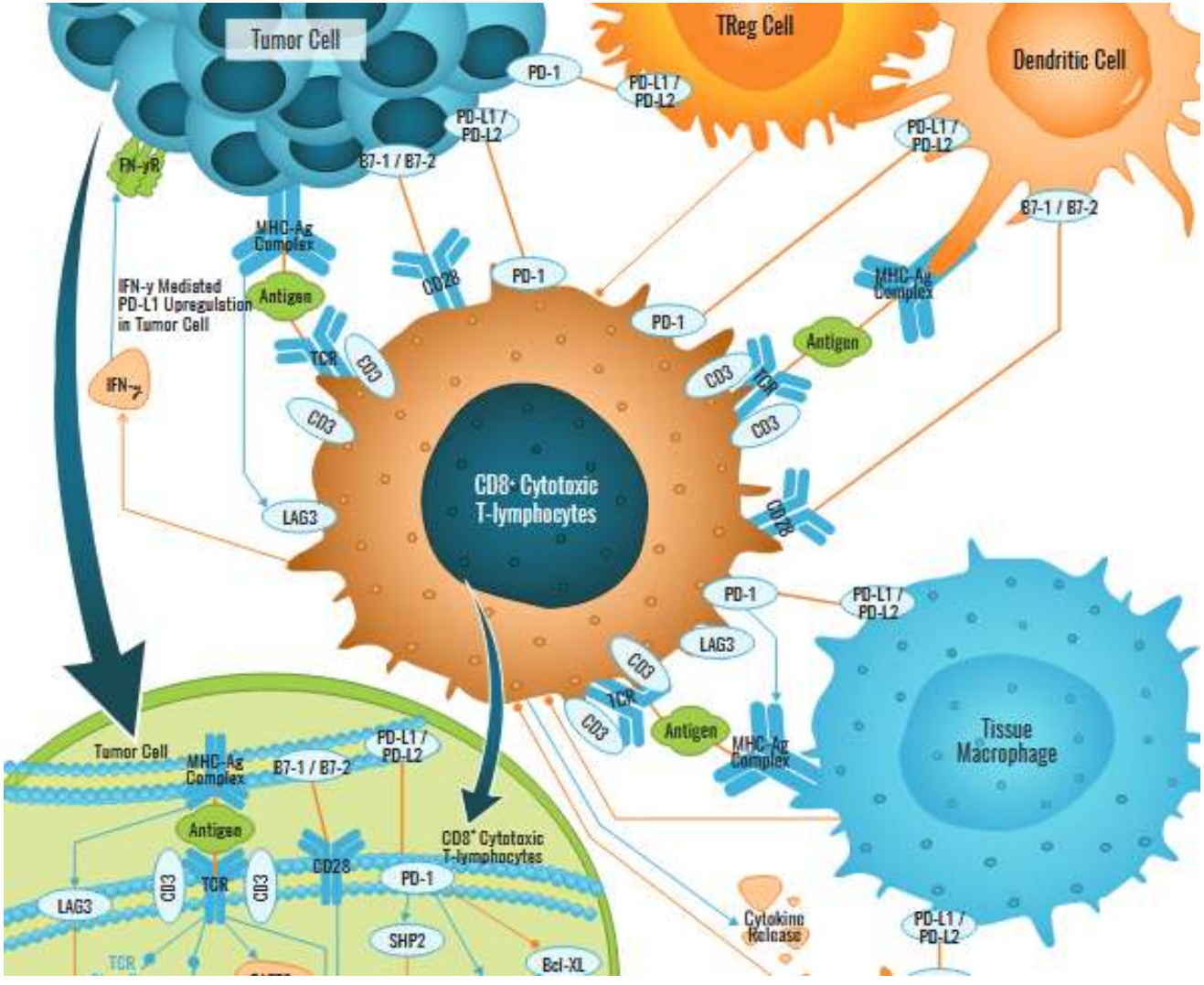
PD-L1

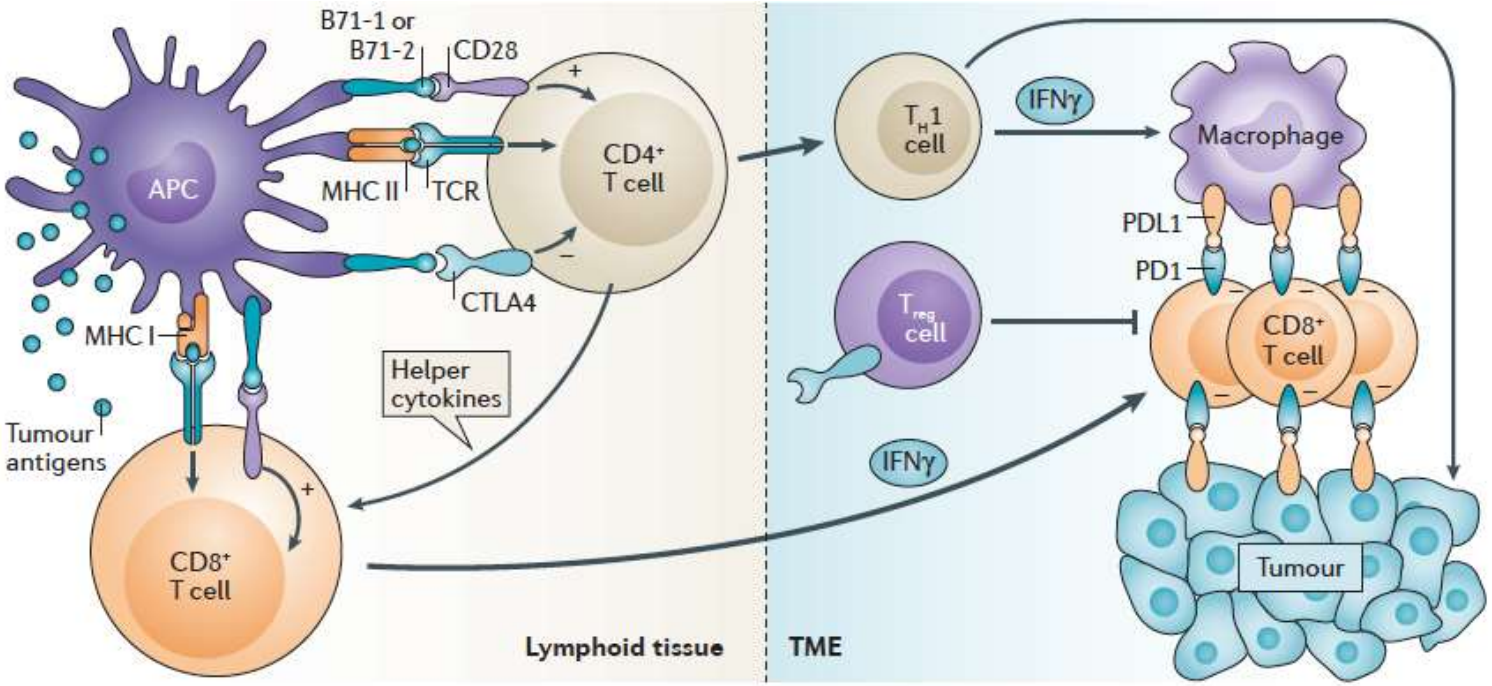
Biomarcador de Inmunoterapias

Reunión Territorial de Andalucía, Ceuta y Melilla de la SEAP-IAP
Cádiz, 13 de Abril de 2018

