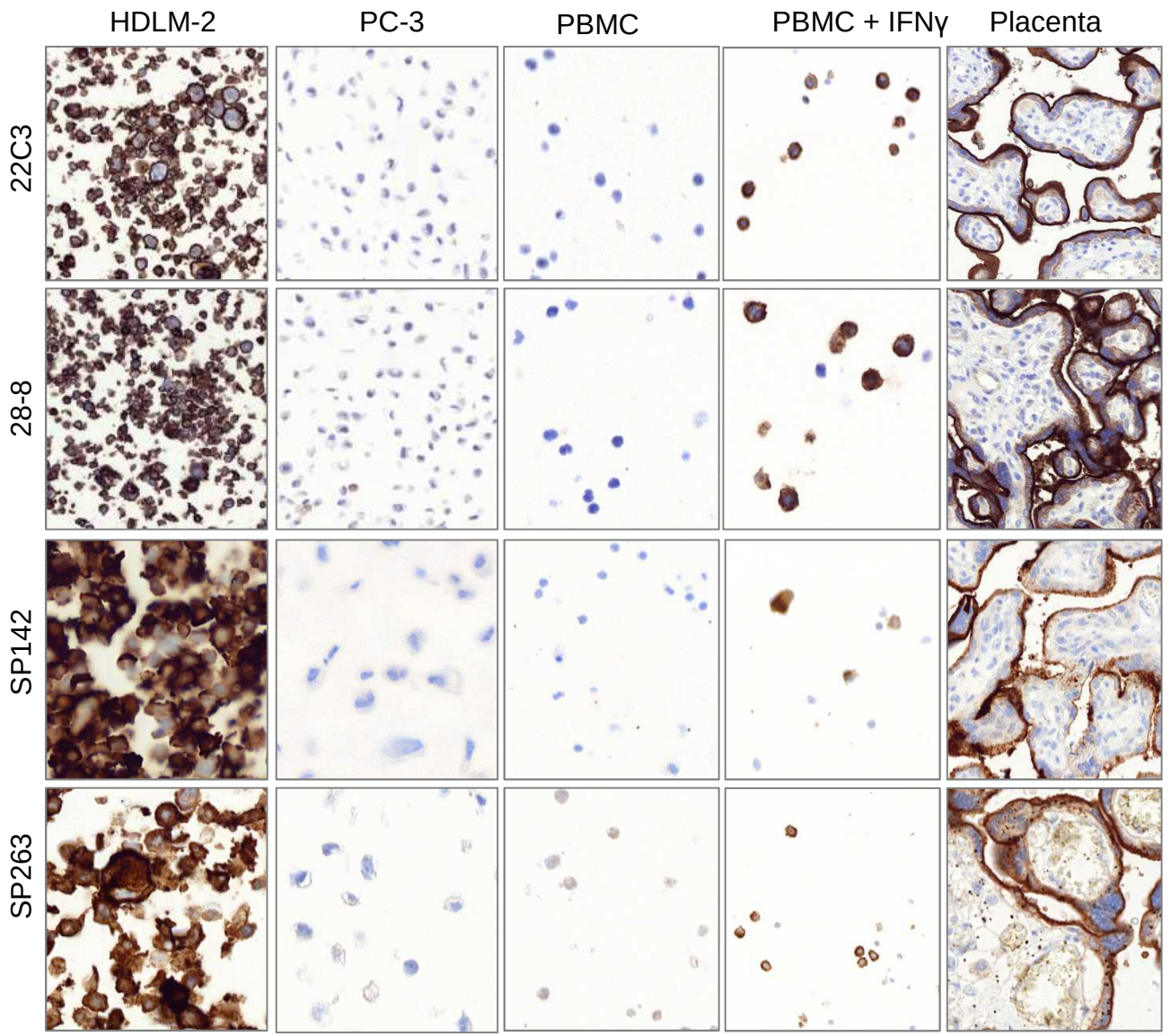
- Commercially available PD-L1 IHC kits

Clone	Evaluation	Pharma	Therapy
28-8 (Rb) PharmDX	TC	FDA Complementary Dx (BMS)	Nivolumab (Opdivo®) BMS-936.559
22C3 (Ms) PharmDX	TC	FDA Companion Dx (MSD)	Pembrolizumab (Keytruda®)
SP142 (Rb)	TC & IC	FDA Complementary Dx (Roche)	Atezolizumab (Tecentriq®)
SP263 (Rb)	TC & IC		Durvalumab Nivolumab (Opdivo®)

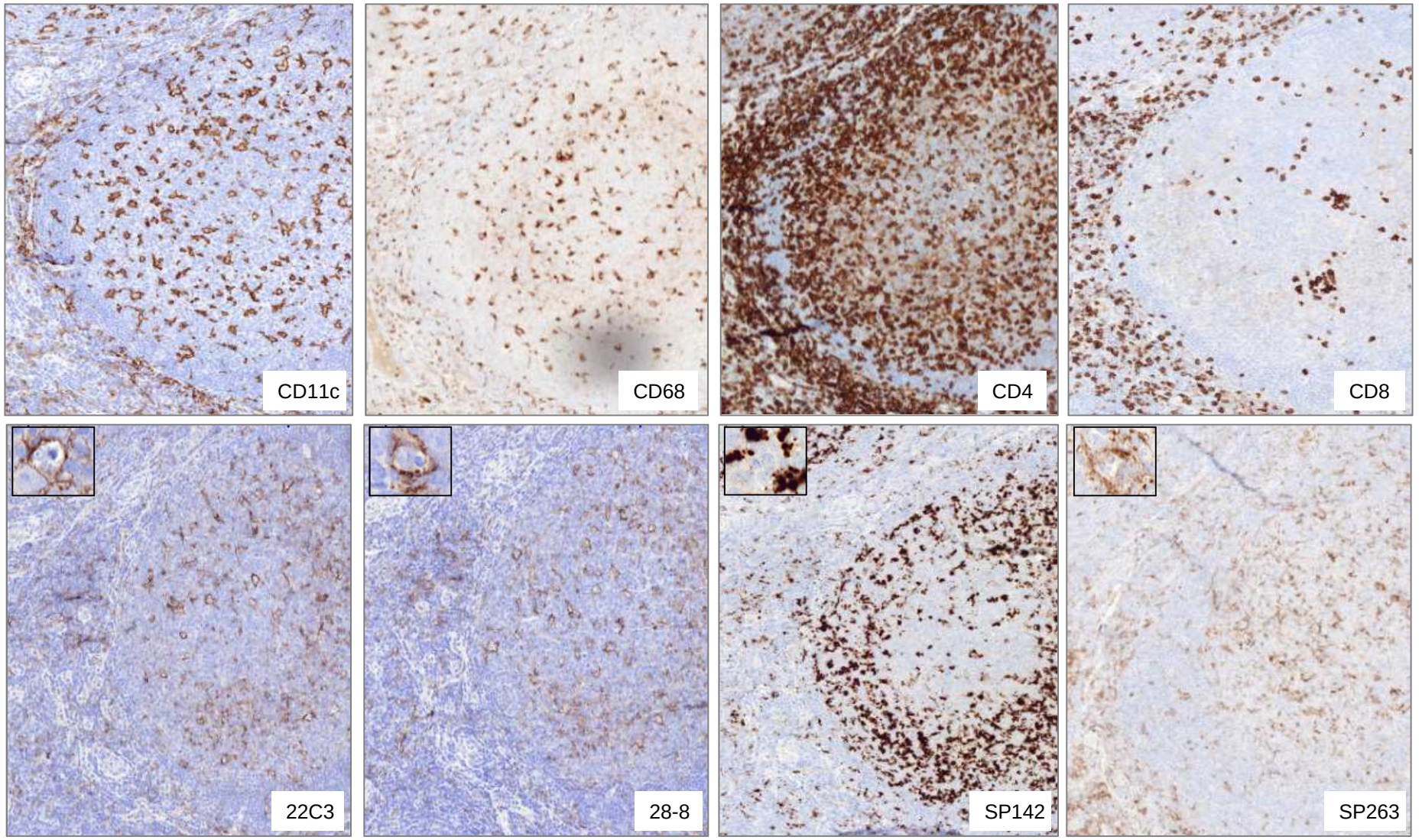
PD-L1 IHC assay implementation in the lab

