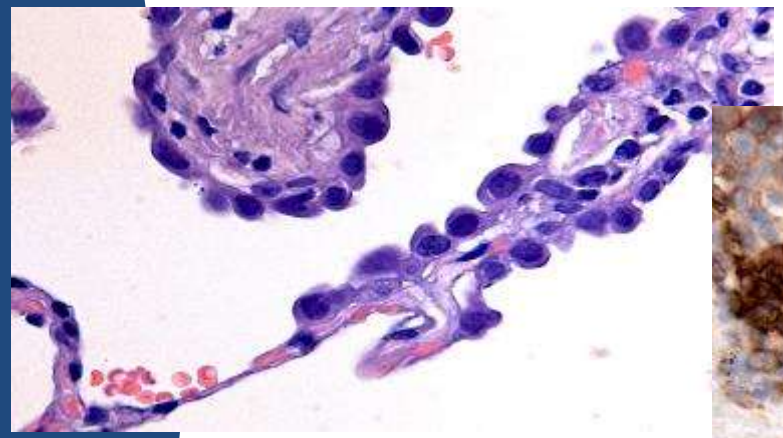

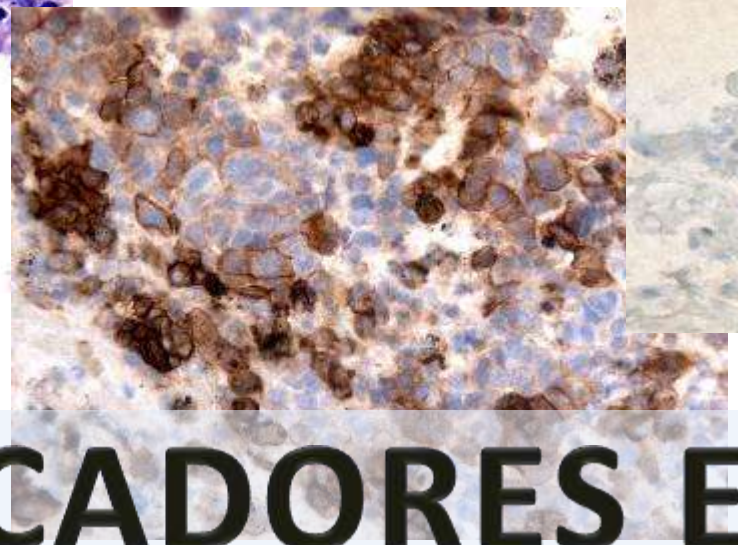

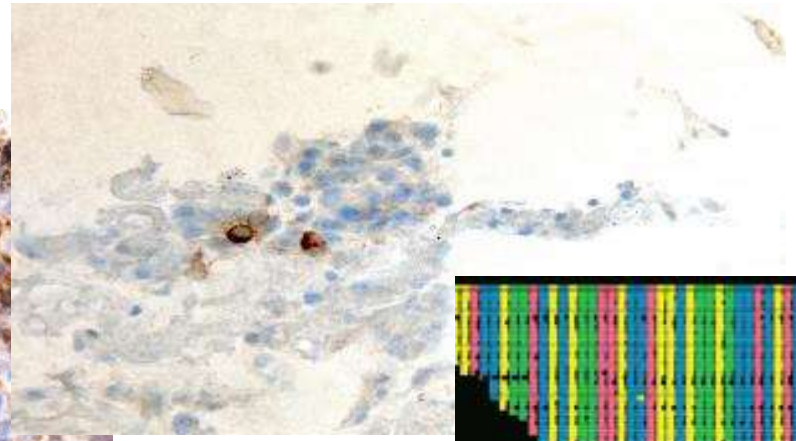

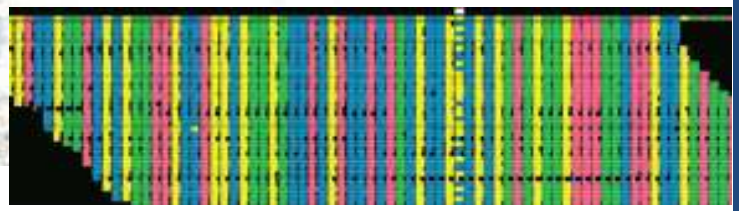

BIOMARCADORES EN INMUNOTERAPIA

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Santander

25 ONKOLOGIA MEDIKOAREN URTEURRENA
ANIVERSARIO ONCOLOGÍA MÉDICA

Valdecilla Instituto de Investigación Sanitaria IDIVAL

Etorkizunari begira
Mirando al futuro

DECLARACIÓN DE POTENCIALES CONFLICTOS DE INTERESES

Título de la presentación: Biomarcadores en inmunoterapia

Autor: Dr José Javier Gómez Román

☒ Declaro no tener ningún conflicto de interés.

☐ Relativas a esta presentación existen las siguientes relaciones que podrían ser percibidas como potenciales conflictos de interés

GUIÓN

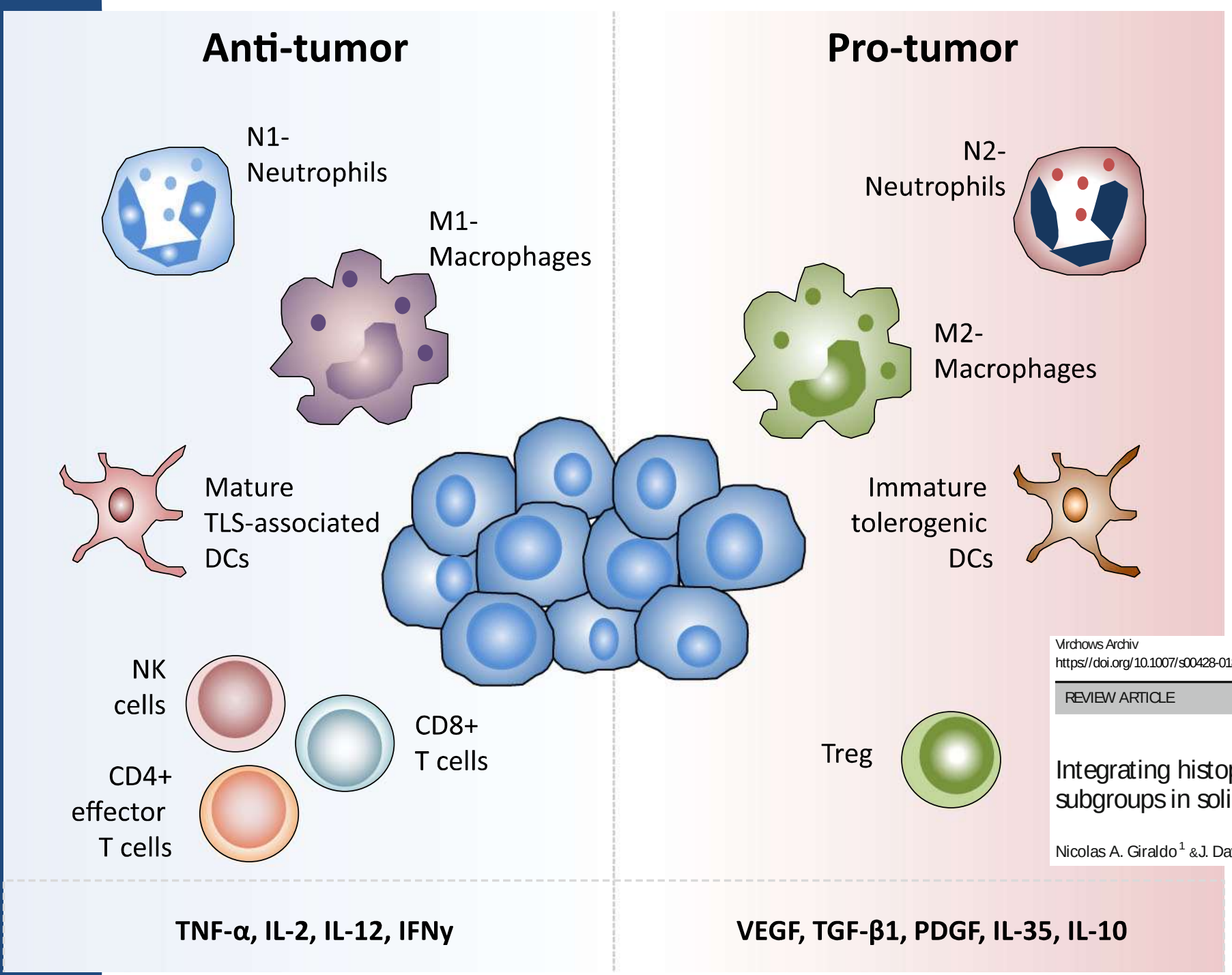
1. Conceptos básicos en PD-L1
2. ¿En qué muestras?
3. Novedades técnicas (TMB)
4. Impacto clínico
5. Retos

GUIÓN

1. **Conceptos básicos en PD-L1**
2. ¿En qué muestras?
3. Novedades técnicas (TMB)
4. Impacto clínico
5. Retos

LA RESPUESTA INMUNE

- **Inmunidad innata**
  - Elementos celulares y extracelulares que proporcionan la primera línea de defensa ante una agresión
  - Poco específica y carece de mecanismos de memoria
  - PMN, Macrófagos y linfocitos NK
    - PMN N1: Citoquinas antitumorales (TNFα, IL12)
    - PMN N2: Proangiogénicas (VEGF) e inmunosupresoras (TGFβ)
    - Macrófagos M1: Proinflamatorios antitumorales (Toll-like e IFNγ)
    - Macrófagos M2: Inmunomoduladores y proliferación de fibroblastos (IL4, IL13, TGFβ, PDGF)



Virchows Archiv
https://doi.org/10.1007/s00428-018-02517-1

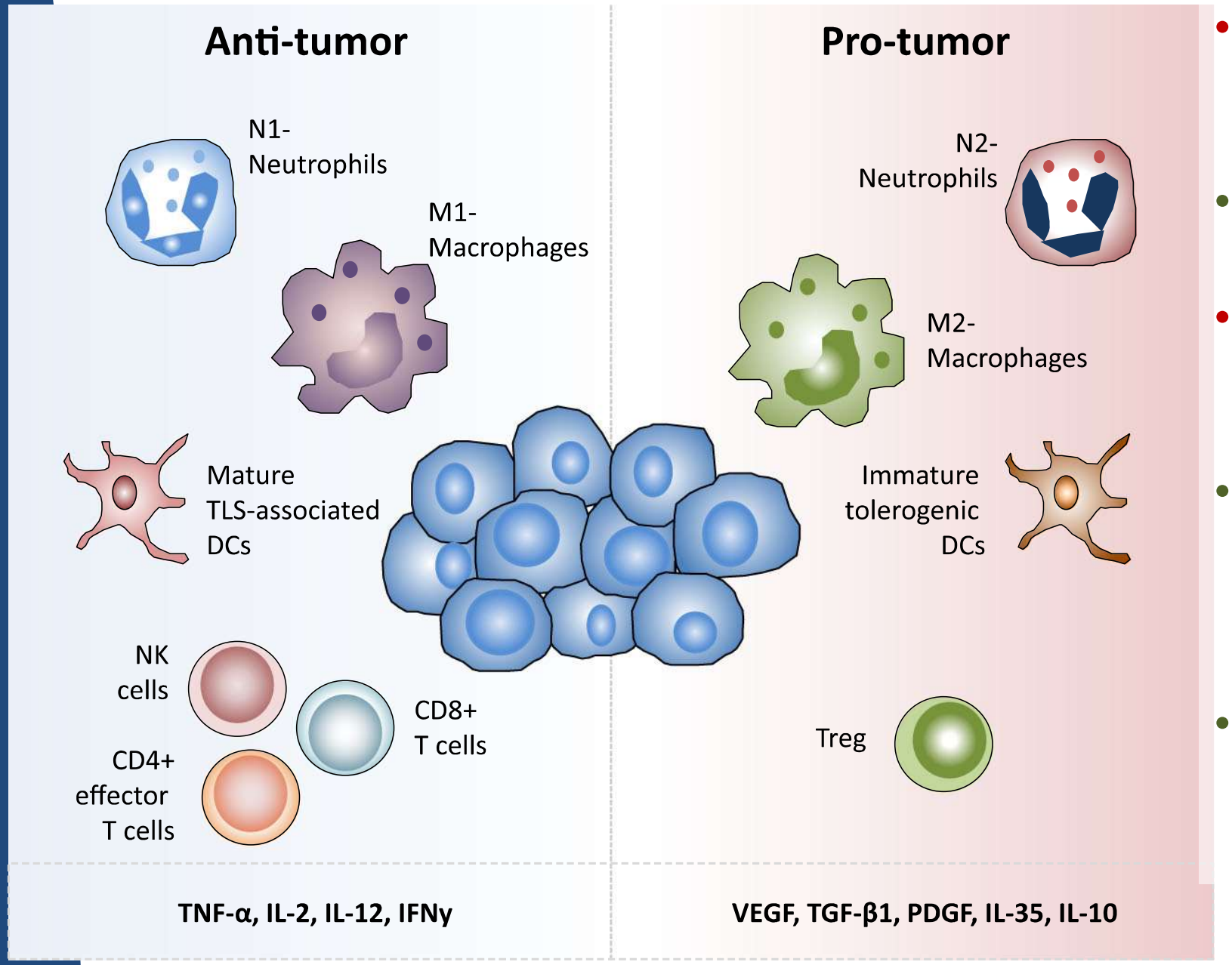
REVIEW ARTICLE

Integrating histopathology, immune biomarkers, and molecular subgroups in solid cancer: the next step in precision oncology

Nicolas A. Giraldo[1] &J. David Peske[1] &Catherine Sautès-Fridman[2,3,4] &Wolf H. Fridman[2,3,4]

LA RESPUESTA INMUNE

- **Inmunidad adaptativa**
  - Células que construyen una respuesta inmune específica de antígeno. Desarrollan memoria
  - Linfocitos T CD4 y CD8 y Linfocitos B
    - CD8 citotóxicos previamente activados destruyen células tumorales
    - CD4 modulan la respuesta. Desde activadores hasta los Treg



- **PMNs** peor pronostico en renal, urotelial, cabeza y cuello o esofágico
- **PMNs** mejor pronóstico en colorrectal y gástrico
- **Macrófagos** peor pronóstico en endometrio, esófago, urotelial, mama y ovario
- **Macrófagos** mejor pronóstico en colorrectal, gástrico, pulmón, hígado, próstata y cérvix
- En general **CD8** mejor pronóstico excepto en Linfomas B y Renal

Prognosis

Virchows Archiv
https://doi.org/10.1007/s00428-018-02517-1

REVIEW ARTICLE

Integrating histopathology, immune biomarkers, and molecular subgroups in solid cancer: the next step in precision oncology