D. Bautista Ojeda
Hospital Regional Carlos Haya de Málaga

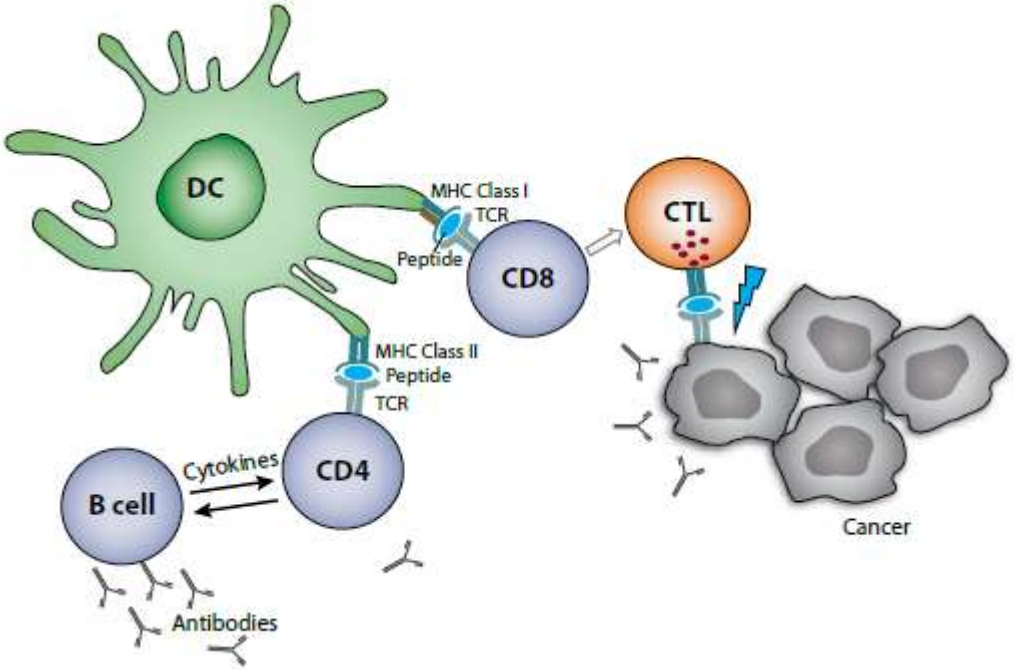

Tumor Cell
TReg Cell
Dendritic Cell
PD-1
PD-L1 / PD-L2
B7-1 / B7-2
IFN-γR
MHC-Ag Complex
Antigen
CD28
IFN-γ Mediated PD-L1 Upregulation in Tumor Cell
IFN-γ
TCR
CD3
LAG3
CD8+ Cytotoxic T-lymphocytes
Tissue Macrophage
Cytokine Release
SHP2
Bcl-XL