- Implementation validation commercial kits
  - Verification of accuracy
  - Precision testing

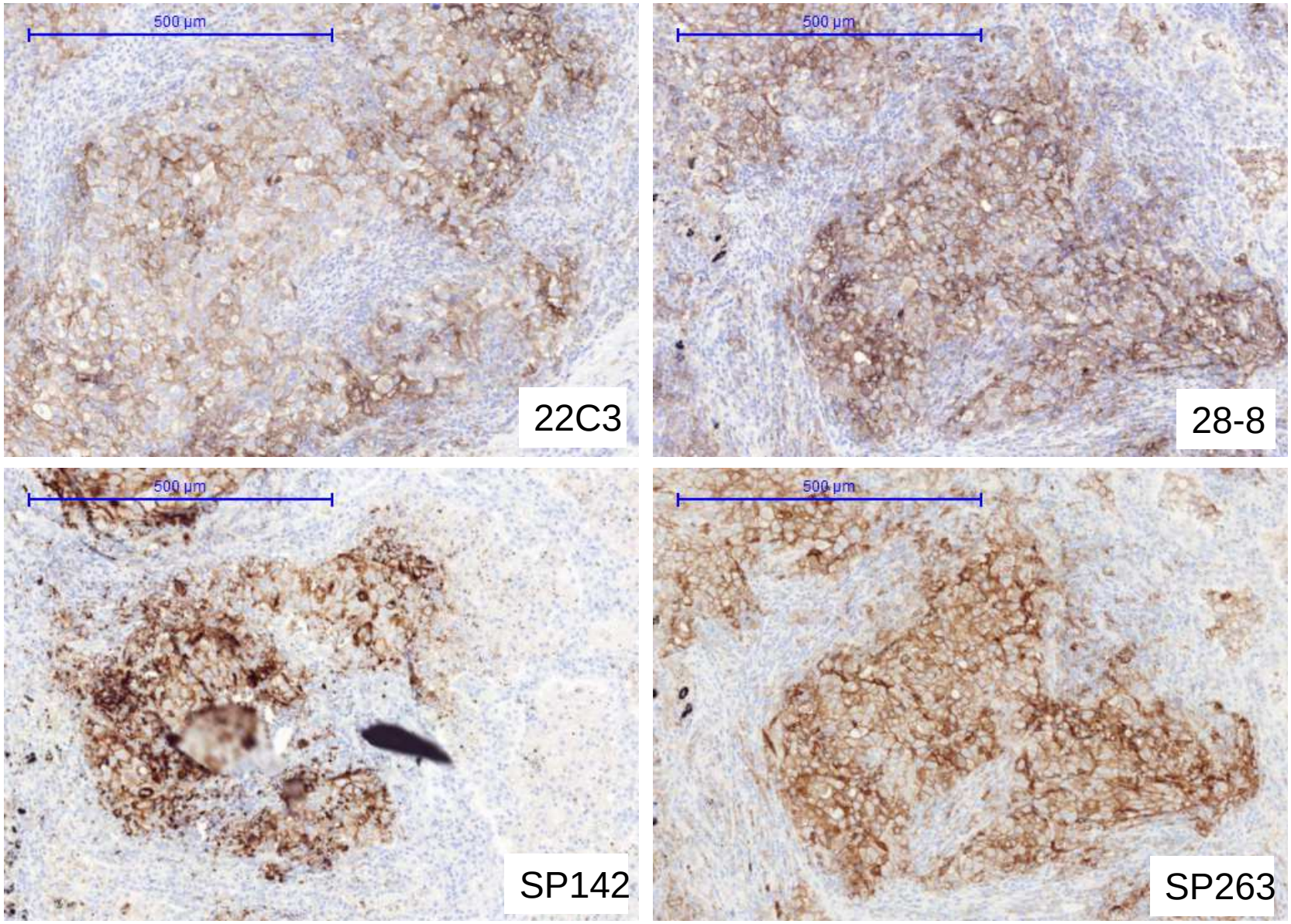
PD-L1 IHC: Accuracy



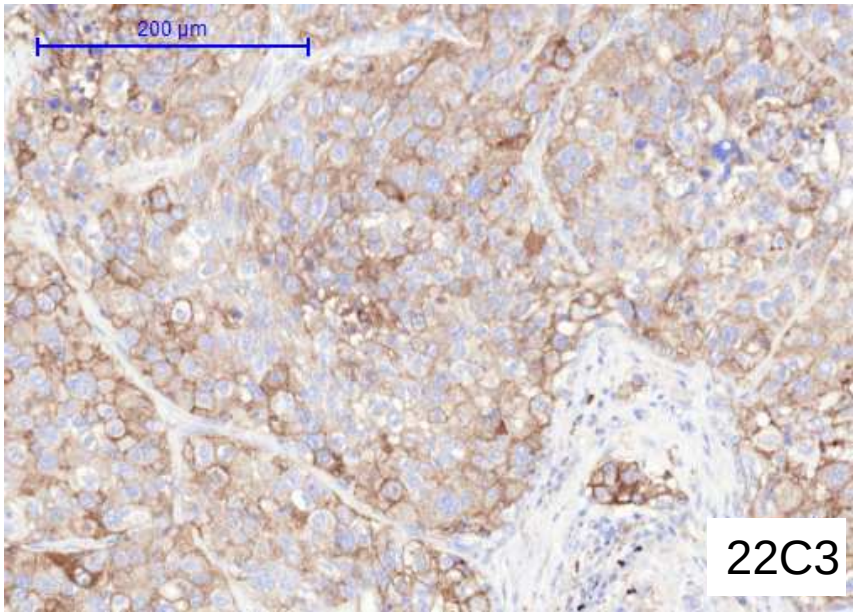
PD-L1 IHC: Tonsil – germinal center

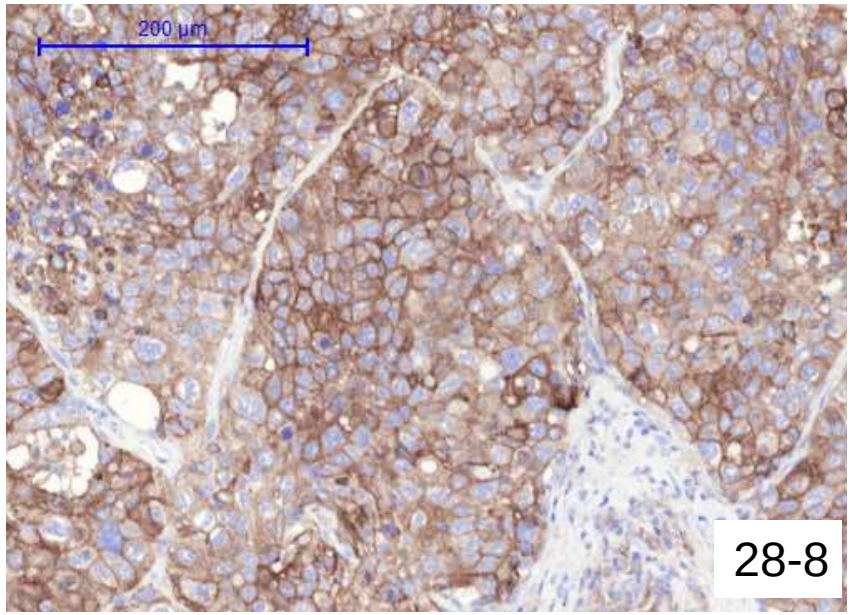


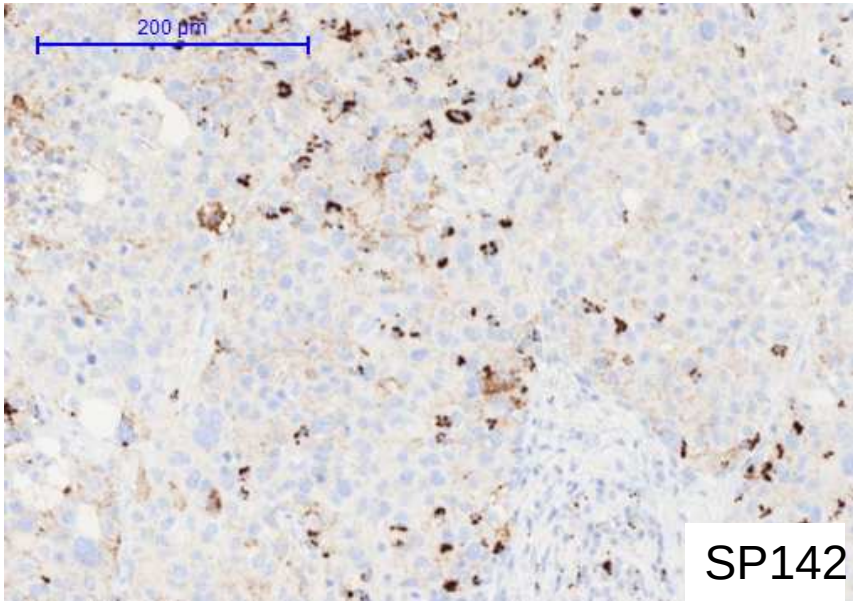
Tumor: Similar PD-L1 TC staining pattern

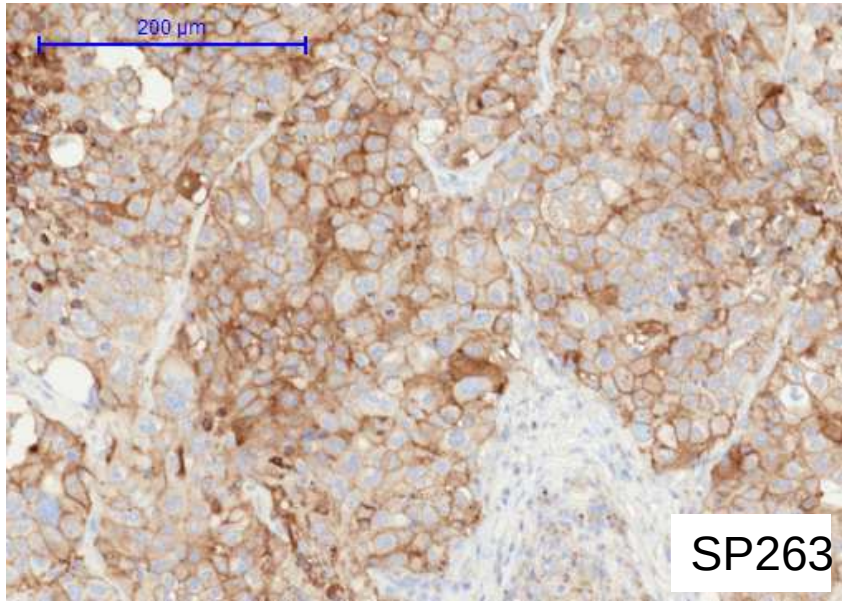


Tumor: Different PD-L1 staining pattern









Different PD-L1 assays

- Further comparison is needed
  - FDA blueprint phase II ongoing
- Clinical relevance of the observed differences?
- Commercial kits VS LDT
  - Always comparison with the approved kit or use the kit without modification.

PD-L1 Expression (IHC) as a Predictive Biomarker

Harmonization Studies

PD-L1 Blueprint Working Group