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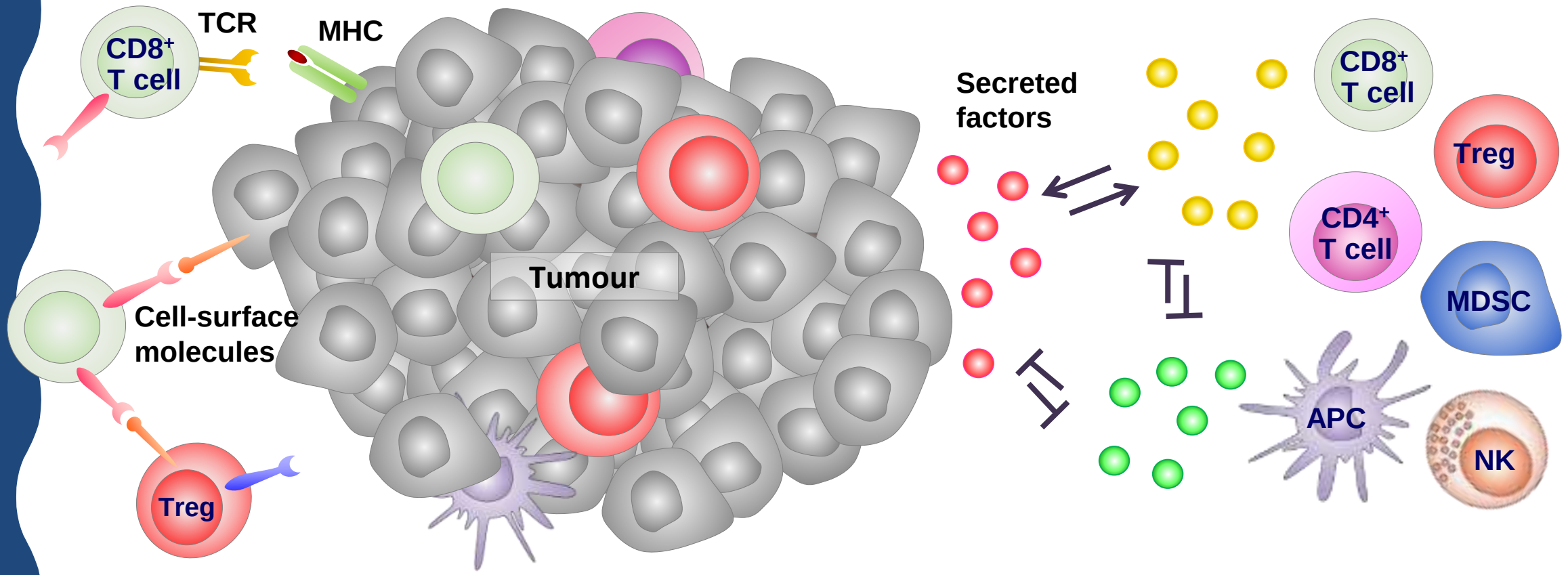
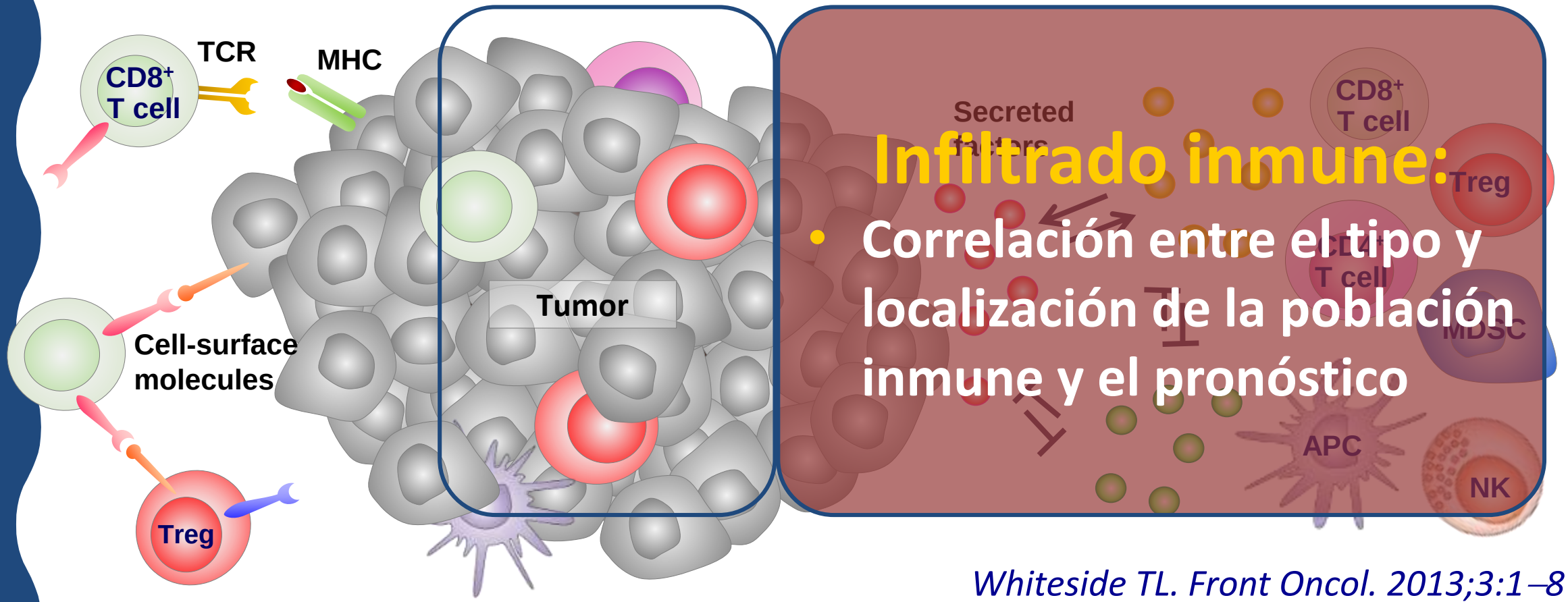
TIPOS DE BIOMARCADORES BASADOS EN LA RESPUESTA INMUNE



Whiteside TL. Front Oncol. 2013;3:1–8

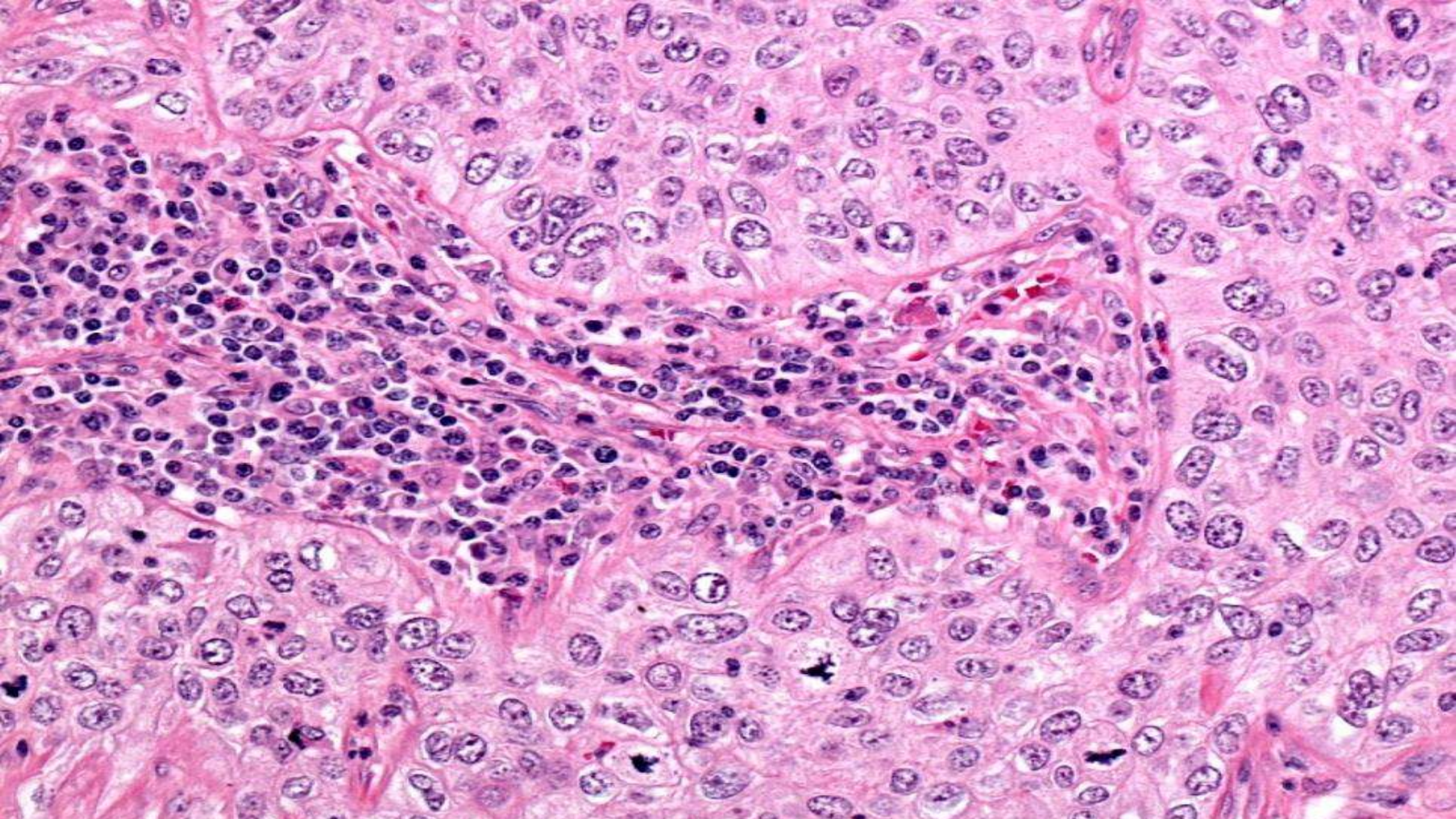
TIPOS DE BIOMARCADORES BASADOS EN LA RESPUESTA INMUNE

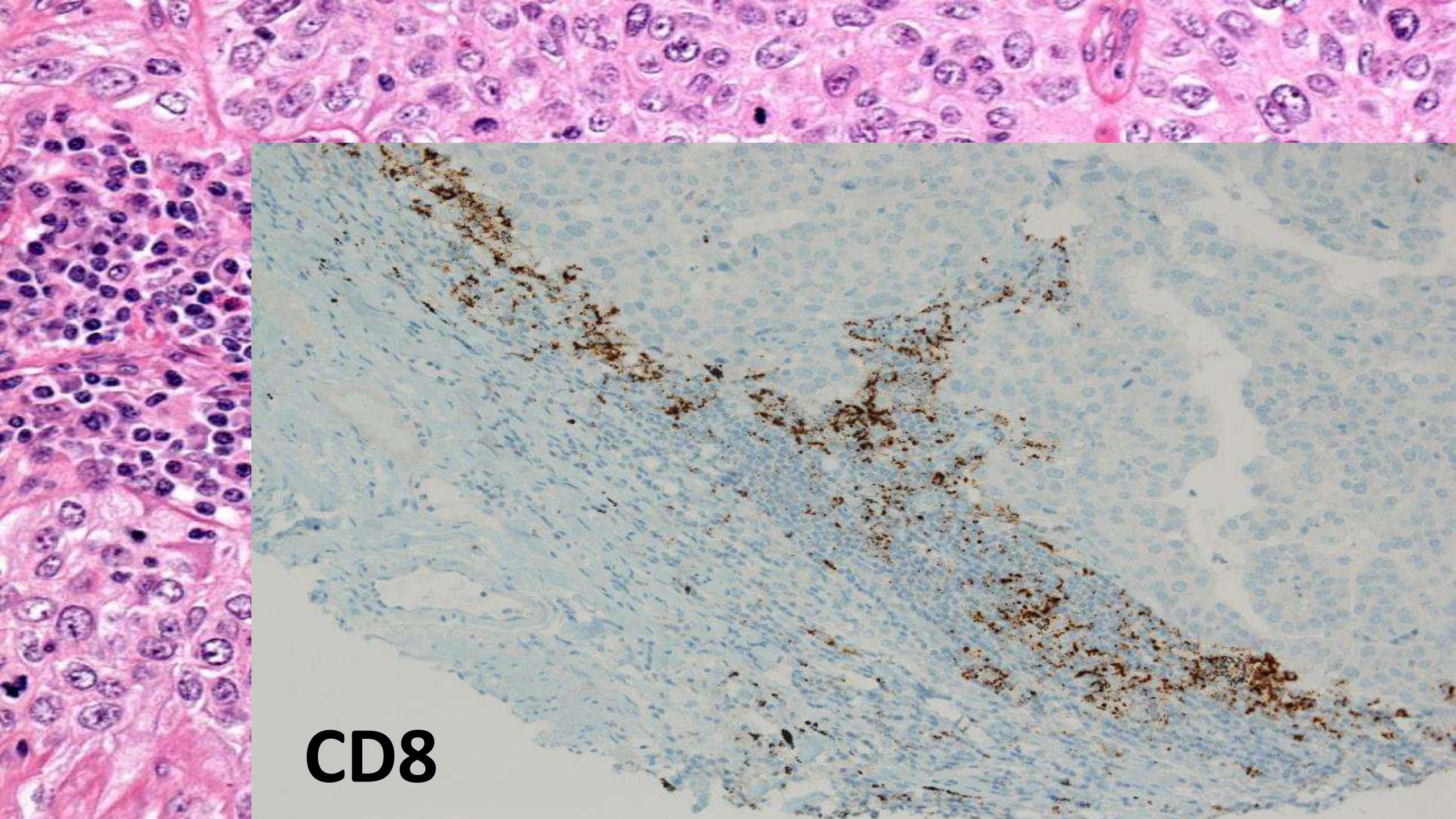


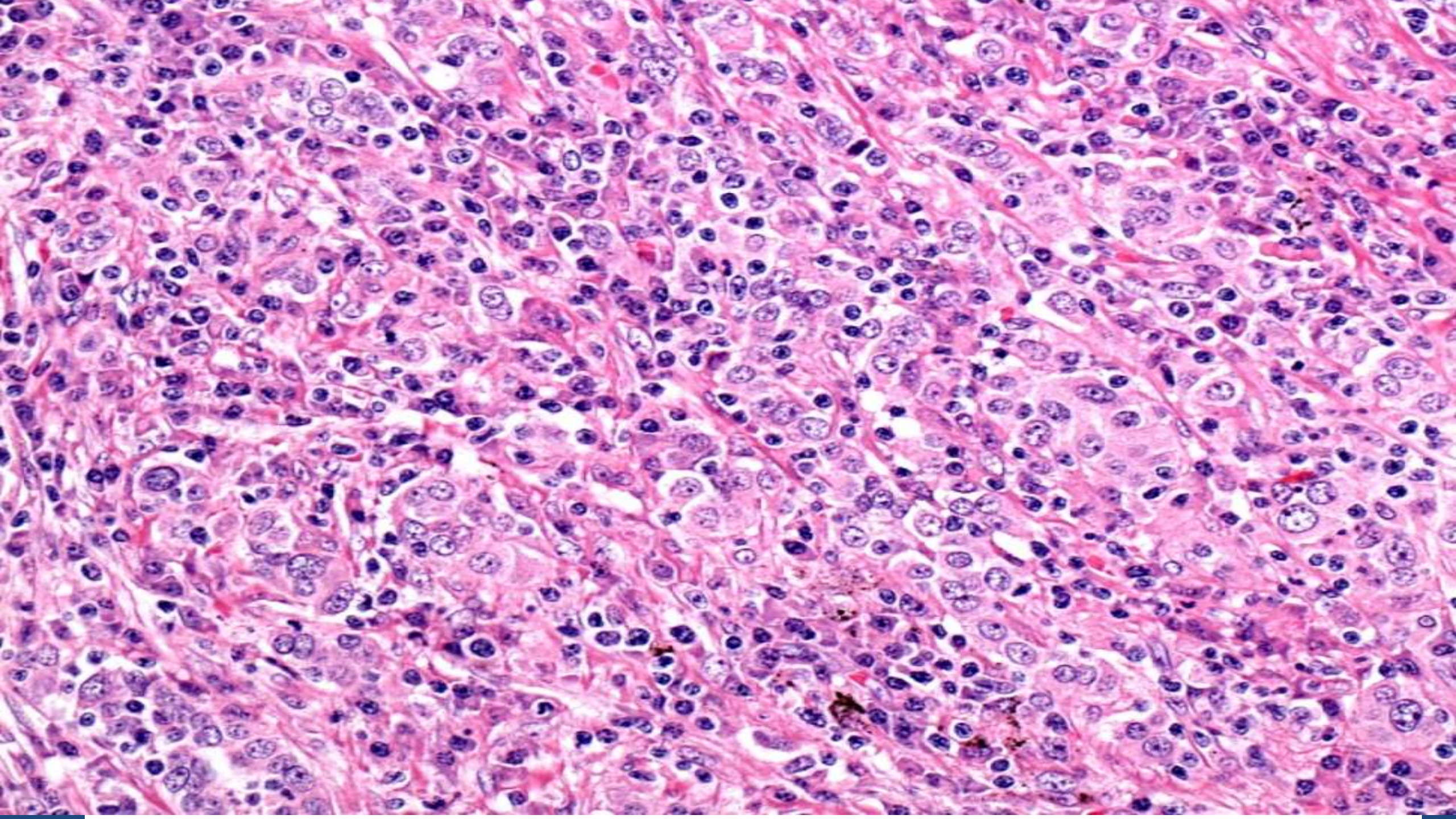
Infiltrado inmune:

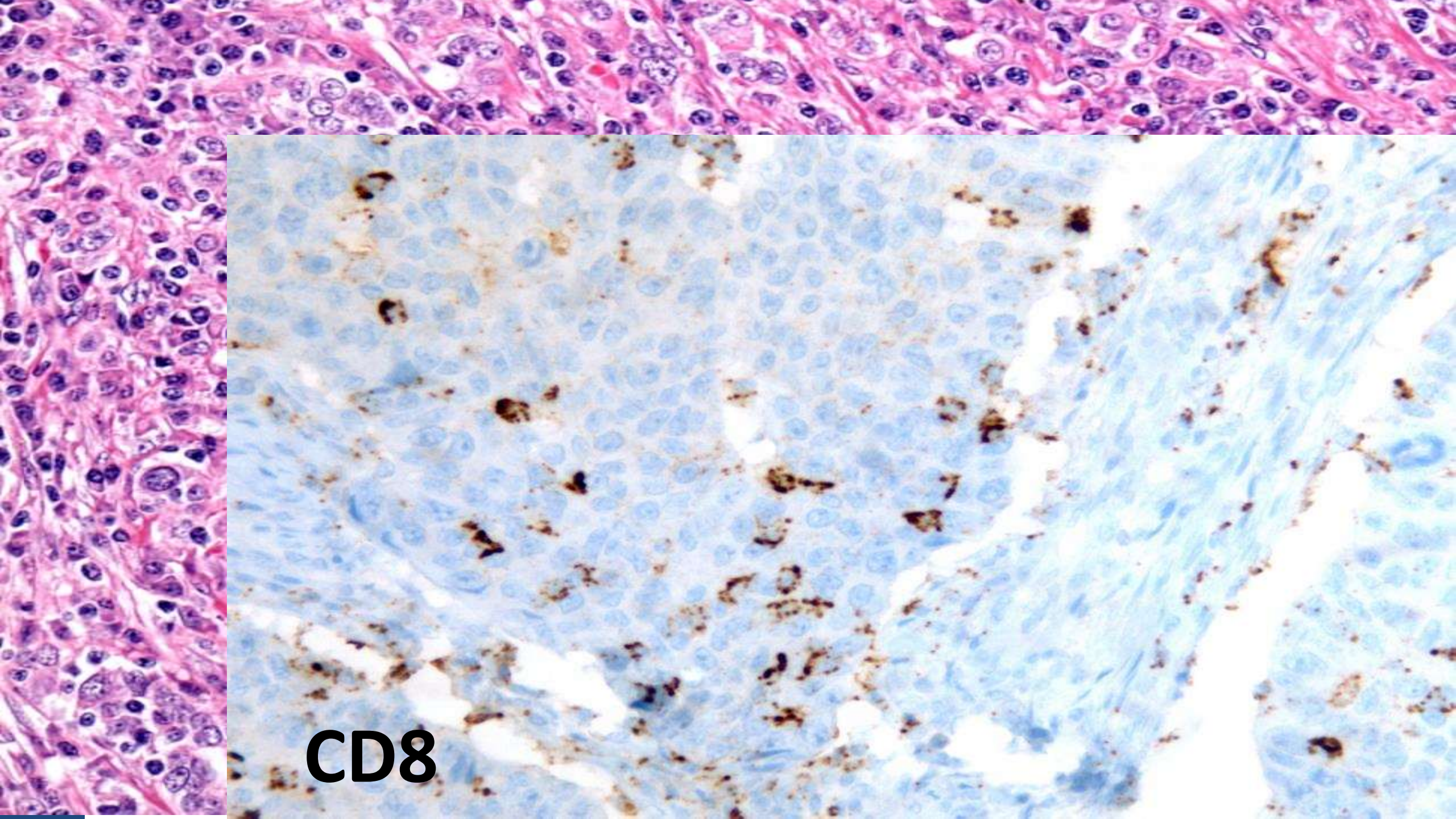
- Correlación entre el tipo y localización de la población inmune y el pronóstico

Whiteside TL. Front Oncol. 2013;3:1–8
Galon J, et al. Immunity. 2013;39:11–26
Fridman WH et al. Nat Rev Cancer. 2012;12:298–306




CD8

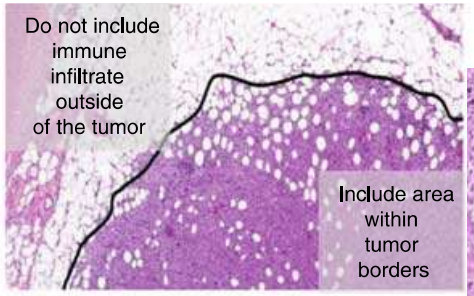


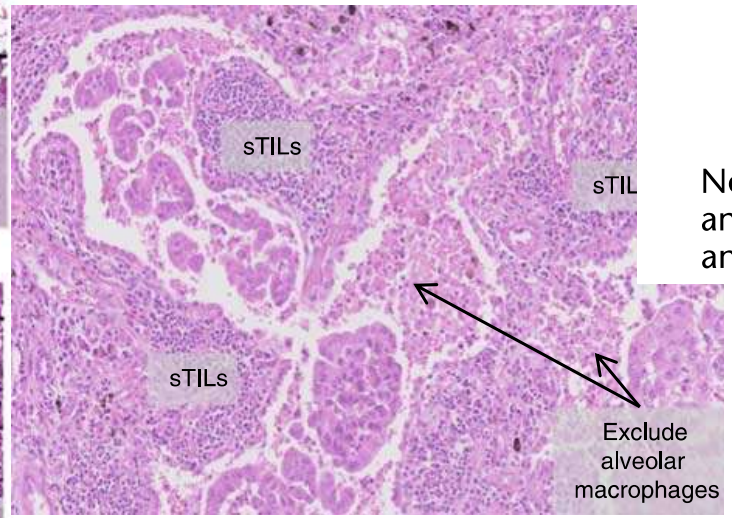

CD8

Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinomas, Non–Small Cell Lung Carcinoma and Mesothelioma, Endometrial and Ovarian Carcinomas, Squamous Cell Carcinoma of the Head and Neck, Genitourinary Carcinomas, and Primary Brain Tumors

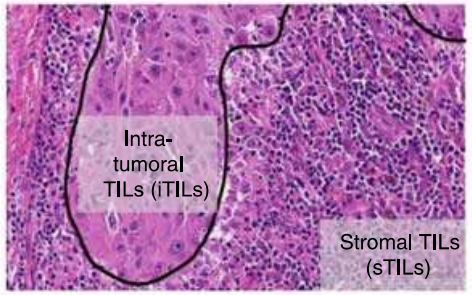
(*Adv Anat Pathol* 2017;00:000–000)

Step 1: Select tumor area

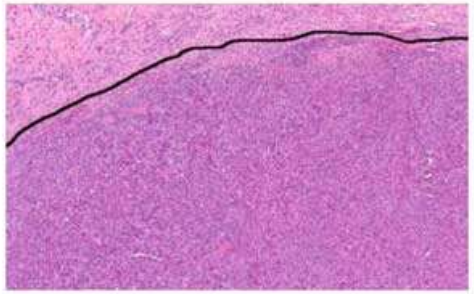




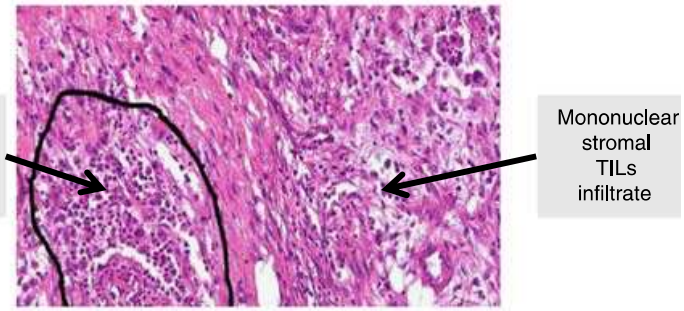
Step 2: Define stromal and intra-tumoral areas



Step 3: Scan at low magnification

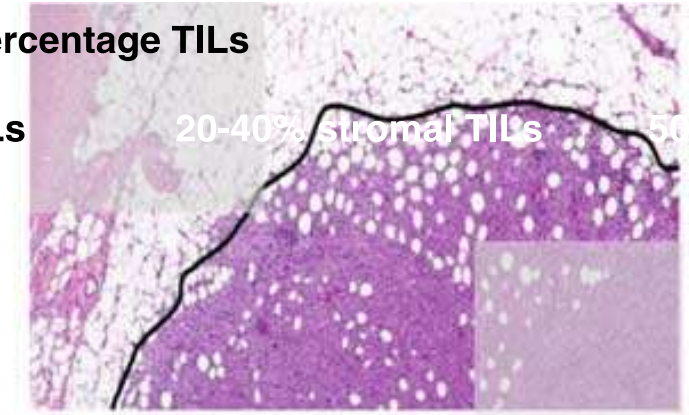


Step 4: Determine type of inflammatory infiltrate



Step 5: Assess the percentage TILs

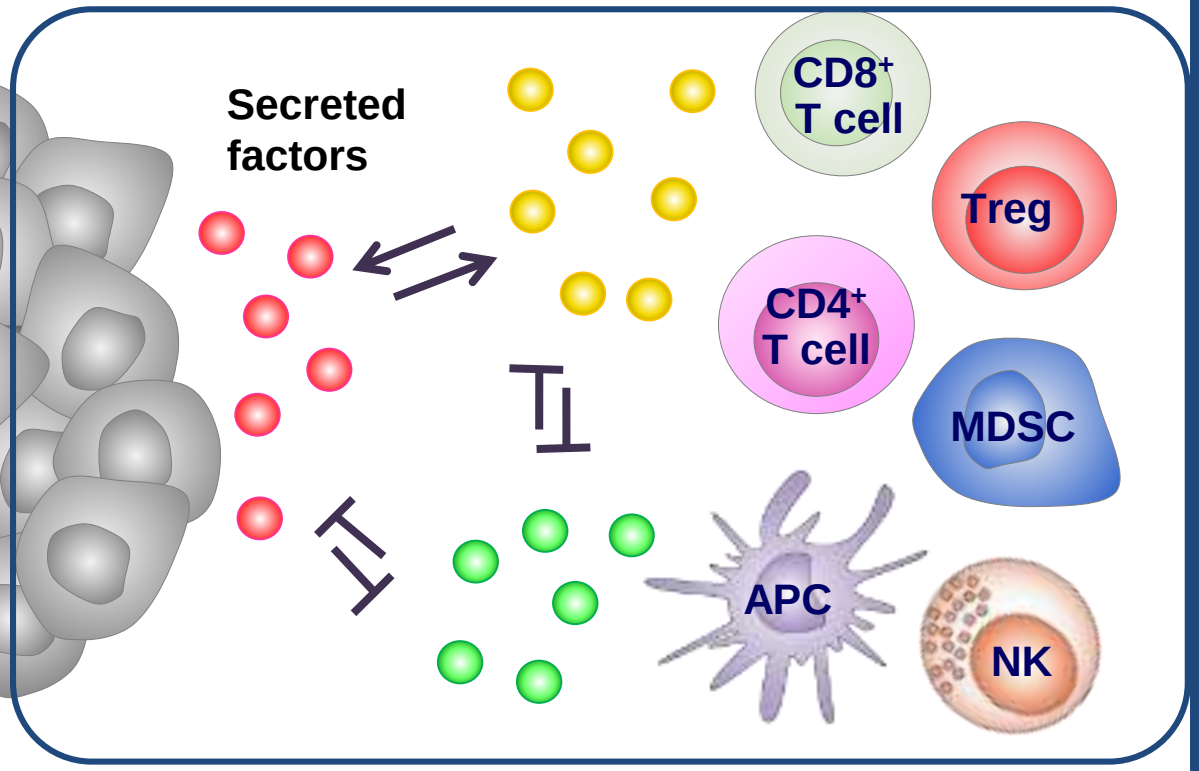
0-10% stromal TILs



TIPOS DE BIOMARCADORES BASADOS EN LA RESPUESTA INMUNE

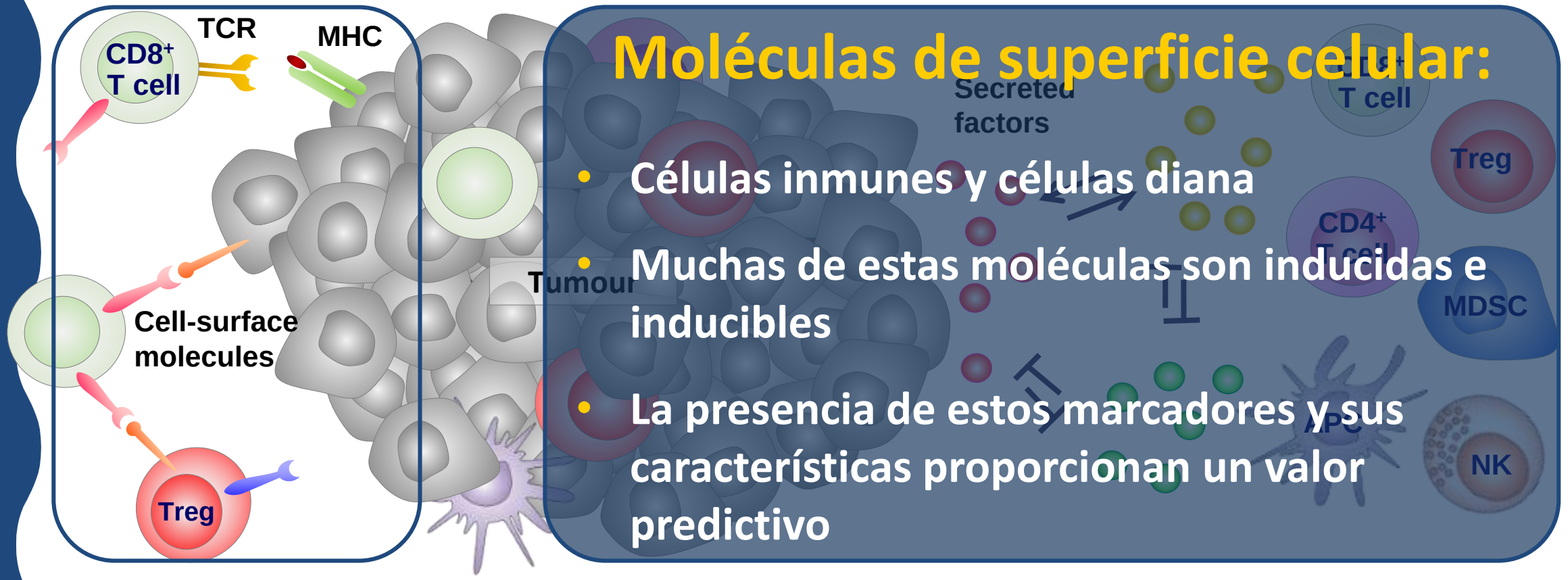
Moléculas segregadas:

- Citoquinas, Factores de crecimiento y otras proteínas que regulan el comportamiento del sistema inmune periférico y también en el interior del tumor
- La actividad se modula por sustancias producidas asimismo por células tumorales



Kwak M, et al. Front Oncol. 2013;3:10
Fridman WH, et al. Nat Rev Cancer. 2012;12:298–306
Alshaker HA, et al. Cancer Cell Int. 2011;11:3

TIPOS DE BIOMARCADORES BASADOS EN LA RESPUESTA INMUNE



Moléculas de superficie celular:

- Células inmunes y células diana
- Muchas de estas moléculas son inducidas e inducibles
- La presencia de estos marcadores y sus características proporcionan un valor predictivo

Ceeraz S, et al. Trends Immunol. 2013;34:556–563
Greaves P, Gribben JG. Blood. 2013;121:734–744
Wang S, Chen L. Cell Mol Immunol. 2004;1:37–42

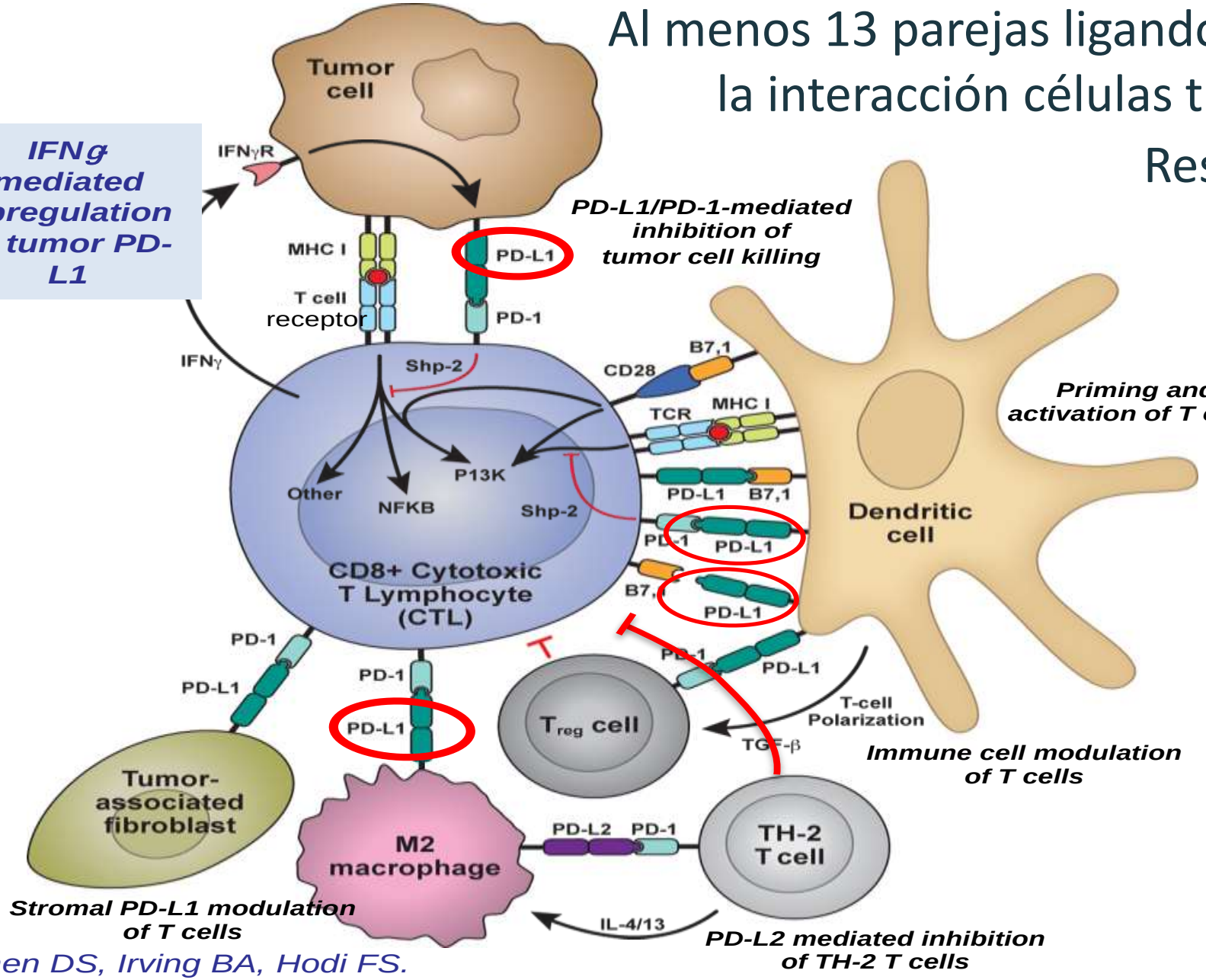
CHECKPOINTS (PUNTOS DE CONTROL)