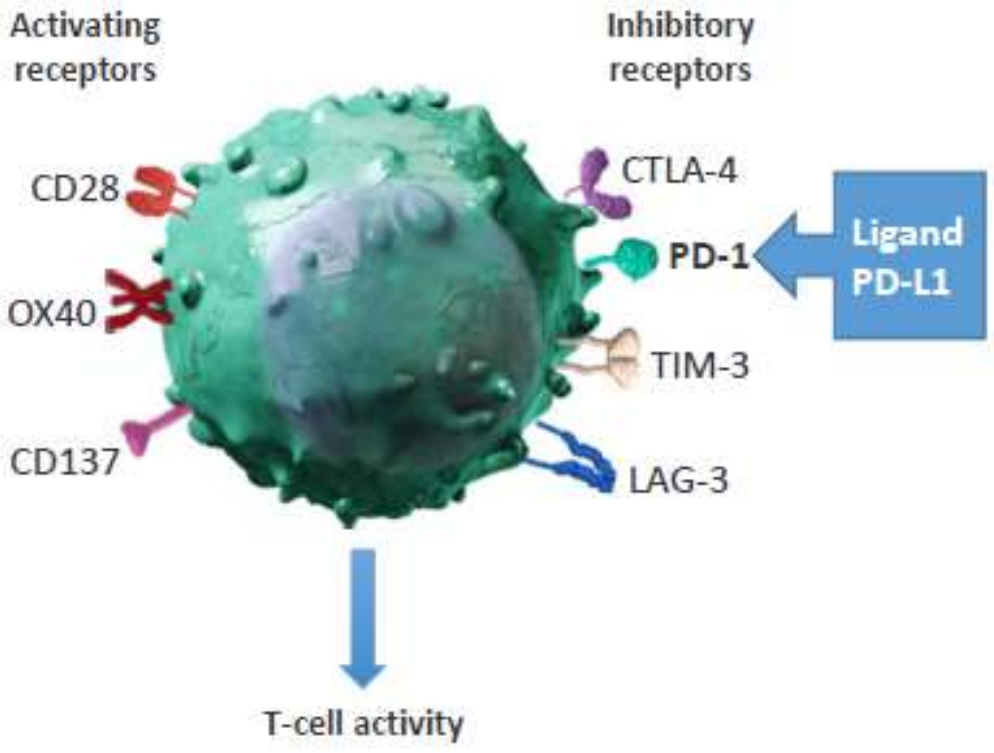
Biomarcadores de respuesta inmune



Topalian et al, Nature Reviews Cancer 2016

Reacción inmune contra las células tumorales

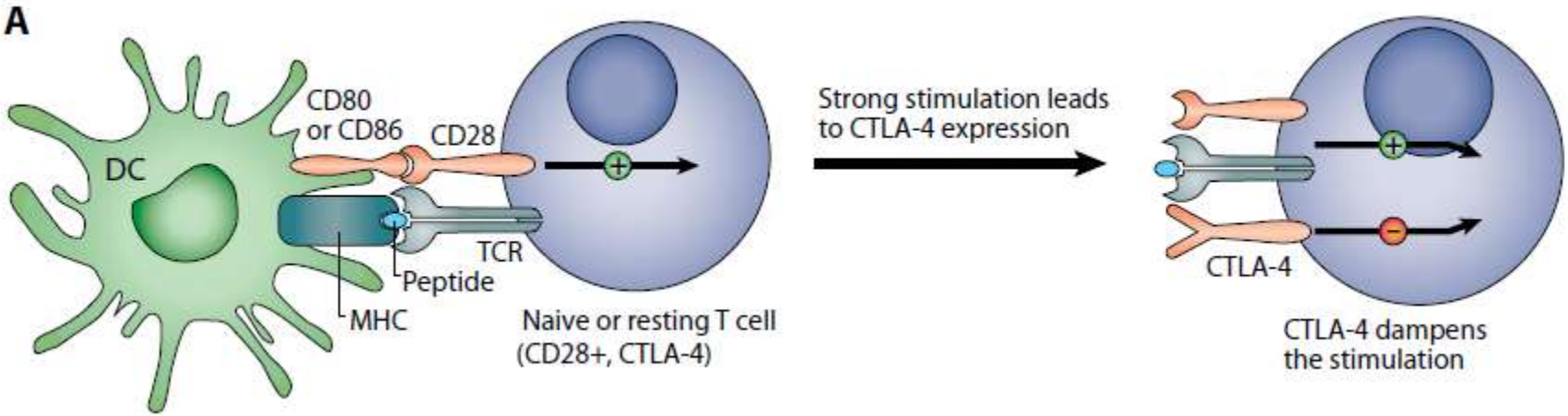




lapted from Mellman I, et al. *Nature* 2011:480;481–9; Pardoll DM. *Nat Rev Cancer* 2012;12:252–64

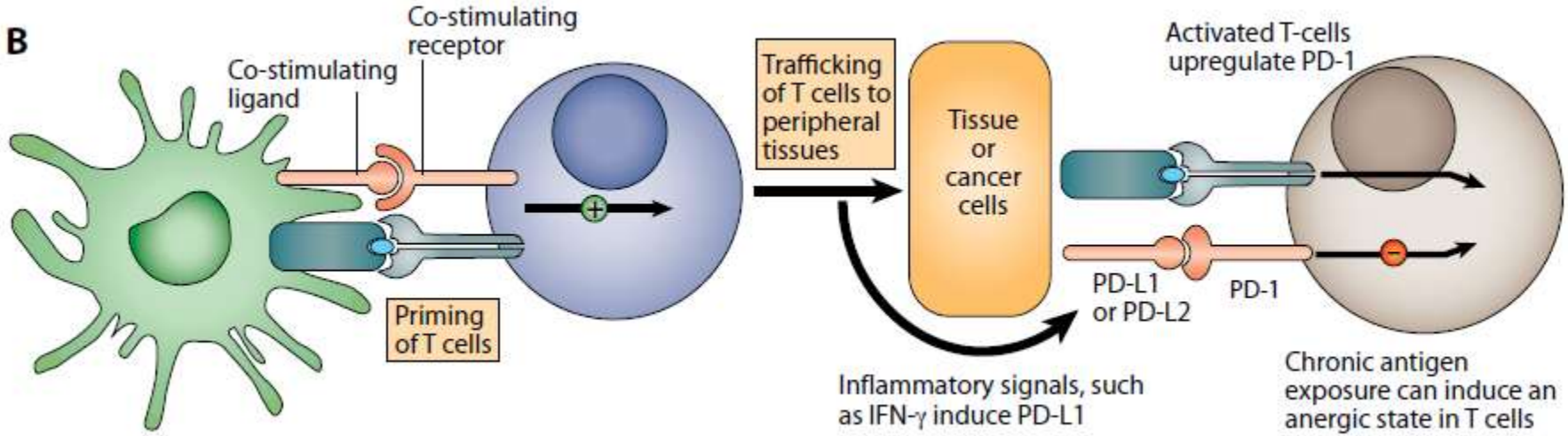
- Estos puntos de control inhibidores probablemente ayudan a los tejidos normales a evitar respuestas autoinmunes.
- Los tumores pueden disregular los puntos de control y vías activadoras y por lo tanteo inhibir la respuesta inmune.
- La inhibición dirigida de estos checkpoints pueden reactivar la respuesta inmune.