PD-L1 IHC ASSAYS FOR LUNG CANCER: RESULTS FROM PHASE 1 OF THE "BLUEPRINT PD-L1 ASSAY COMPARISON PROJECT"

Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas

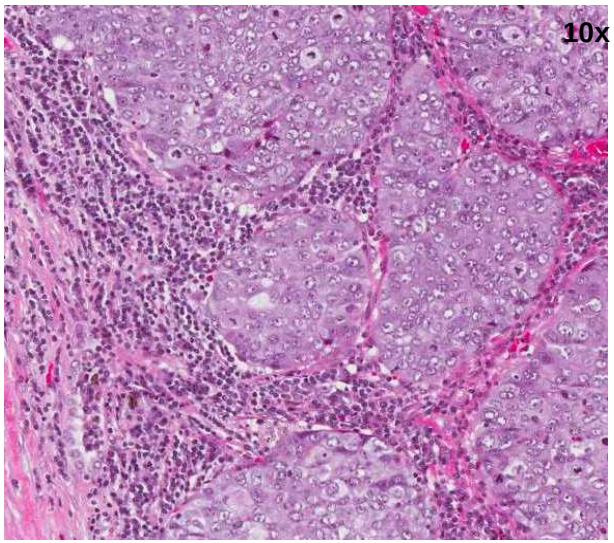
- Scores in tumor cells (TCs) were similar for 22C3, 28-8, and SP263; lower for SP142
- SP263 and SP142 stained immune cells (ICs) more intensely
- Higher agreement when assessing TCs than when assessing ICs

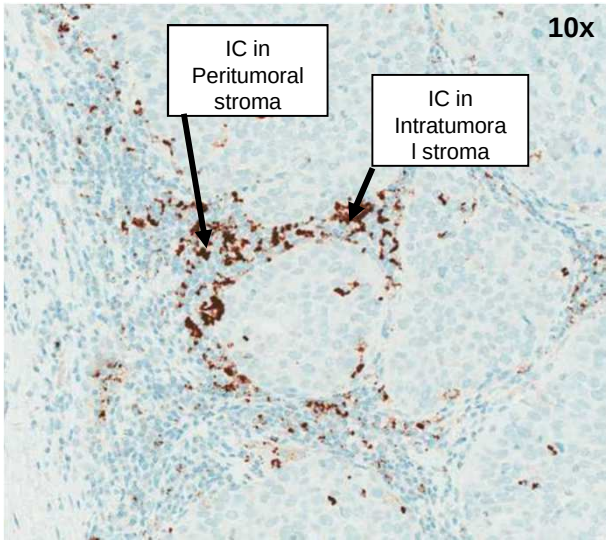
PD-L1 IHC Evaluation by the Pathologist

- Pathologist
  - » **% PD-L1 + in tumor zone**
    - » **PD-L1 tumor signal (membranous/any intensity)**
    - » **PD-L1+ tumor cells and/or PD-L1+ immune cells (SP-142)**
- Different cut-offs for PD-L1 positivity
  - Per kit
  - Per tumor type
    - NSCLC
      - » 22C3 : ≥ 50% TC
      - » 28-8 : ≥ 1% TC
      - » SP142 : ≥ 50% TC (TC3) or ≥ 10% IC (IC3)