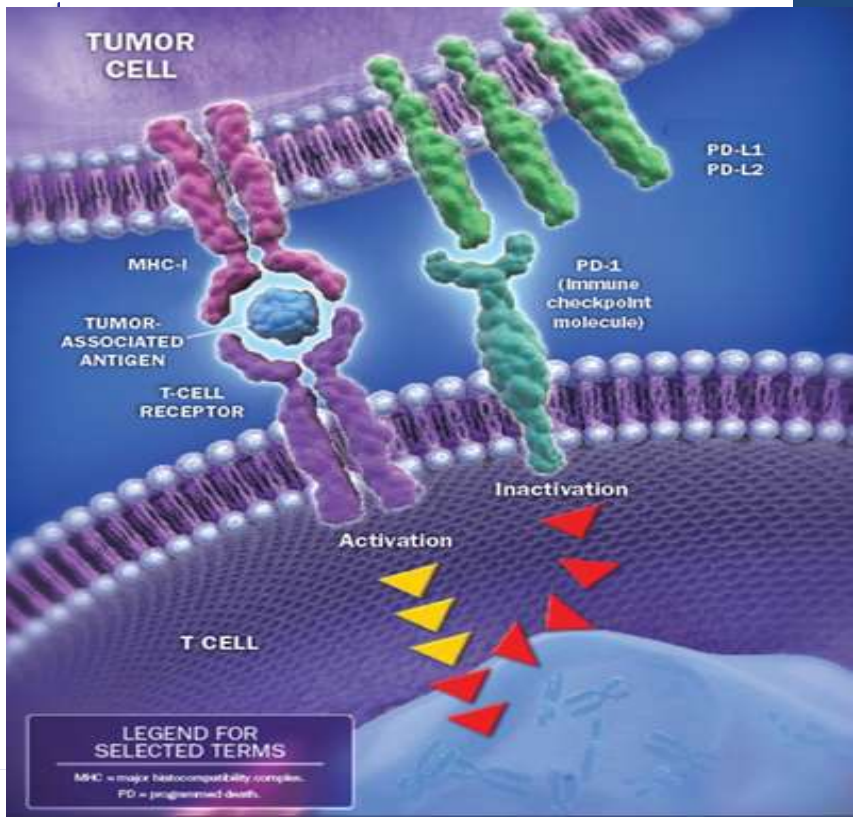
- **Mecanismo fundamental de evasión de la respuesta inmune:**
  - Cuando se activa un checkpoint finaliza la respuesta inmune
- **Moléculas implicadas en este mecanismo:**
  - Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) y CD28
  - Programmed cell death protein 1 (PD-1) y sus ligandos PD-L1 (B7-H1) y PD-L2 (B7-DC)
  - T-cell immunoglobulin mucin-3 (TCIM3)
  - Lymphocyte activation gene-3 (LAG3)
  - Otras

Al menos 13 parejas ligando-receptor son evidentes en la interacción células tumorales – células inmunes

Resistencia inmune adaptativa

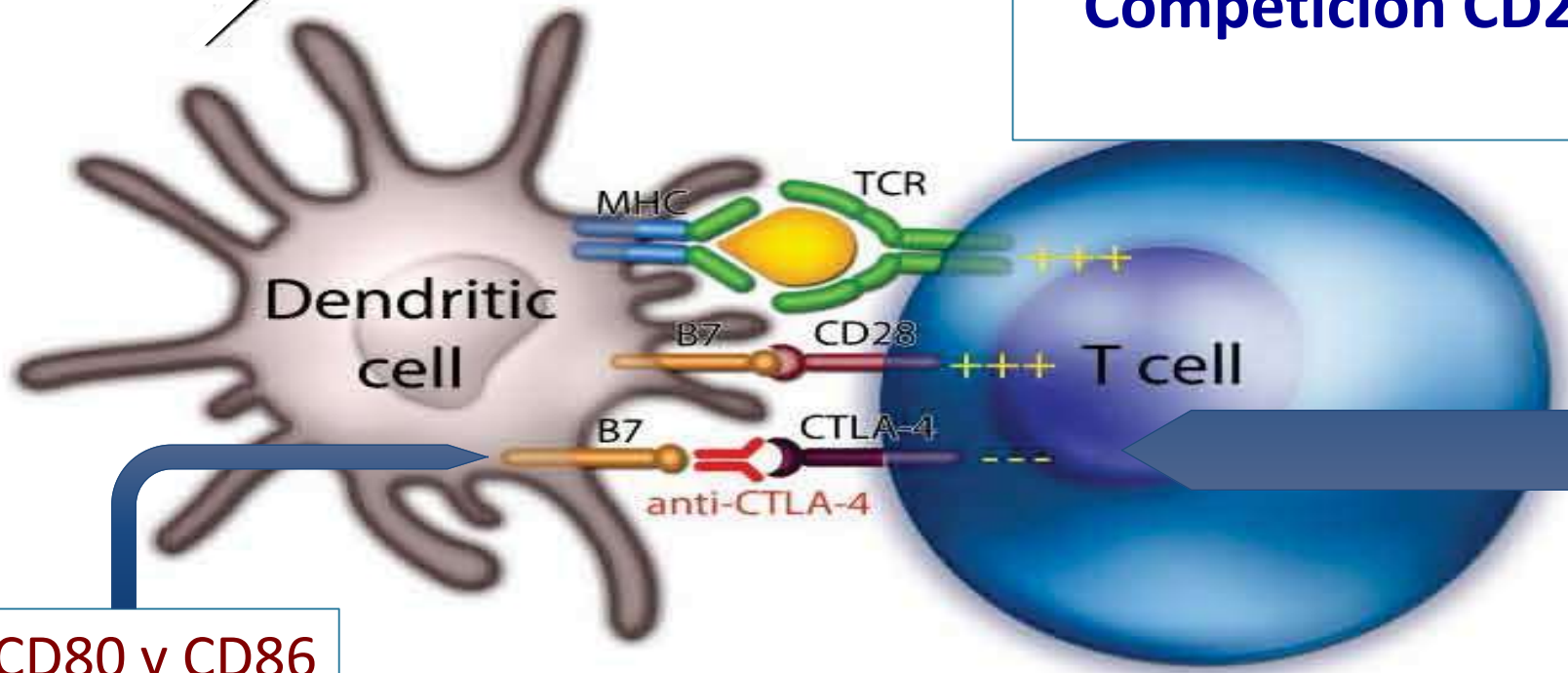
IFNg mediated upregulation of tumor PD-L1





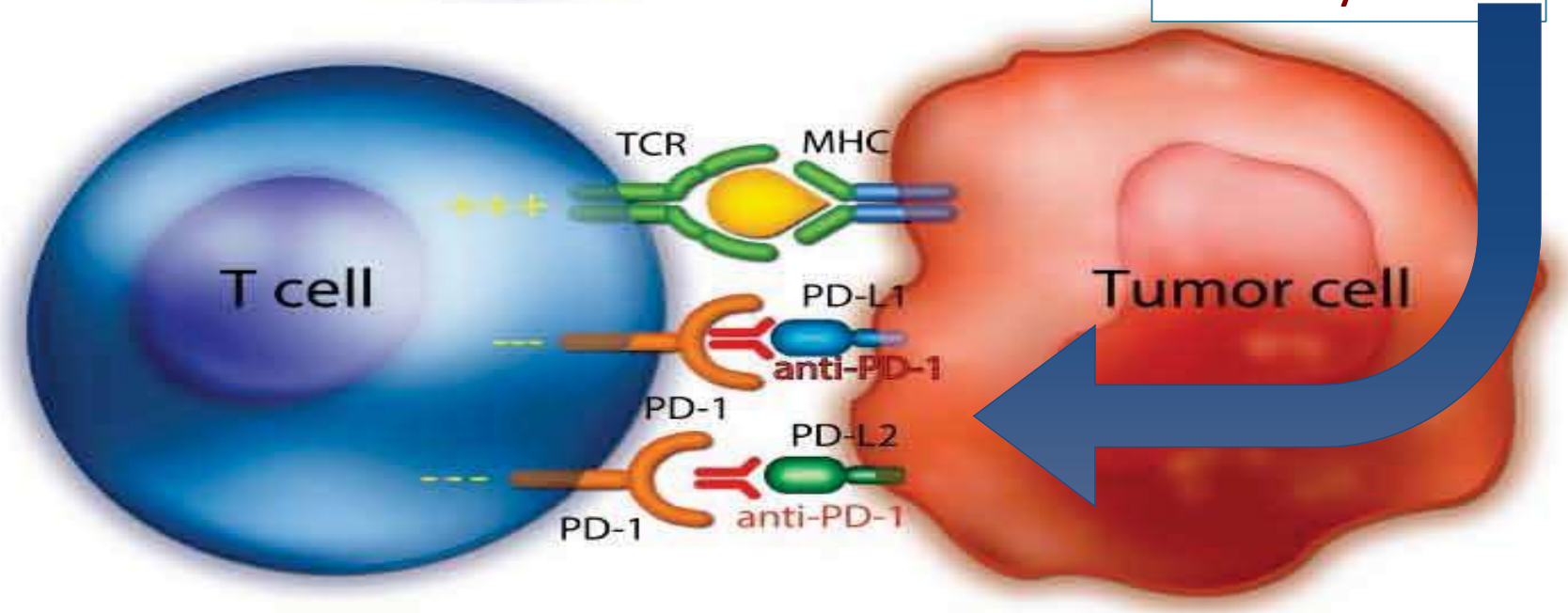
Chen DS, Irving BA, Hodi FS. Clin Cancer Res. 2012;18:6580.

Competición CD28 (estimulador) y CTLA-4 (inhibidor)



CD80 y CD86

B7-H1 y B7-DC



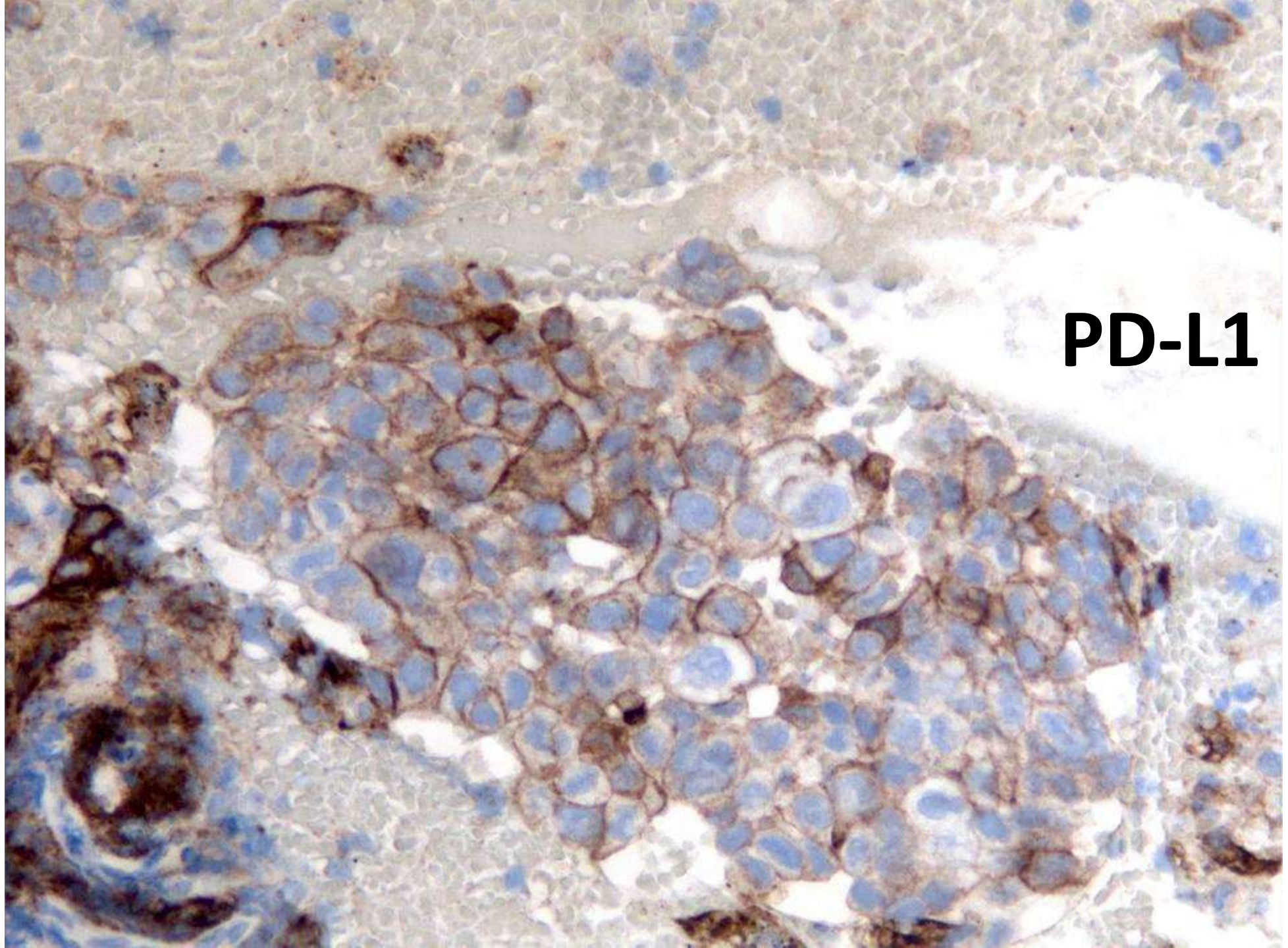
CURRENT ONCOLOGY
A Canadian Cancer Research Journal

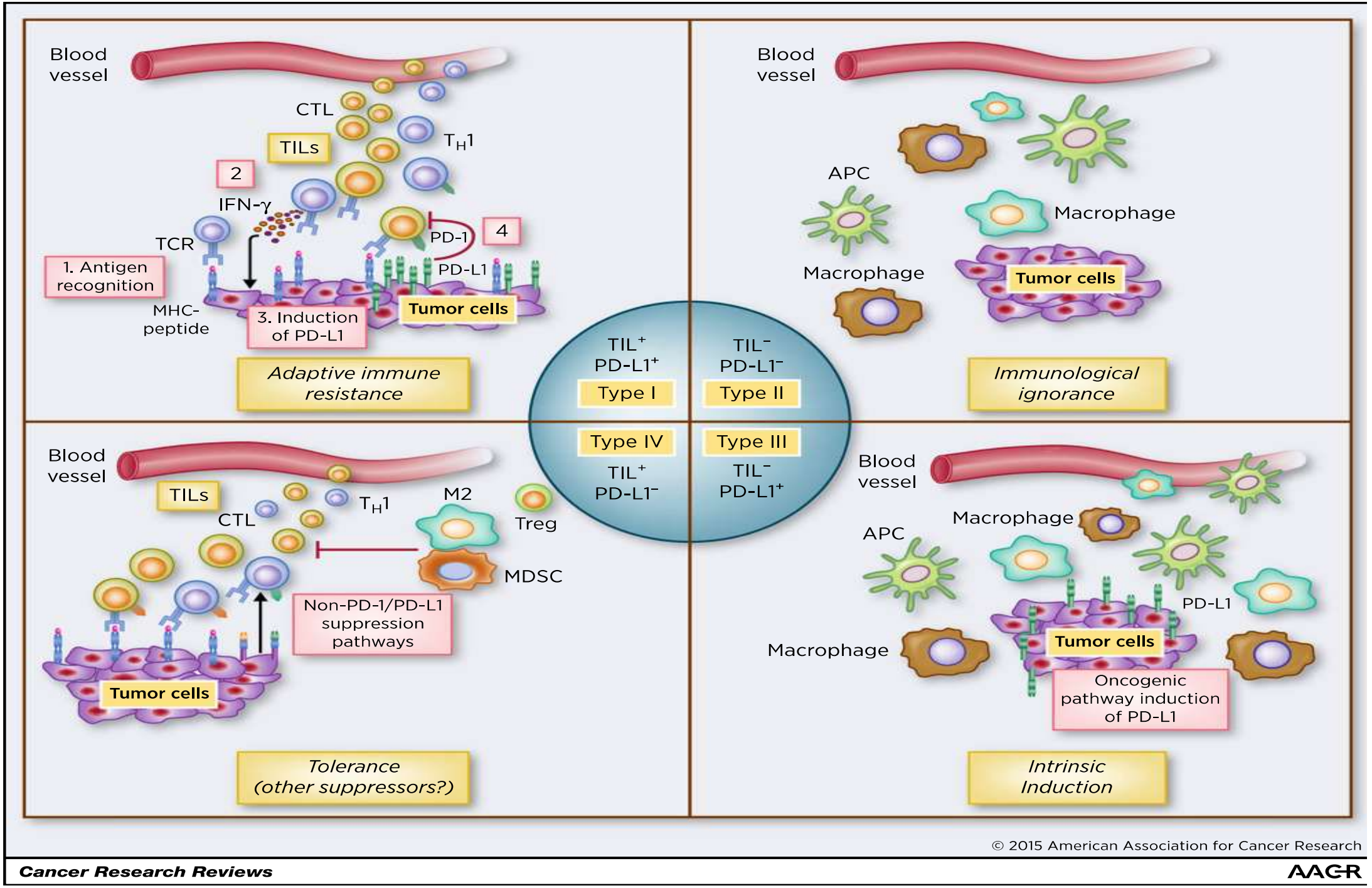
REVIEW ARTICLE

Harmonization of PD-L1 testing in oncology: a Canadian pathology perspective

D.N. Ionescu MD,* M.R. Downes MB BCh BAO MD,†‡ A. Christofides MSc RD,§ and M.S. Tsao MD‖¶

- **El principal mecanismo de inducción de la expresión de PD-L1 es por IFNγ**
- **Pero existen mecanismos alternativos**
  - Amplificación génica de la región 9p24.1
    - Hodgkin, gástrico, colon, mama triple negativa, glioblastoma
  - Deleciones en PTEN, mutaciones PI3K, AKT, EGFR
  - Amplificación de MYC


PD-L1



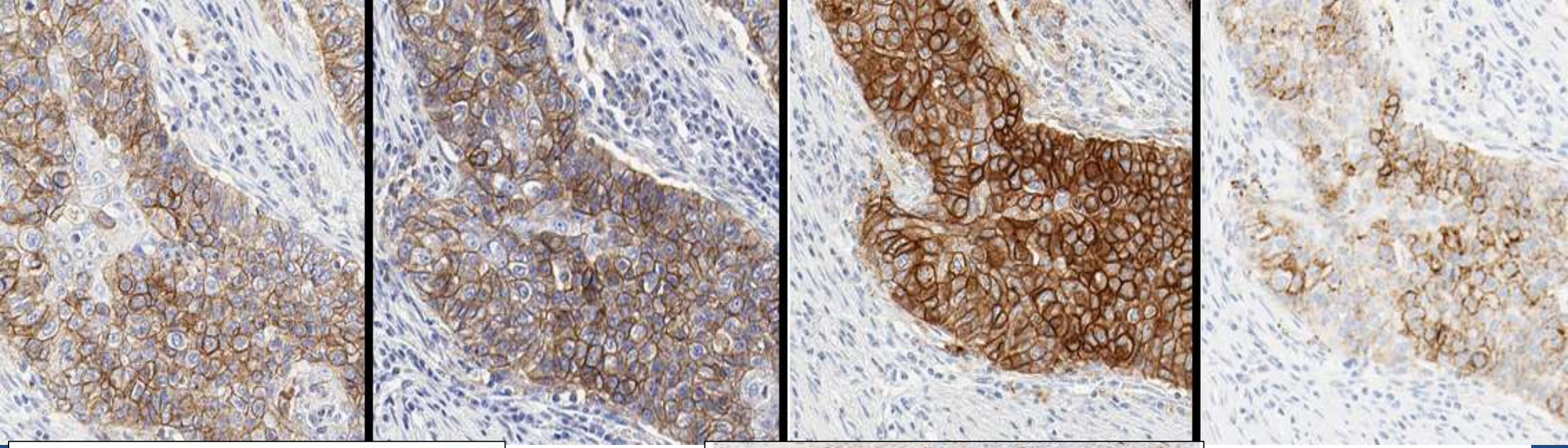
Programmed Death Ligand-1 Inhibitors					
Drug	**Company**	**FDA Approval**	**mAb/Platform**	**Scoring Criteria**	**Comment**
Pembrolizumab (Keytruda)	Merck (Kenilworth, New Jersey)	FDA approved for NSCLC	22C3 (DAKO pharmDx)/ Link 48 Autostainer (Dako, Carpenteria, California)	≥50% tumor cells	Companion diagnostic[a] (as of October 2015)
Nivolumab (Opdivo)	Bristol-Myers Squibb (New York, New York)	FDA approved for squamous and nonsquamous NSCLC	28-8 (DAKO pharmDx)/ Link 48 Autostainer	≥1% tumor cells	Complementary diagnostic[b] (as of October 2015); predictive only in nonsquamous carcinomas
Atezolizumab (MPDL3280)	Roche (Basel, Switzerland)	Expected in 2016	SP142 (Ventana, Tucson, Arizona)	Tumor cells and/ or tumor-infiltrating immune cells	In development
Durvalumab (MEDI4736)	Astra-Zeneca (London, United Kingdom)	Expected in 2016	SP263 (Ventana)	≥25% tumor cells	In development

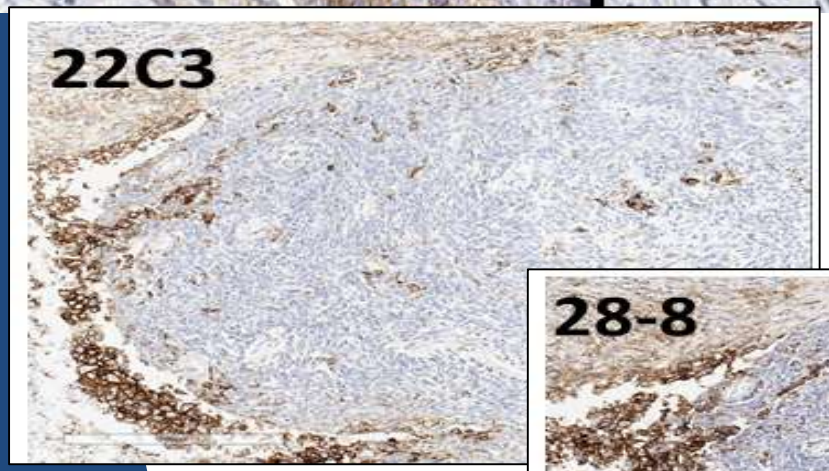
Abbreviations: FDA, US Food and Drug Administration; mAb, monoclonal antibody; NSCLC, non–small cell lung cancer.

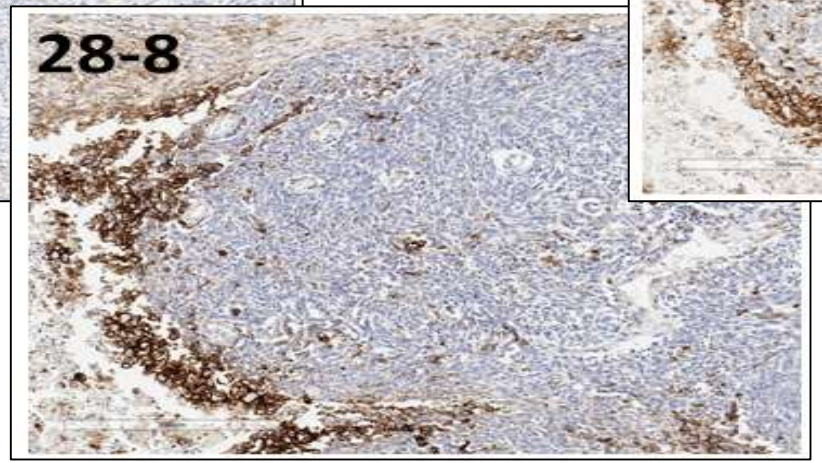
[a] According to the FDA, a *companion diagnostic* is one whose use is "essential to the safe and effective use of a corresponding therapeutic product" and is typically developed contemporaneously with the clinical development of that particular therapeutic. See "In Vitro Comparison Diagnostic Devices." [21]

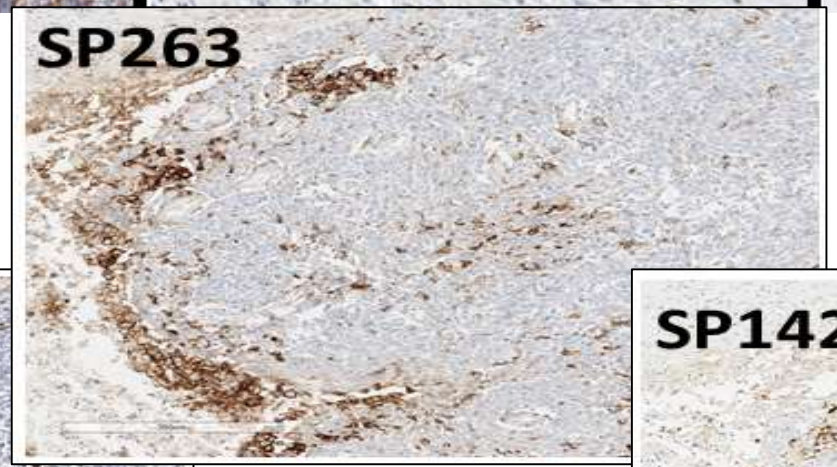
[b] The definition of *complementary diagnostic* has not been formally established by the FDA at the time of this writing. One proposed definition is that of a test that is not required for use of a therapeutic product, but that may be used to identify patients most likely to derive benefit. See Ray's[22] article on the FDA's recent approval of Opdivo.

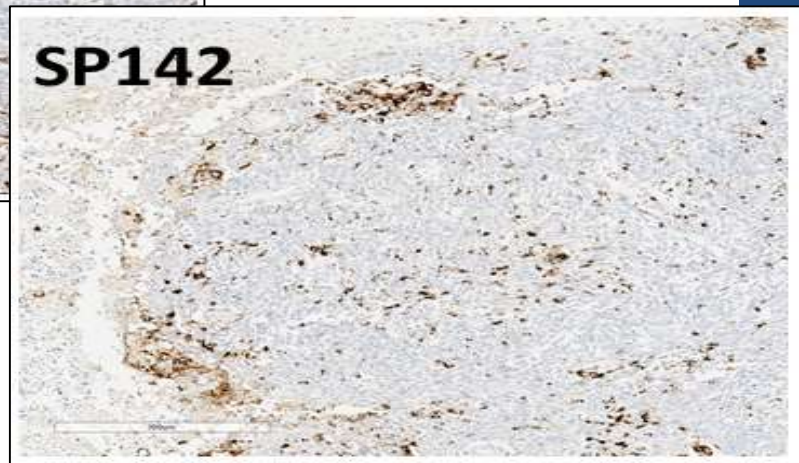
PD-L1 antibody	Platform	Detection system	Agent	Cut-off		
				NSCLC	HNSCC	UC
SP263[20]	Ventana BenchMark Ultra[a]	OptiView DAB IHC Detection Kit[b]	Durvalumab[h]	TC: ≥25%	TC: ≥25%	TC or IC: ≥25%
22C3[21]	Dako Autostainer Link 48[c]	EnVision FLEX visualization system[d]	Pembrolizumab[i]	TC: ≥1%, ≥50% TPS	TC or IC: >1%, >50%	TC and IC: ≥10% TPS
28-8[22]	Dako Autostainer Link 48	EnVision FLEX visualization system	Nivolumab[j]	TC: >1%, >5%	>1%, >5%	TC: >1%, >5%
SP142[23]	Ventana BenchMark Ultra	OptiView DAB IHC Detection Kit and OptiView Amplification Kit[e]	Atezolizumab[k]	TC: ≥10%, IC: ≥50%	TC: ≥5%, IC: ≥5%	IC: ≥5%
73-10[24]	Dako Autostainer Link 48	EnVision FLEX visualization system	Avelumab[l,m]	TC: ≥1%, 50%, 80%	NA	TC: ≥5%




22C3


28-8


SP263


SP142

Clinical Cancer Research

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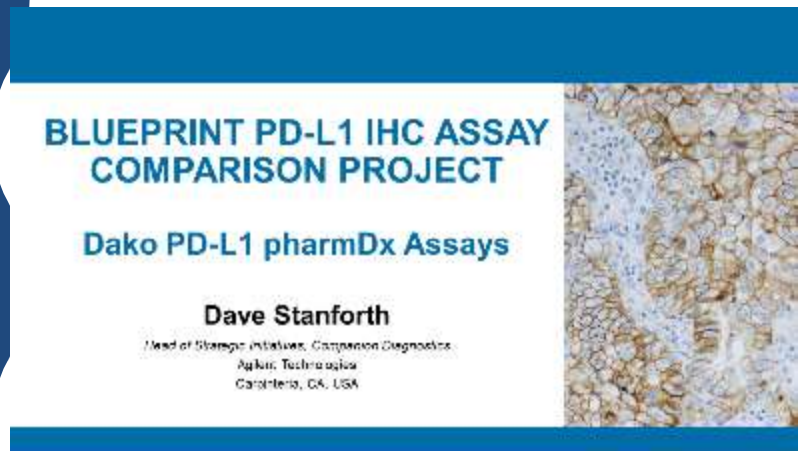
Cancer Therapy: Clinical

Agreement between Programmed Cell Death Ligand-1 Diagnostic Assays across Multiple Protein Expression Cut-Offs in Non-Small Cell Lung Cancer

Marianne J Ratcliffe, Alan Sharpe, Anita Midha, Craig Barker, Marietta Scott, Paul Scorer, Hytham Al-Masri, Marion Rebelatto, and Jill Walker

DOI: 10.1158/1078-0432.CCR-16-2375 Published January 2017



PD-L1 Blueprint Working Group

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

Fred R. Hirsch, Abigail McElhinny, Dave Stanforth, James Ranger-Moore, Malinka Jansson, Karina Kulangara, William Richardson, Penny Towne, Debra Hanks, Bharathi Vennapusa, Amita Mistry, Rasika Kalamegham, Steve Averbuch, James Novotny, Eric Rubin, Kenneth Emancipator, J. Andrew Williams, Jill Walker, John Longshore, Ming S. Tsao, Keith Kerr

Study	Assays	Samples (*n*)	Findings
Gaule *et al.*, 2016[25]	28-8 22C3 SP263 SP142 E1L3N 9A11	30	▪ When using chromogenic staining in isogenic cell lines, high levels of agreement were observed for all antibodies (R^2=0.76–0.99).
Adam *et al.*, 2017[27] (French study)	28-8 22C3 SP263 LDTs	41	▪ Assays 22C3, 28-8, SP263 performed in several centres were highly concordant. ▪ Using a 4-category scale with 1%, 5%, and 10% thresholds, immune cell staining agreement was low. ▪ Laboratory-developed tests demonstrated variable levels of agreement, with SP263 being most concordant.
Brunnstrom *et al.*, 2017[28] (Swedish study)	28-8 22C3 SP263 SP142	55	▪ The highest values for comparisons were seen between 22C3 and 28-8; the lowest were seen between SP142 and the other assays. ▪ Agreement was fairly good for 28-8, 22C3, and SP263, but not as good for SP142.
Hirsch *et al.*, 2017[29] (Blueprint 1)	28-8 22C3 SP263 SP142	39	▪ The percentage of PD-L1–stained tumour cells was comparable with 22C3, 28-8, and SP263, but the SP142 assay showed fewer stained tumour cells. ▪ Variability of staining was higher for immune cells than for tumour cells.
Rimm *et al.*, 2017[30] (Bristol–Myers Squibb, NCCN)	28-8 22C3 SP142 E1L3N	90	▪ SP142 was an outlier, detecting significantly less PD-L1 expression in tumour cells and immune cells. ▪ Compared with either 28-8 or E1L3N, 22C3 showed slightly, yet statistically significantly, lower staining. ▪ The immune cell score showed poor agreement with any antibody.
Saito *et al.*, 2017[31]	22C3 28-8	420	▪ Percentage agreement between assays was fair. ▪ Positive agreement was suboptimal when the cut-off was ≥25% and ≥50%.
Scheel *et al.*, 2017[32]	28-8 22C3 SP263 SP142 LDTs	21	▪ The SP142 staining pattern was distinct. ▪ Staining with 22C3, 28-8, SP263 was similar. ▪ Of 11 laboratory-developed tests, 6 showed staining similar to that with 22C3 and 28-8.
Scott *et al.*, 2017[33], Ratcliffe *et al.*, 2017[18] (AstraZeneca study)	28-8 22C3 SP263 SP142	493	▪ SP263, 22C3, and 28-8 showed strong agreement in immune cell scoring. ▪ Immune cell agreement and PD-L1 staining or agreement were both less with SP142 than with SP263.
Tsao *et al.*, 2017[24] (Blueprint 2, abstract)	28-8 22C3 SP263 SP142 73-10	81 (from routine practice)	▪ Results of Blueprint phase 1 were affirmed. ▪ The 22C3, 28-8, and SP263 assays are comparable; SP142 detects fewer cells, and 73-10 stains more PD-L1–positive tumour cells. ▪ Variability of staining was higher for immune cells than for tumour cells.
Velcheti *et al.*, 2017[34]	28-8 22C3 SP142 LDTs	1728 (retrospective database)	▪ No differences in PD-L1 expression using 22C3, 28-8, and laboratory-developed tests. ▪ SP142 was an outlier.