PDL1 SP-142 IC Staining - Aggregates

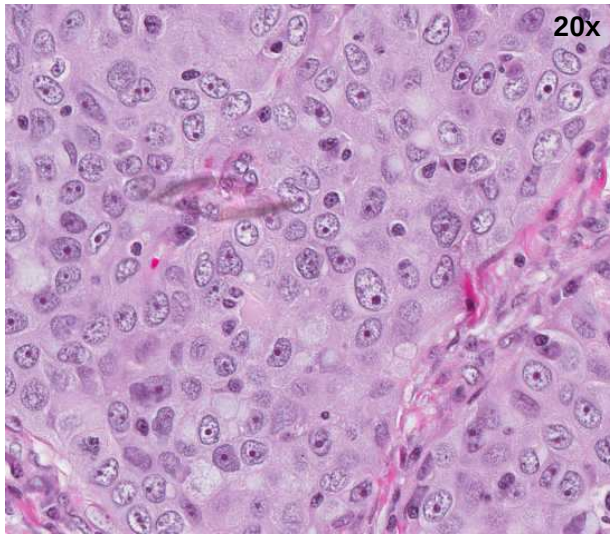
HistoPattern: Often at the interface tumor strands and stroma

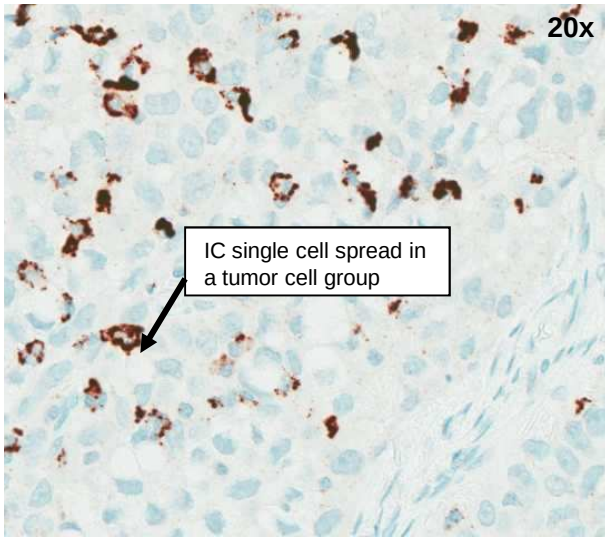




PDL1 SP-142 IC Staining – Single Cell Spread

HistoPattern: Often intra-epithelial immune cells

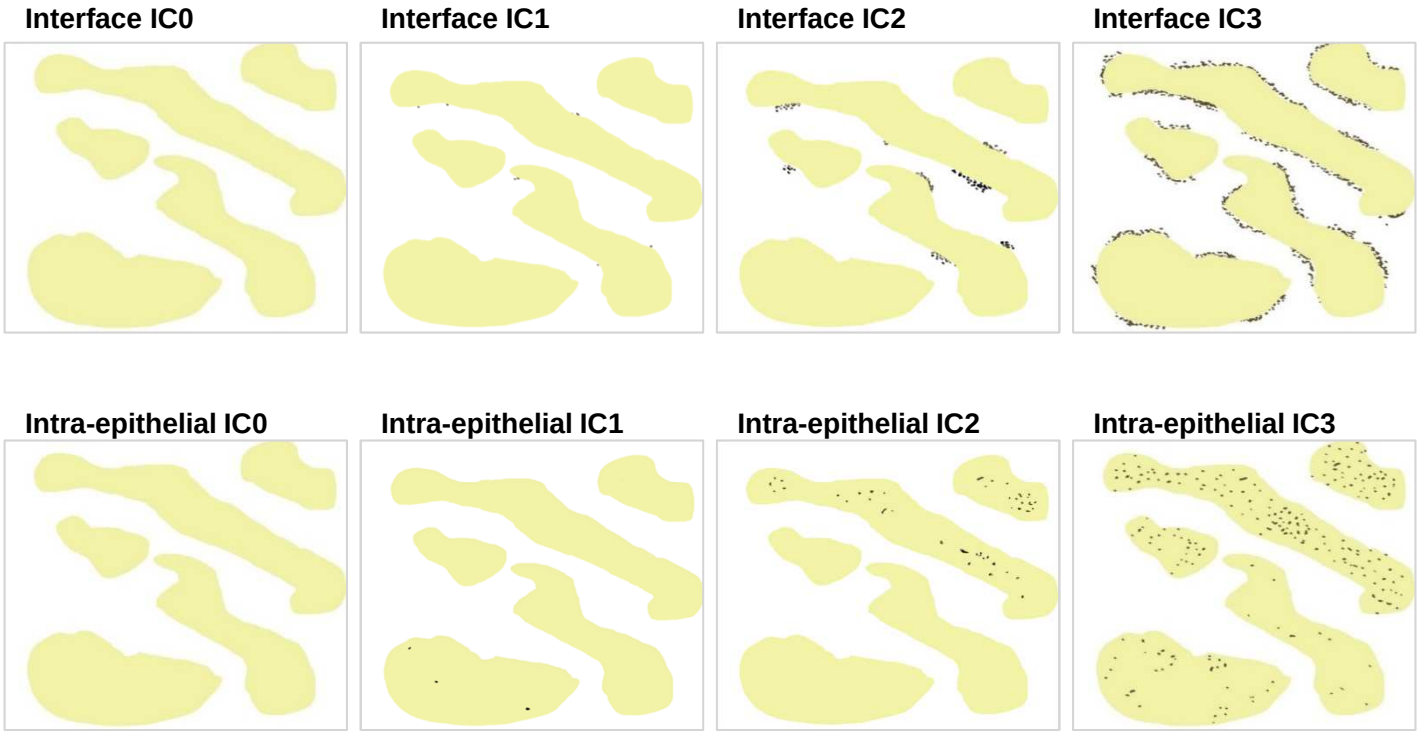




The Pattern estimation is the translated to the the final % IC score

PD-L1 IC Staining Criteria	
IC Score	**% of PD-L1–Expressing IC**
IC3	≥ 10%
IC2	≥ 5% and < 10%
IC1	≥ 1% and < 5%
IC0	< 1%