ASPECTOS A ACLARAR

- **¿Qué nivel de expresión es significativo desde el punto de vista clínico? El 1, el 25, el 50%?**
- **¿Dónde observar la positividad? ¿Únicamente en células tumorales? ¿TPS? ¿CPS? ¿En TIL?**
- **El biomarcador usado para uso en rutina debe tener en cuenta:**
  - **La variabilidad en el tiempo de recepción de la muestra**
  - **El tipo de muestra (primario, metástasis) (citología, biopsia)**
  - **El tipo de anticuerpo y la plataforma**
  - **La definición de la tinción (membrana, citoplasmática)**
  - **Reproducibilidad**

¡¡¡Seguimos igual desde 2016!!!

GUIÓN

1. Conceptos básicos en PD-L1
2. **¿En qué muestras?**
3. Novedades técnicas (TMB)
4. Impacto clínico
5. Retos

CITOLOGÍA Y PD-L1

- 30-40% de todos los carcinomas pulmonares avanzados se diagnostican sólo por citología
- El uso de muestras citológicas sólo para determinación de PD-L1 no ha sido contemplado
  - Sin embargo, no hay nada desde el punto de vista técnico en contra
  - Cuidado con el anticuerpo SP-142 ya que no permite discriminar entre células inmunes vecinas o incluídas en el estroma tumoral
- Existen buenos datos en la literatura acerca de la utilidad de la citología para el diagnóstico de PD-L1 (Consenso)
- Detallar el clon usado y los controles utilizados

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

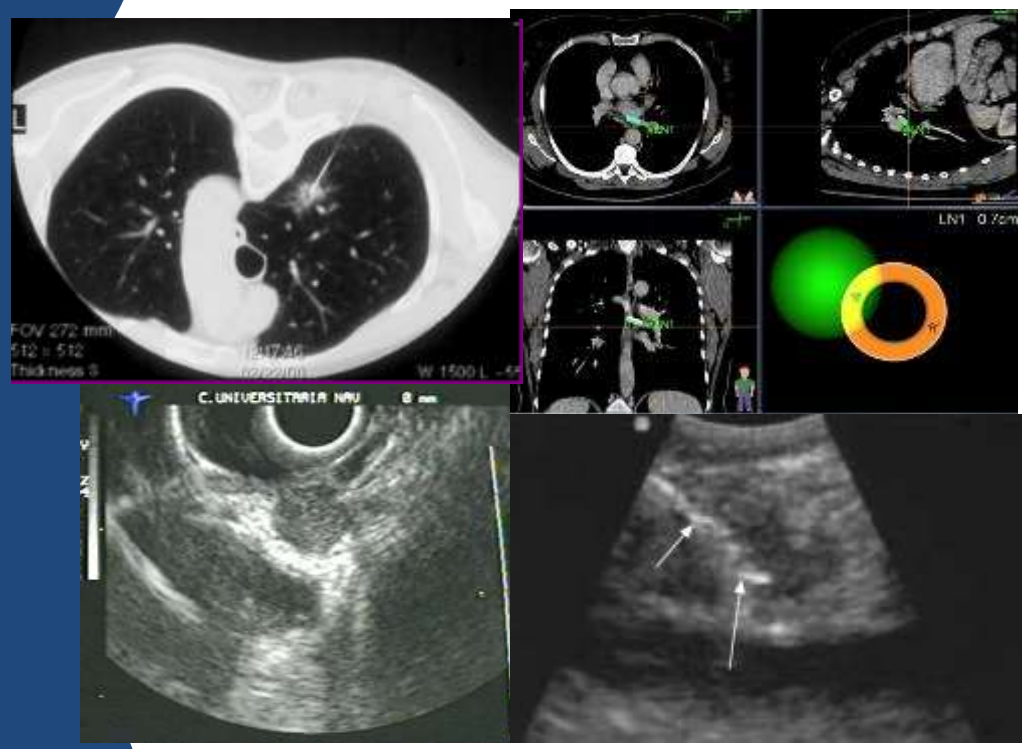
"Imfinzi as monotherapy is indicated for the treatment of locally advanced, unresectable non-small cell lung cancer (NSCLC) in adults whose tumours express PD-L1 on ≥ 1% of tumour cells and whose disease has not progressed following platinum-based chemoradiation therapy."

Immunohistochemistry of Pulmonary Biomarkers

A Perspective From Members of the Pulmonary Pathology Society

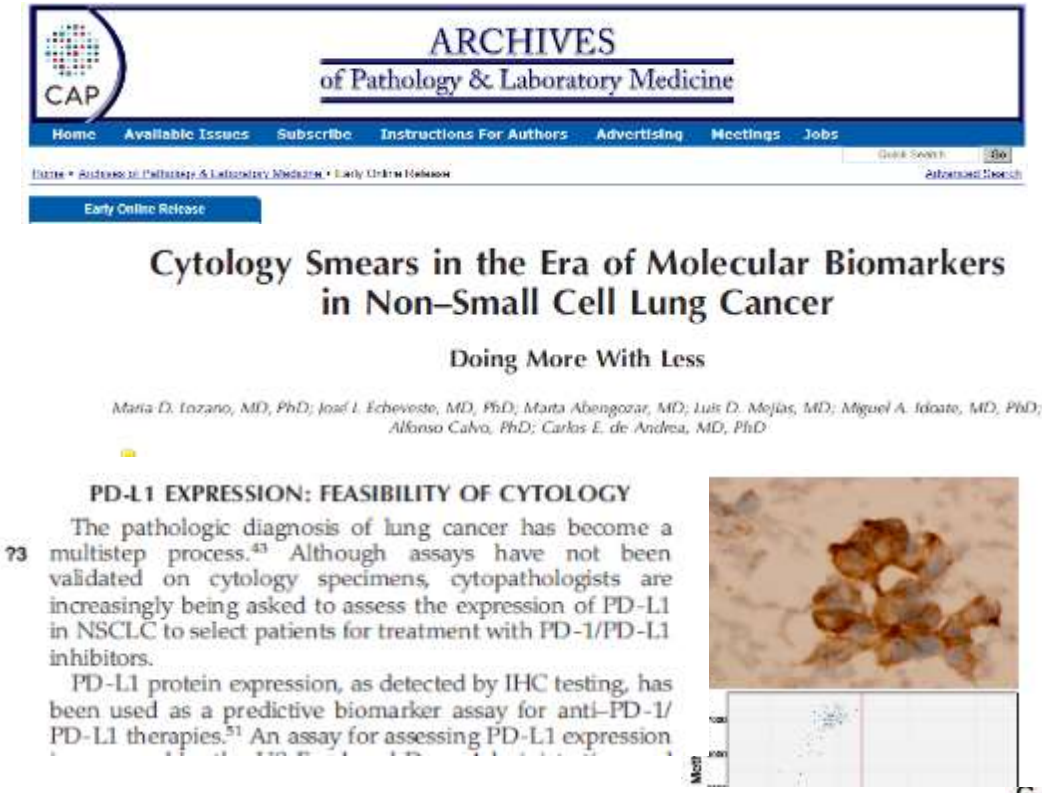
Erik Thunnissen, MD; Timothy Craig Allen, MD, JD; Julien Adam, MD; Dara L. Aisner, MD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Philip T. Cagle, MD; Vera Luiza Capelozzi, MD, PhD; Wendy Cooper, MD; Lida P. Hariri, MD, PhD; Izidor Kern, MD; Sylvie Lantuejoul, MD, PhD; Ross Miller, MD; Mari Mino-Kenudson, MD; Teodora Radonic, MD, PhD; Kirtee Raparia, MD; Natasha Rekhtman, MD, PhD; Sinchita Roy-Chowdhuri, MD, PhD; Prudence Russell, MBBS(Hons), FRCPA; Frank Schneider, MD; Lynette M. Sholl, MD; Ming Sound Tsao, MD; Marina Vivero, MD; Yasushi Yatabe, MD, PhD

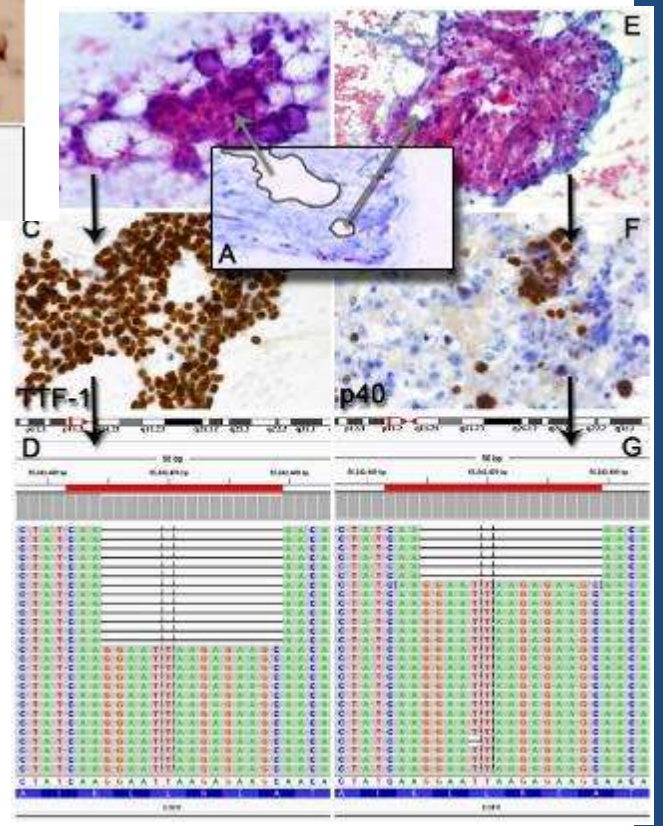
(***Arch Pathol Lab Med.*** **doi: 10.5858/arpa.2017-0106-SA)**



Although 30% to 40% of all advanced NSCLCs are diagnosed by cytology alone, the use of cytology samples for determination of PD-L1 is currently not advocated, as none of the assays has been validated for this purpose. However, since immunostaining of tumor cells is standard diagnostic practice in many institutions,[100] PD-L1 staining and quantitation should be technically feasible in principle, being irrelevant for PD-L1 scoring. Emerging data from cell blocks and matched histologic specimens suggest that cytologic material is as good as histologic material for PD-L1 IHC tumor cell analysis.[101] However, preexisting

Cortesía Dra Lozano CUN





- **Existen instituciones donde se usa la citología como primer recurso para las pruebas moleculares**
- **Importancia del manejo correcto de la citología para generar multiples materiales susceptibles de NGS, FISH ó IHQ**

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

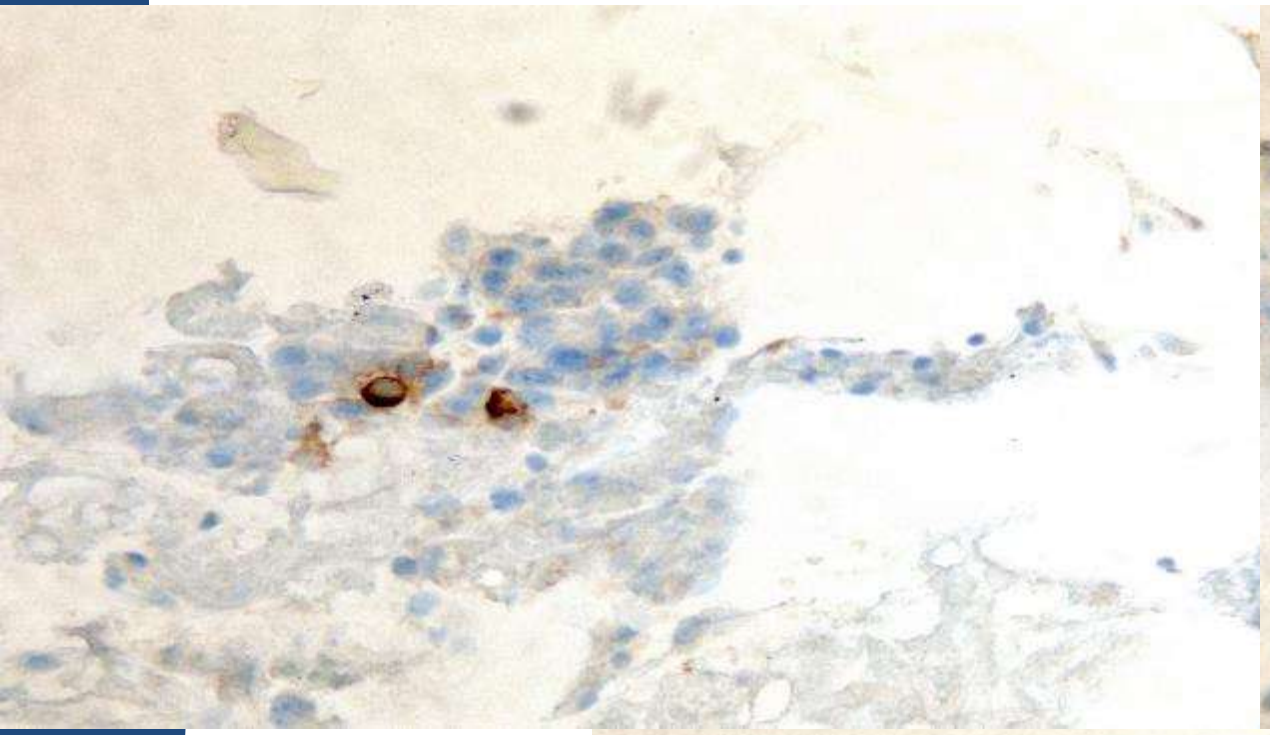
Neal I. Lindeman, MD; Philip T. Cagle, MD; Dara L. Aisner, MD, PhD; Maria E. Arcila, MD; Mary Beth Beasley, MD; Eric Bernicker, MD; Carol Colasacco, MLIS, SCT(ASCP); Sanja Dacic, MD, PhD; Fred R. Hirsch, MD, PhD; Keith Kerr, MB, ChB; David J. Kwiatkowski, MD, PhD; Marc Ladanyi, MD; Jan A. Nowak, MD, PhD; Lynette Sholl, MD; Robyn Temple-Smolkin, PhD; Benjamin Solomon, MBBS, PhD; Lesley H. Souter, PhD; Erik Thunnissen, MD, PhD; Ming S. Tsao, MD; Christina B. Ventura, MPH, MT(ASCP); Murry W. Wynes, PhD; Yasushi Yatabe, MD, PhD

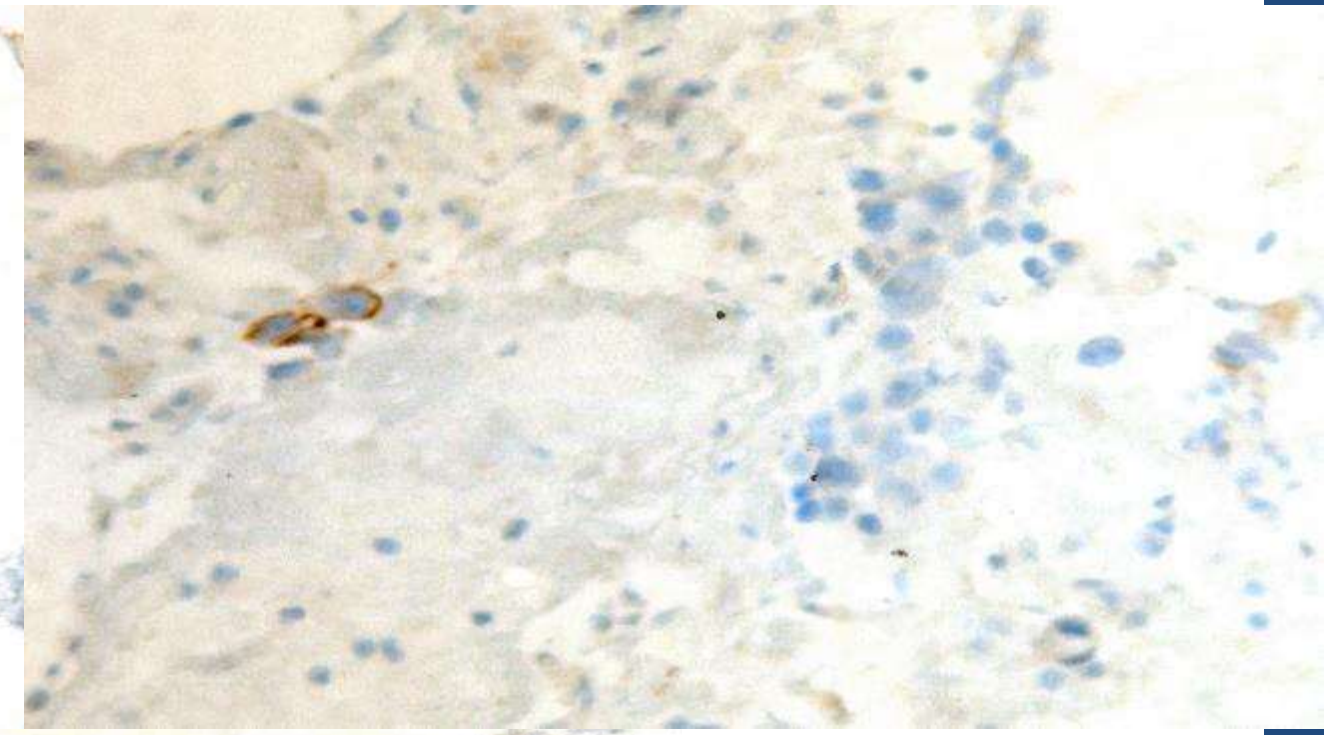
ARCHIVES of Pathology & Laboratory Medicine