Graphical representation of interface IC scores and intra-epithelial IC scores



Sample Types Used for PD-L1 Analysis in NSCLC & Bladder Cancer

NSCLC

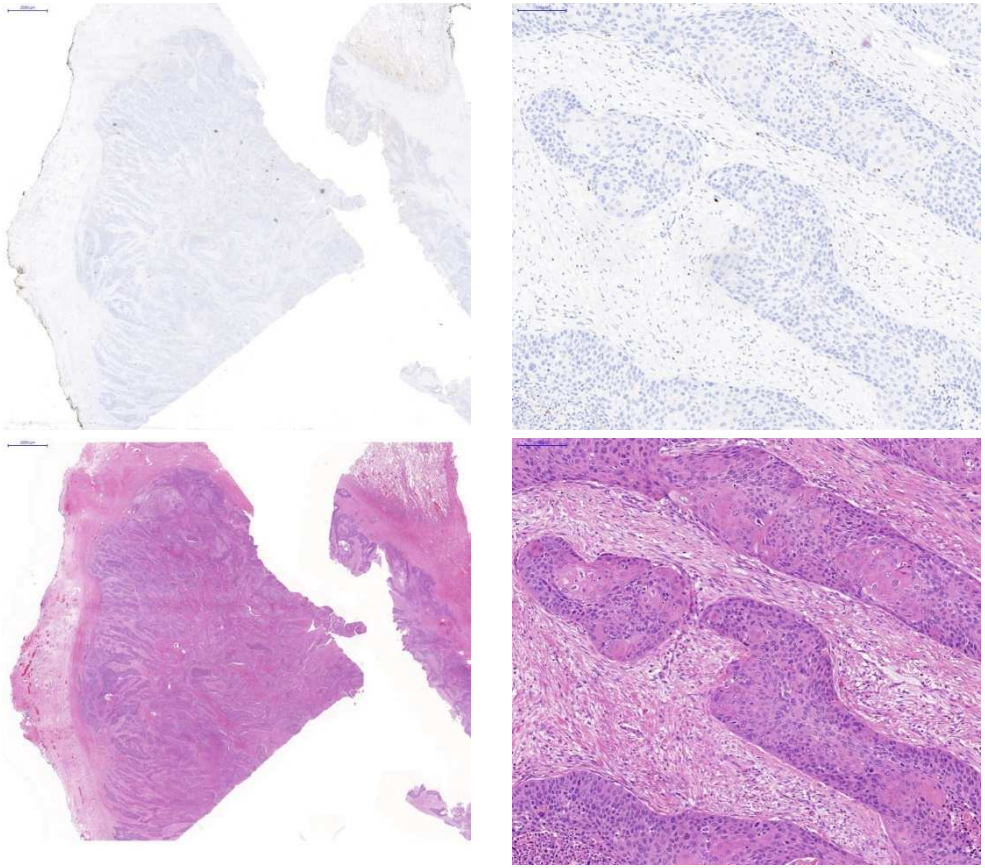
- Bronchoscopic Biopsies
- Tru Cut Biopsies
- Resections
- Metastatic Lesions

Bladder

- TransUrethral Resection (TUR)
- Resections
- Metastatic Lesions

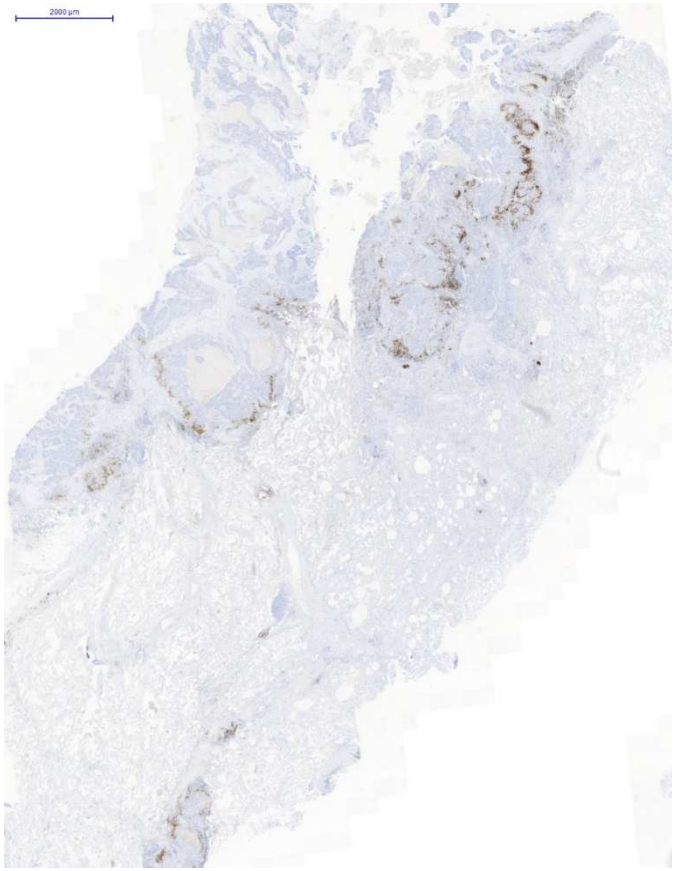
PDL1 SP142

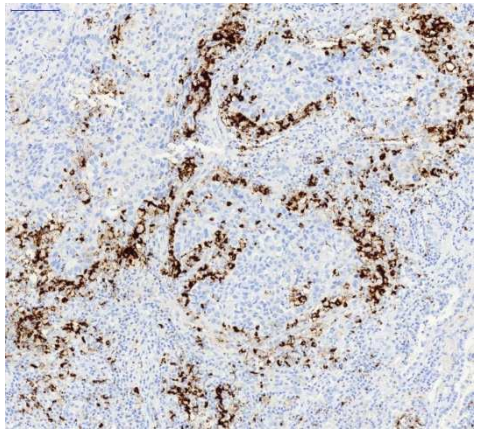
	IC0	Negative

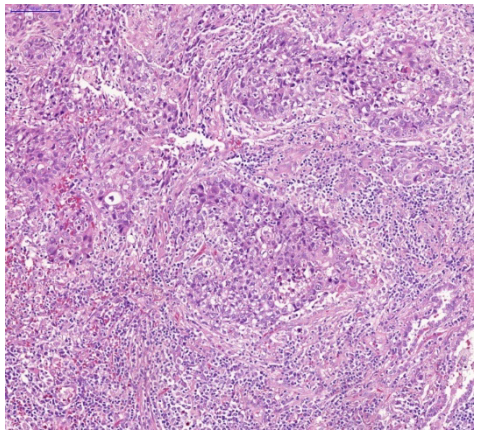


PDL1 SP142

IC aggregate type	IC2	**Pattern in less then half of tumor area**

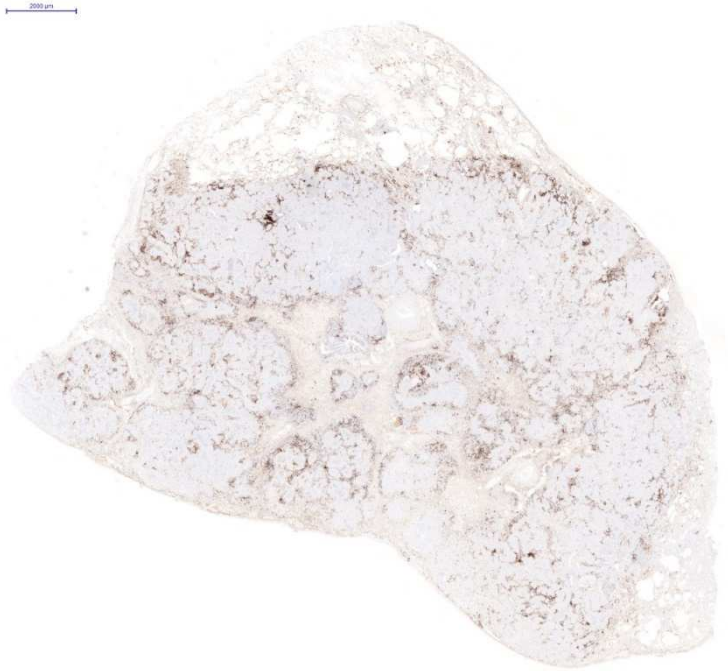


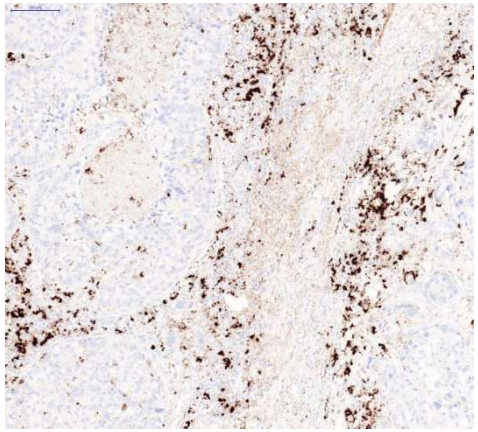


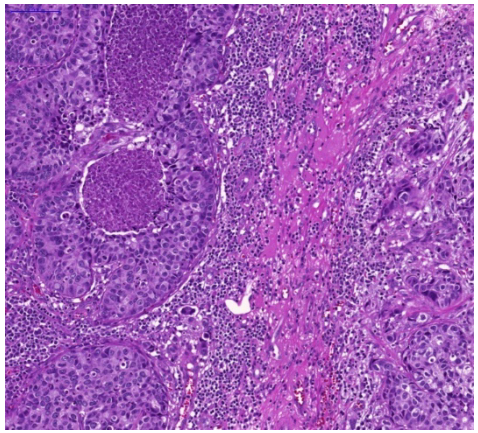


PDL1 SP142

IC aggregate type	IC3	**Pattern in more then half of the tumor area**

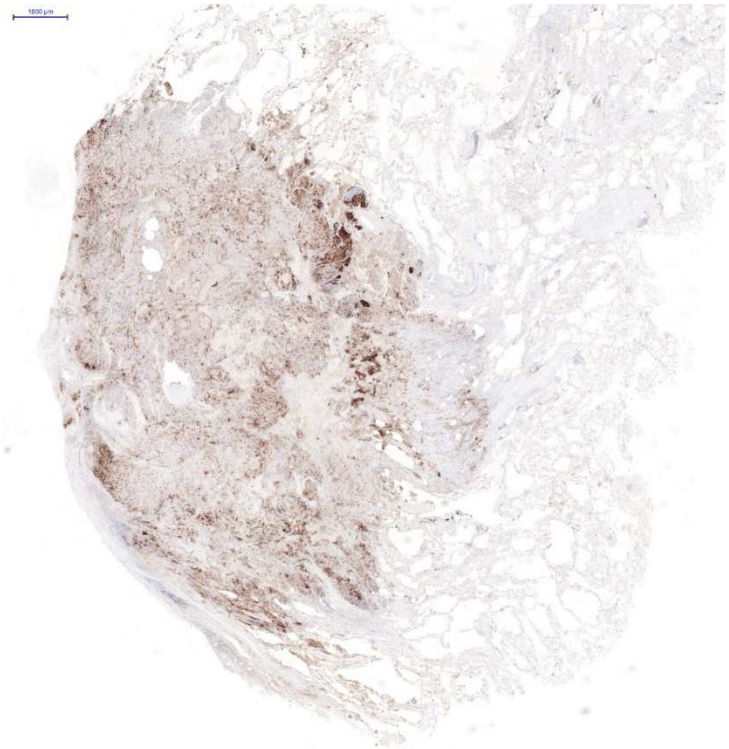


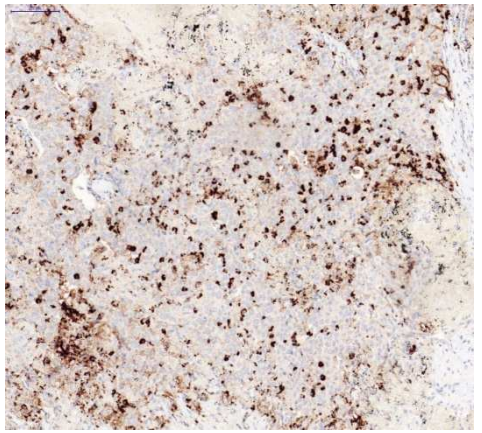


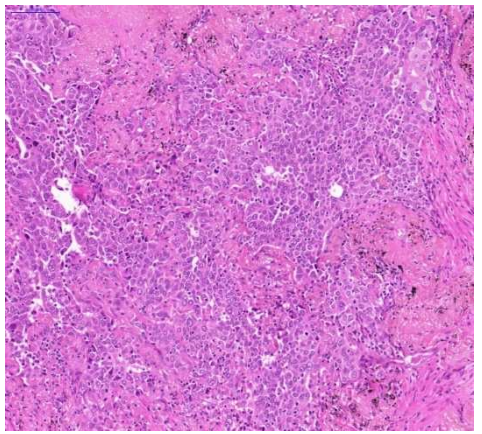


PDL1 SP142

IC single cell spread	**IC3**	**Patten in more then half of tumor area**

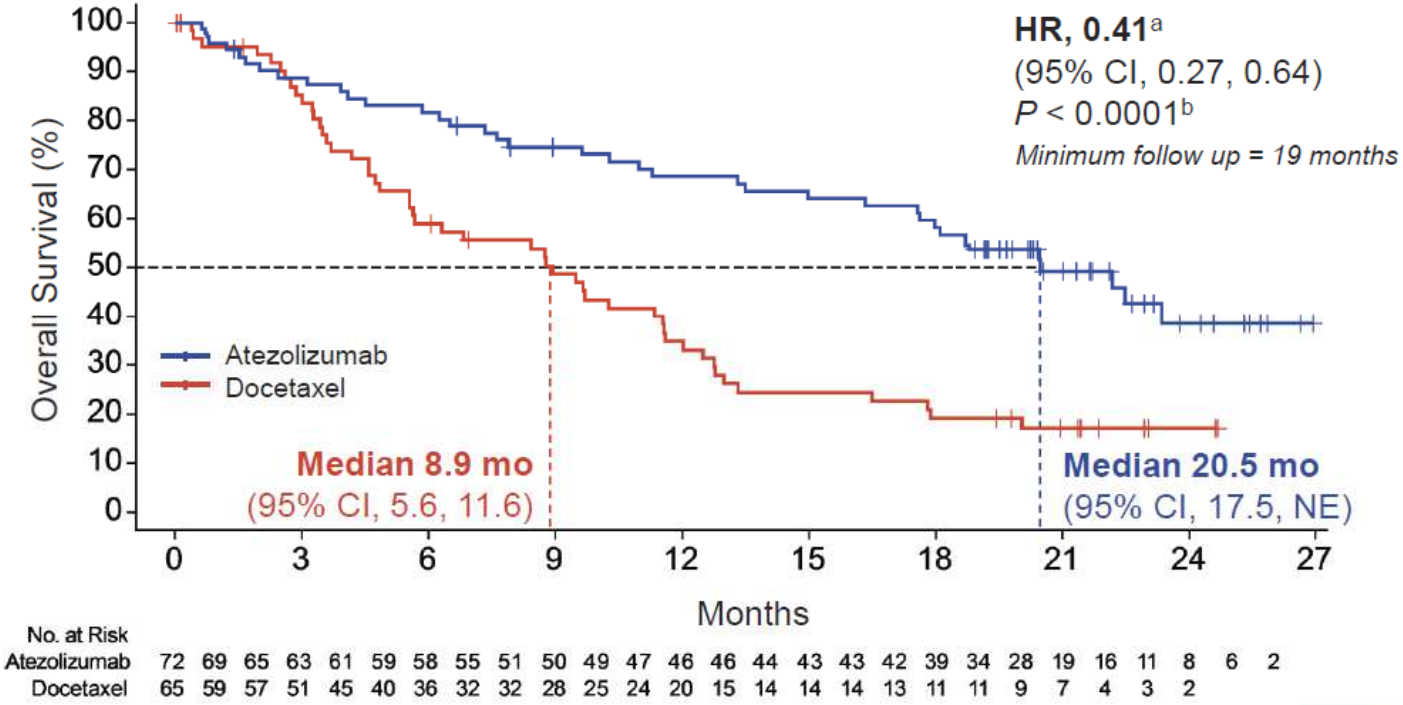






OS, PD-L1 EXPRESSION ON ≥ 50% TC OR ≥ 10% IC

TC3 OR IC3; 16% OF PATIENTS



[a]Unstratified HR.
[b]*P* values for descriptive purpose only.
TC, tumor cells; IC, tumor-infiltrating immune cells; OS, overall survival.