doi: 10.5858/arpa.2017-0388-CP

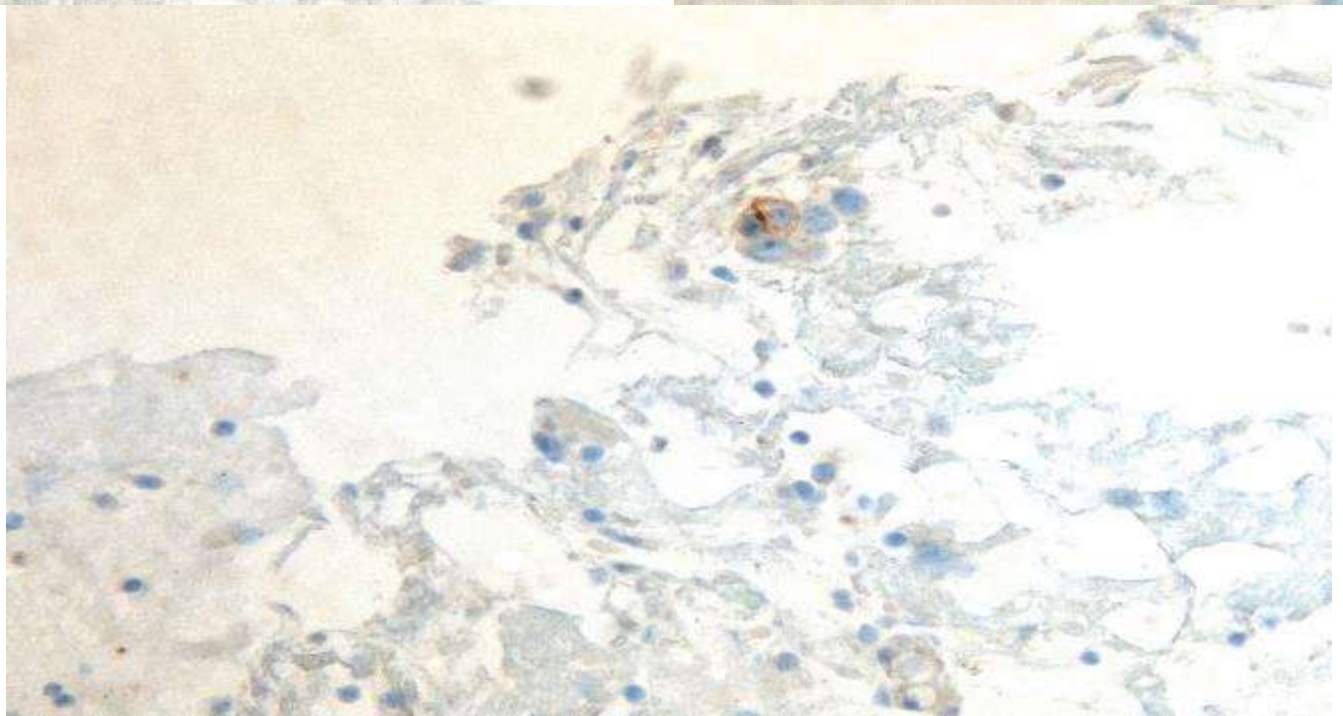
Table 3. Summary of the Updated Statements With Strength of Recommendations[a]

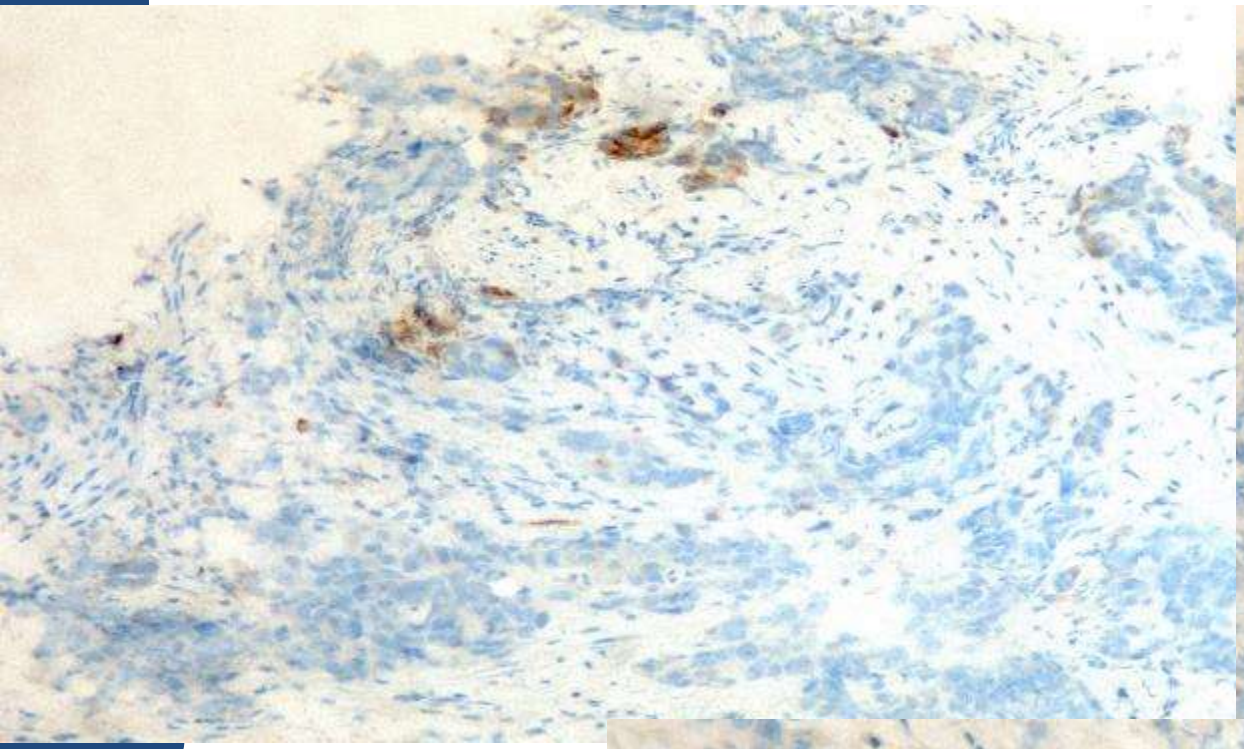
2013 Statement	2017 Statement
Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.	Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing.
Expert consensus opinion: Laboratories should use EGFR test methods that are able to detect mutations in specimens with at least 50% cancer cell content, although laboratories are strongly encouraged to use (or have available at an external reference laboratory) more sensitive tests that are able to detect mutations in specimens with as little as 10% cancer cells.	Expert consensus opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells.
Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.	Strong recommendation: Laboratories should not use total EGFR expression by IHC testing to select patients for EGFR-targeted TKI therapy.

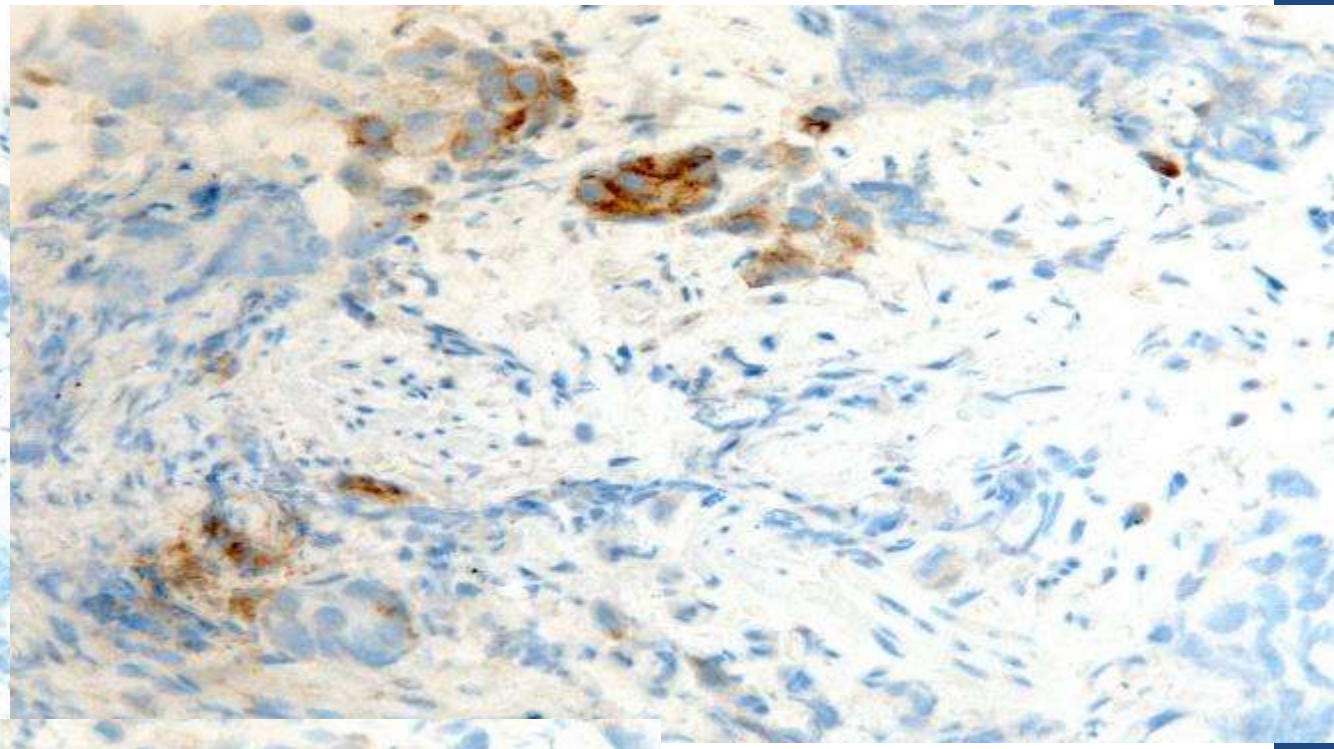


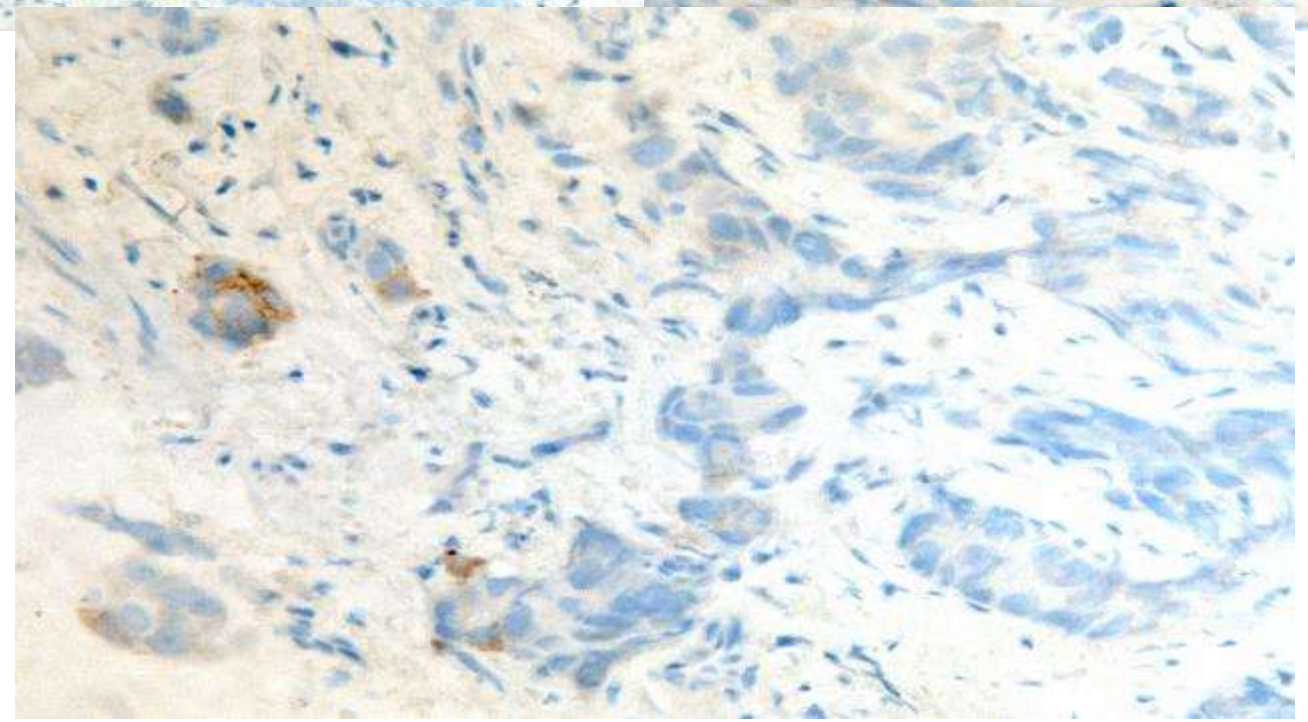


EBUS SP263 10%





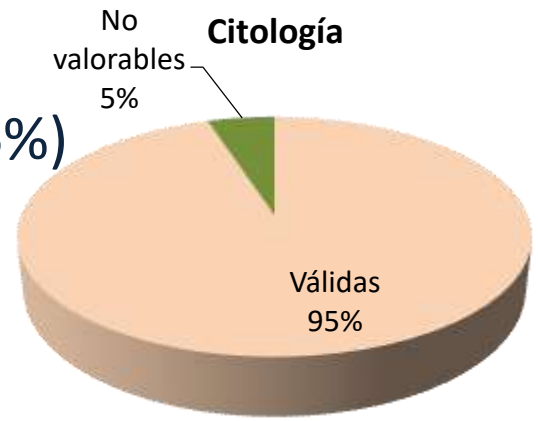


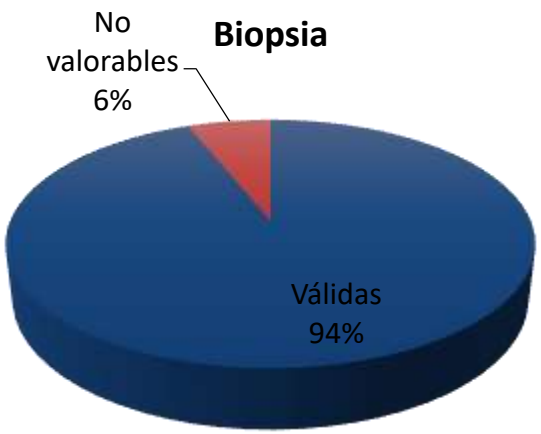


Primario
SP263
10%

NUESTRA CASUÍSTICA

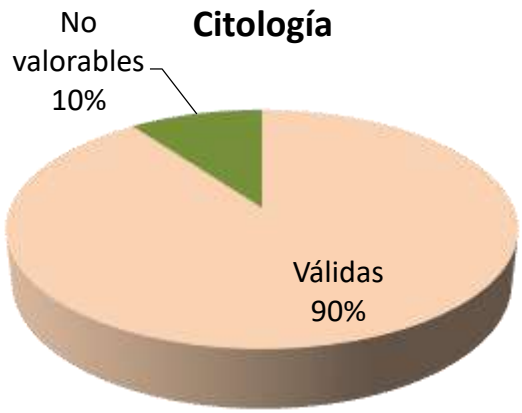
- Nuestra casuística 2017
  - 198 biopsias pequeñas 12 nv (<6%)
  - 40 citologías 2 nv (5%)

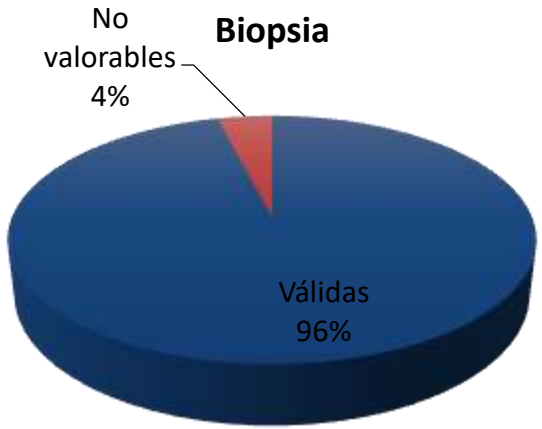


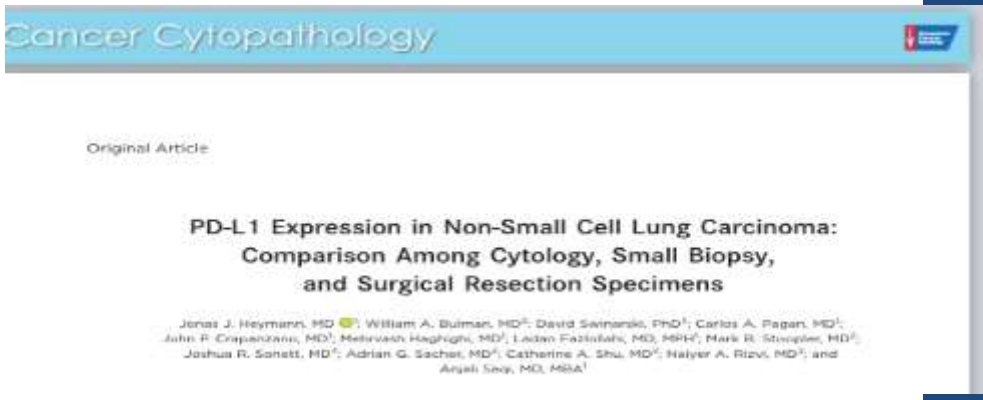


36 de 40 citologías (90%) adecuadamente celulares

- 69/72 biopsias pequeñas (96%)