Barlesi et al, Atezolizumab Phase III OAK Study. http://tago.ca/9Hh

Sample Types Used for PD-L1 Analysis in NSCLC & Bladder Cancer

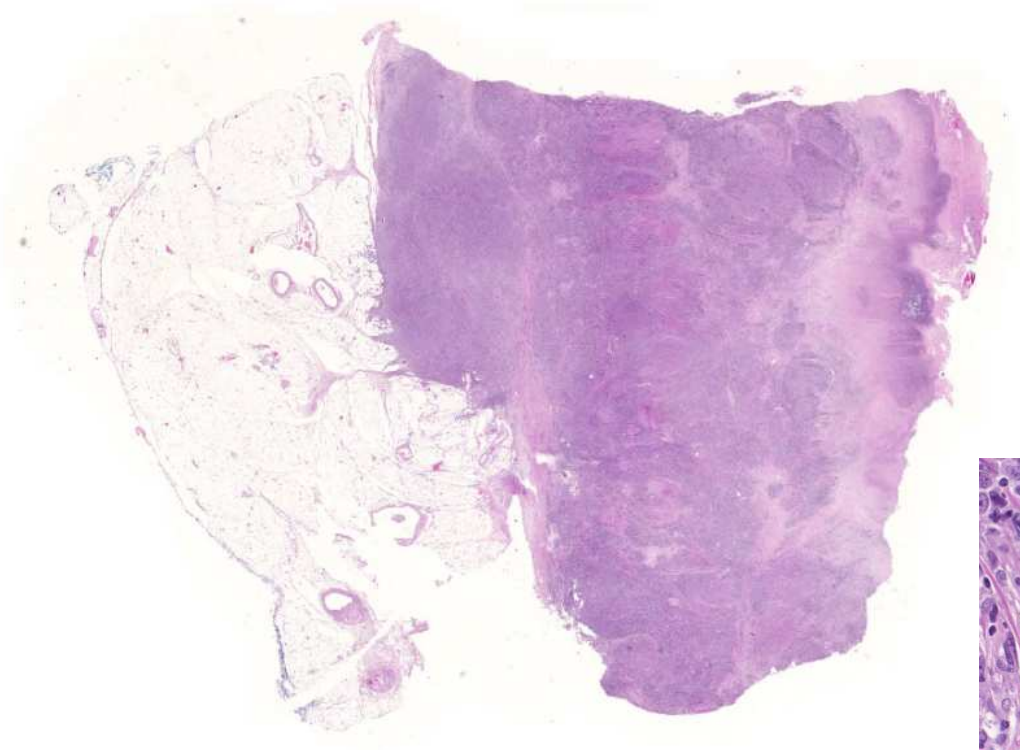
NSCLC

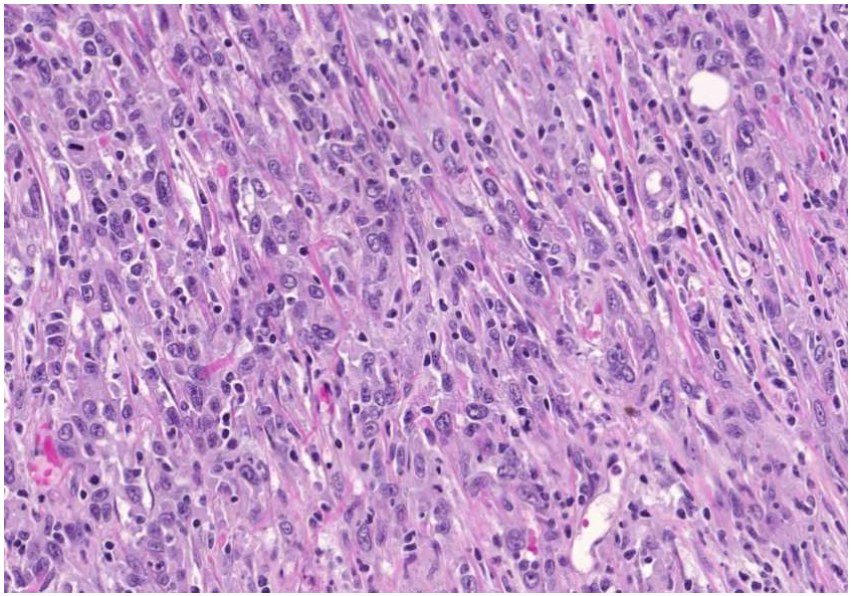
- Bronchoscopic Biopsies
- Tru Cut Biopsies
- Resections
- Metastatic Lesions

Bladder

- TransUrethral Resection (TUR)
- Resections
- Metastatic Lesions

Bladder Cancer Resection

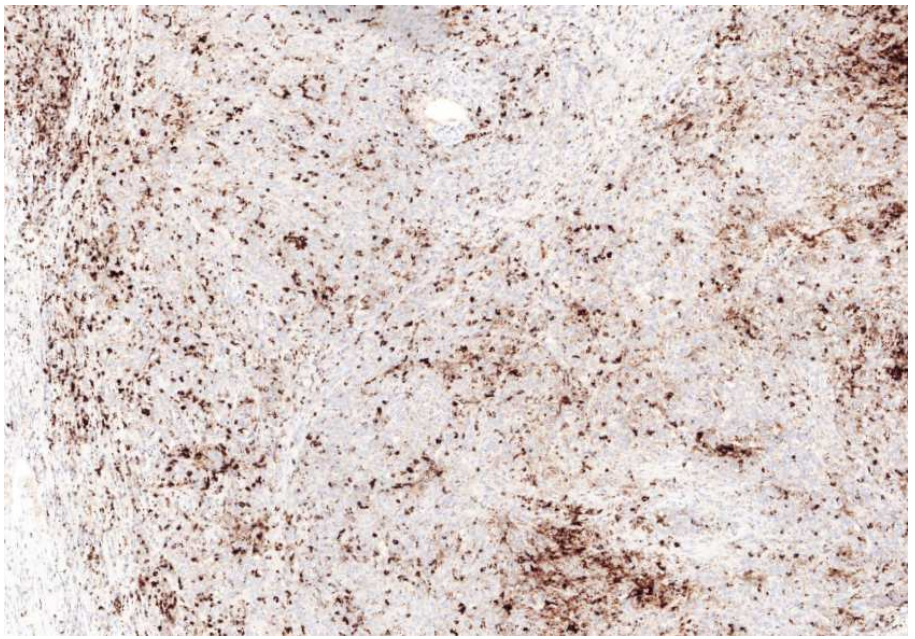




Bladder Cancer Resection

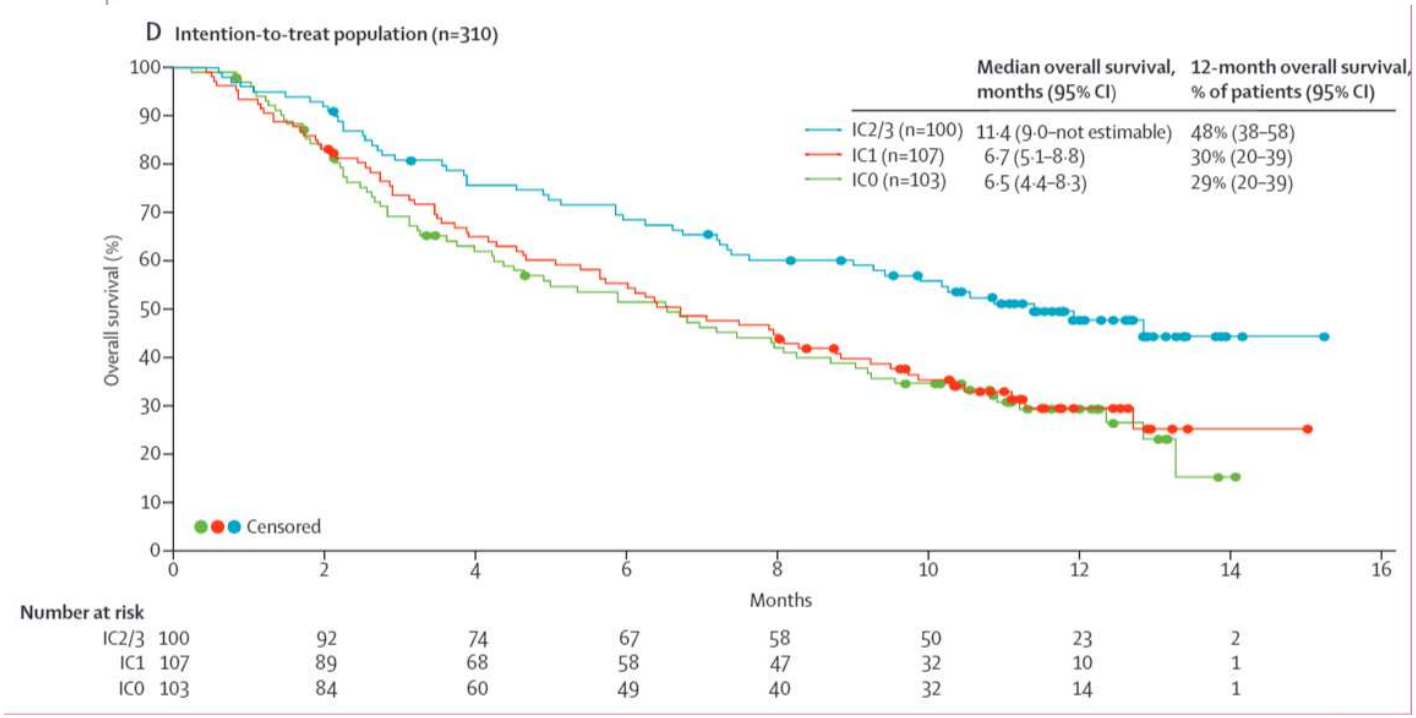
PD-L1 Positive TC and IC Staining

PDL1 SP142



Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial

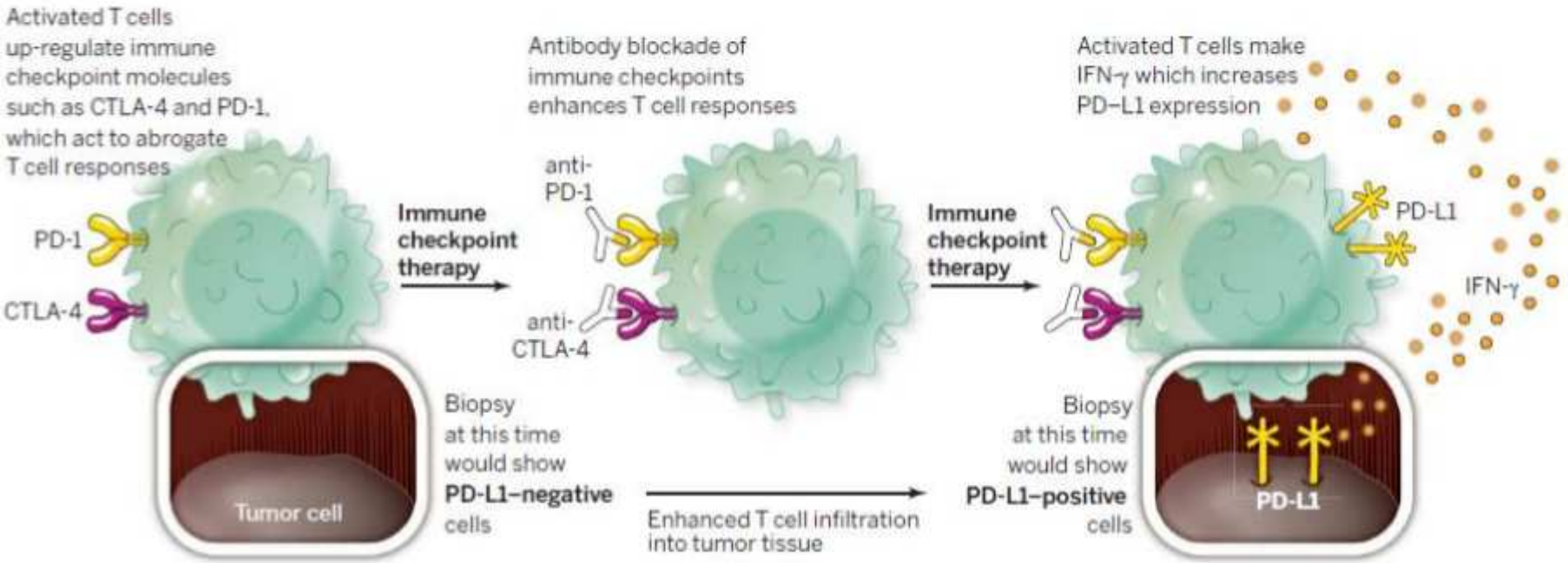
Jonathan E Rosenberg, Jean Hoffman-Censits, Tom Powles, Michiel S van der Heijden, Arjun V Balar, Andrea Necchi, Nancy Dawson, Peter H O'Donnell, Ani Balmanoukian, Yohann Loriot, Sandy Srinivas, Margitta M Retz, Petros Grivas, Richard W Joseph, Matthew D Galsky, Mark T Fleming, Daniel P Petrylak, Jose Luis Perez-Gracia, Howard A Burris, Daniel Castellano, Christina Canil, Joaquim Bellmunt, Dean Bajorin, Dorothee Nickles, Richard Bourgon, Garrett M Frampton, Na Cui, Sanjeev Mariathasan, Oyewale Abidoye, Gregg D Fine, Robert Dreicer



Lancet 2016

PD-L1 Expression (IHC) as a Predictive Biomarker

Spatiotemporal Heterogeneity



Conclusions

- The PDL1 Tumor cell (TC) staining of 28-8, 22C3 and SP263 show a good correlation if done with the correct detection system
- This indicates that selection of patients for PDL1 TC expression can be done by one of these validated assays.
- The PDL1 SP142 shows lower TC staining but a crisp IC staining
- PD-L1 scoring SP142 will be the first PDL1 IC assay.
- Pattern approach helps for IC scoring

HistoGeneX