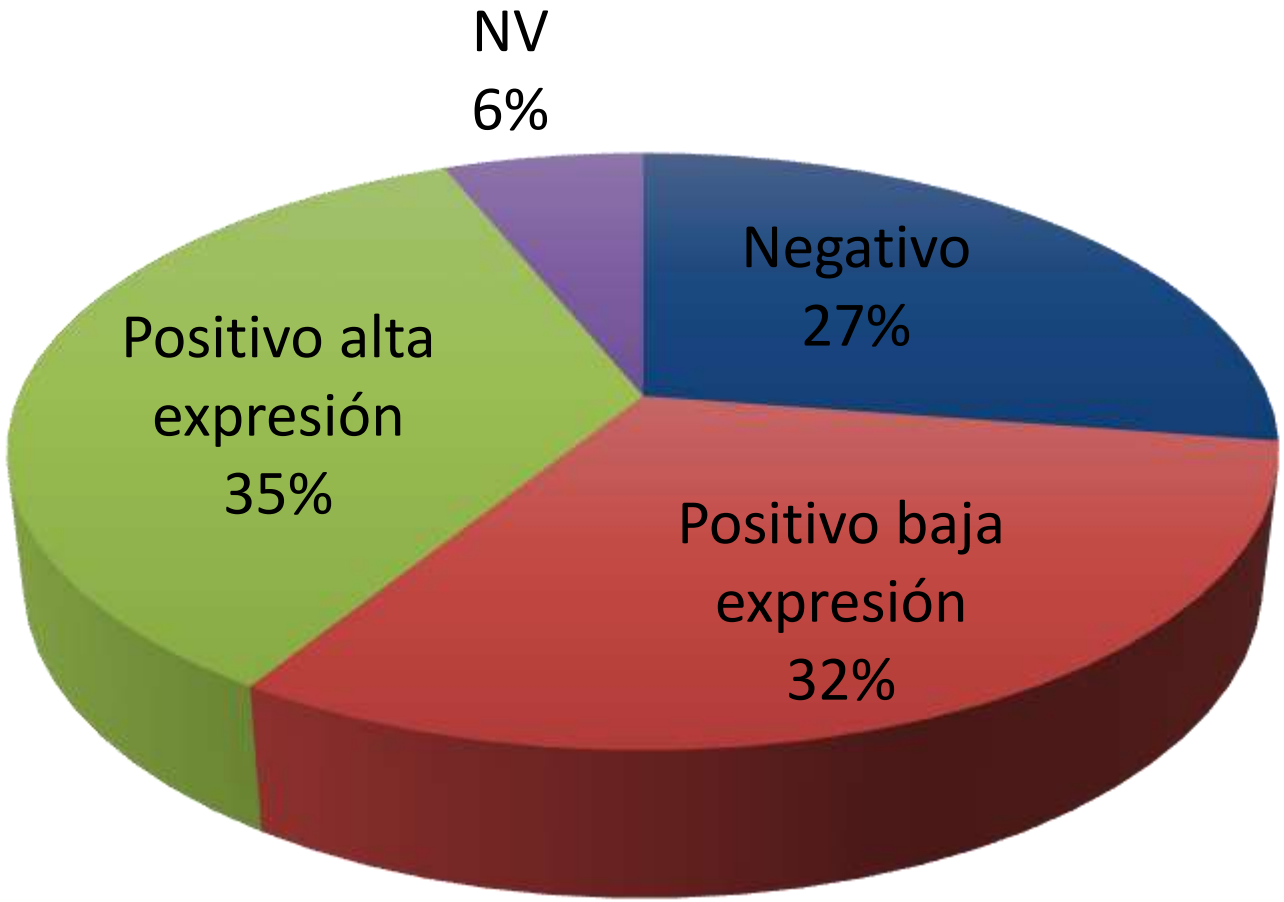

Cancer Cytopathology

Original Article

PD-L1 Expression in Non-Small Cell Lung Carcinoma: Comparison Among Cytology, Small Biopsy, and Surgical Resection Specimens

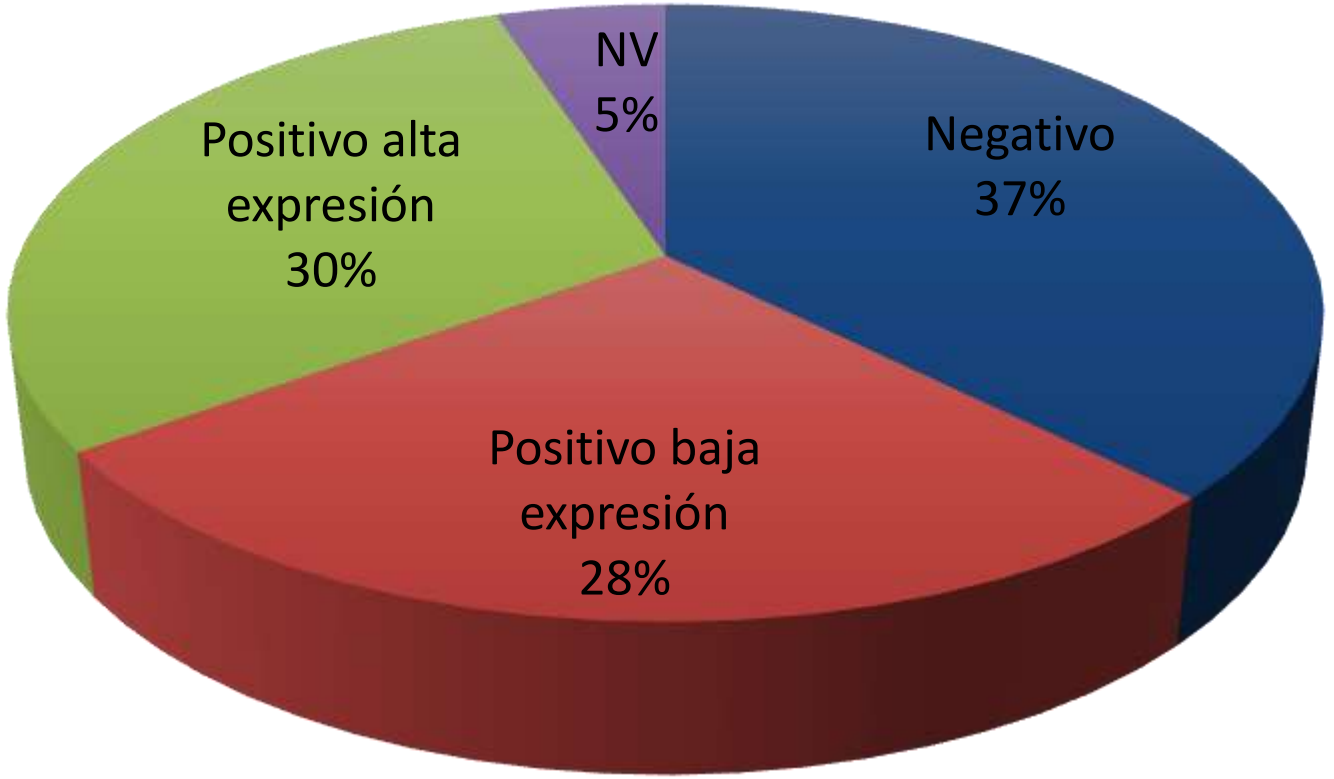
CASUÍSTICA 2017 (198 CASOS)

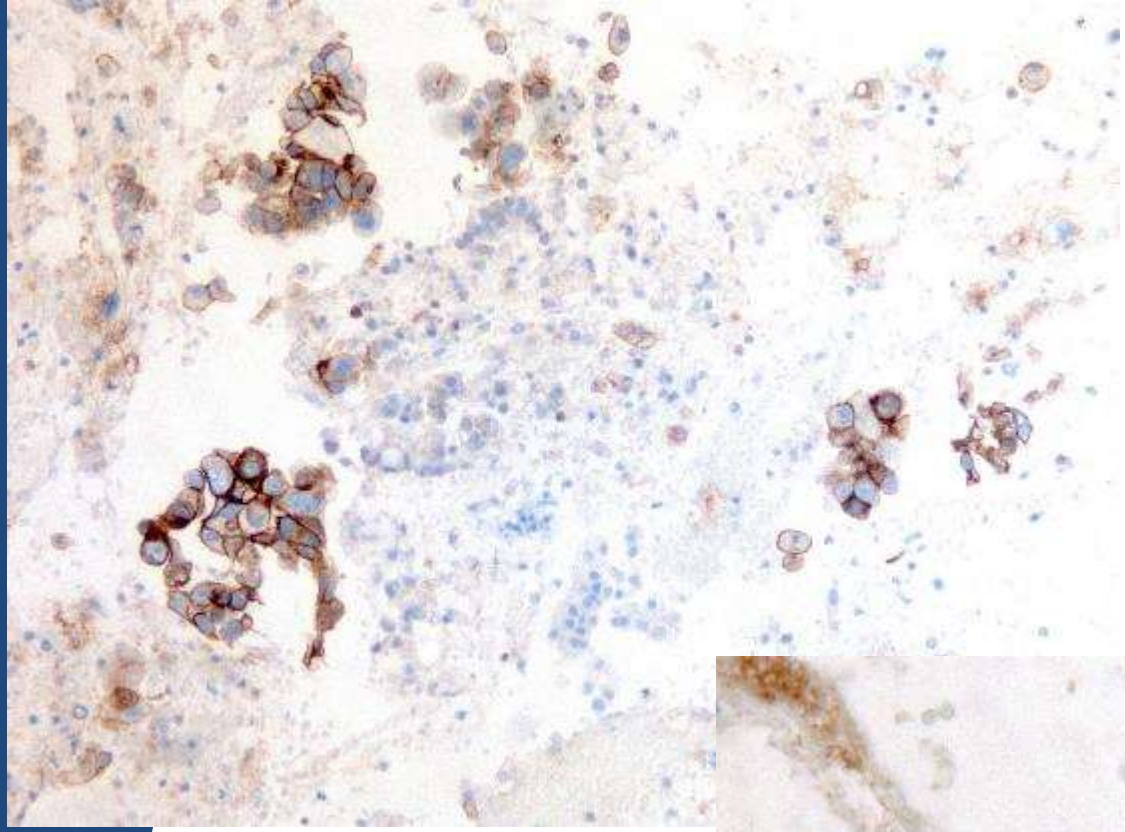
Biopsias 22C3

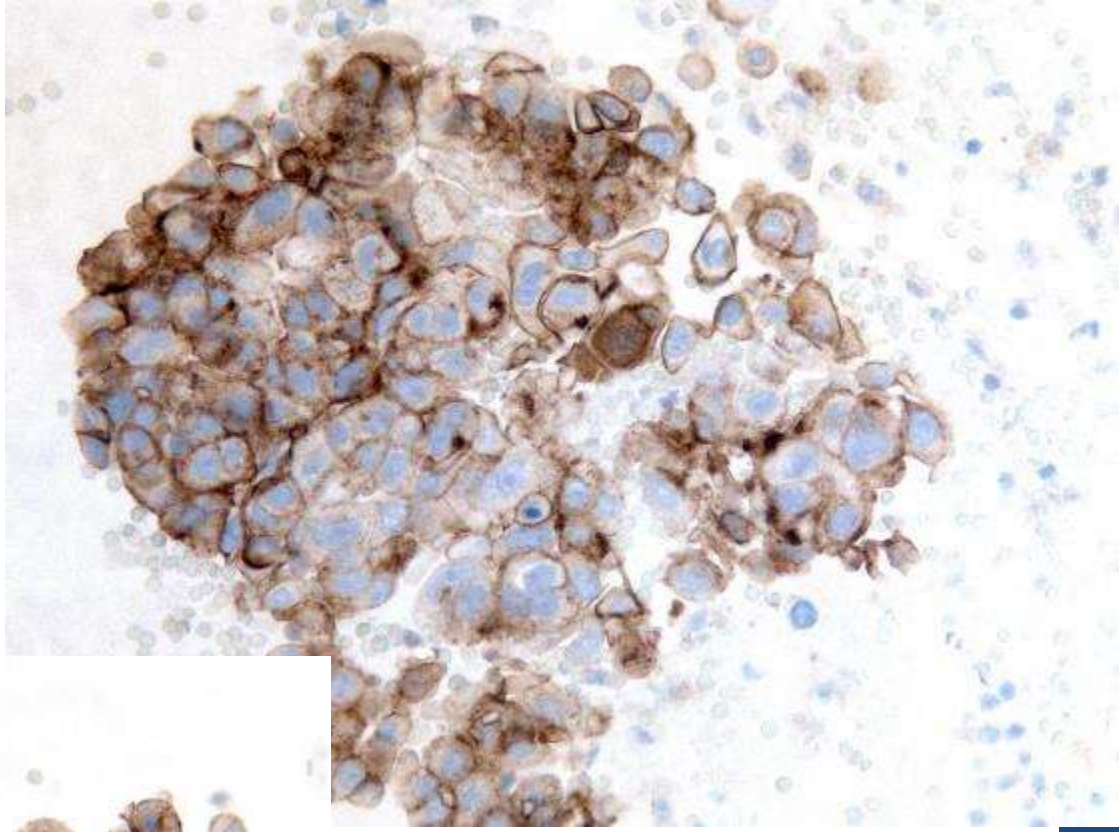


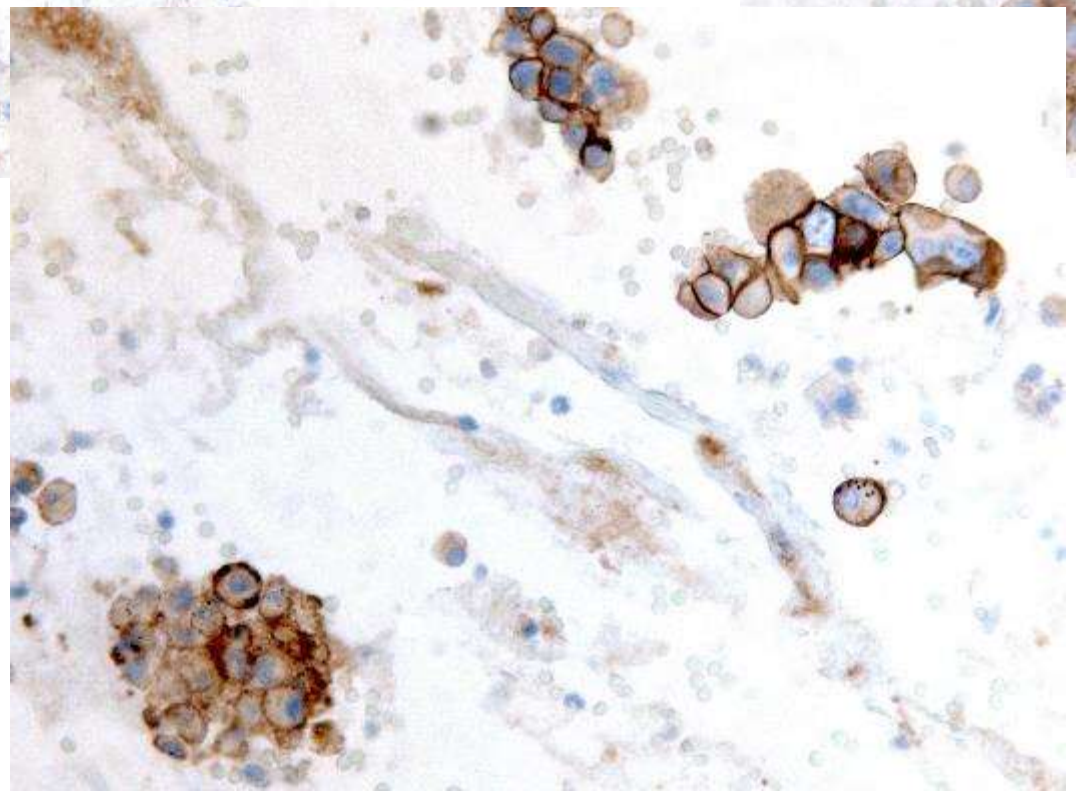
CASUÍSTICA 2017 (40 CASOS)

Citología 22C3









EBUS

22C3

90%