Los dos principales reguladores de checkpoints inmunes y las respuestas inmunes.
A: **CTLA-4 (Cytotoxic T-lymphocyte-associate antigen-4)**



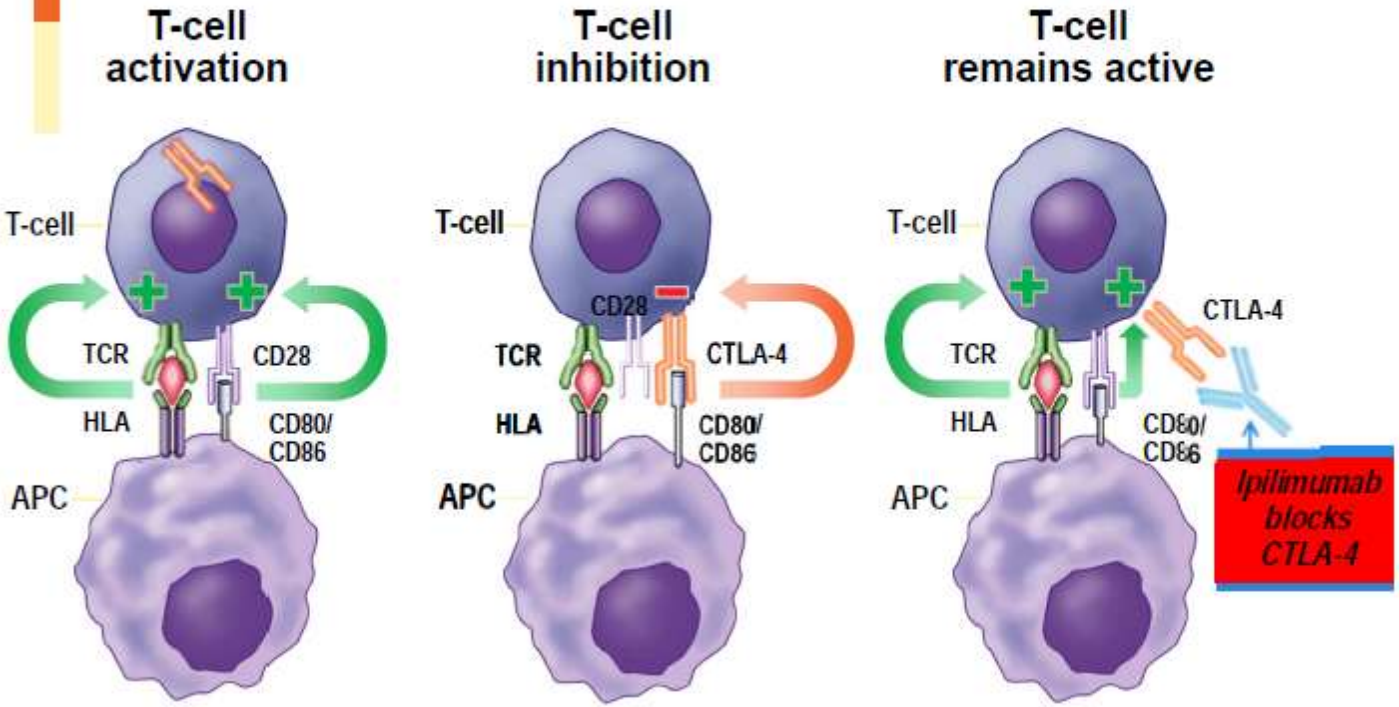
Pardoll DM. Nat. Rev. Cancer. 2012,12(4):252-264.

B: **PD-1 (Programmed cell death receptor)**



Pardoll DM. Nat. Rev. Cancer. 2012,12(4):252-264.

Ipilimumab Augments T-Cell Activation and Proliferation

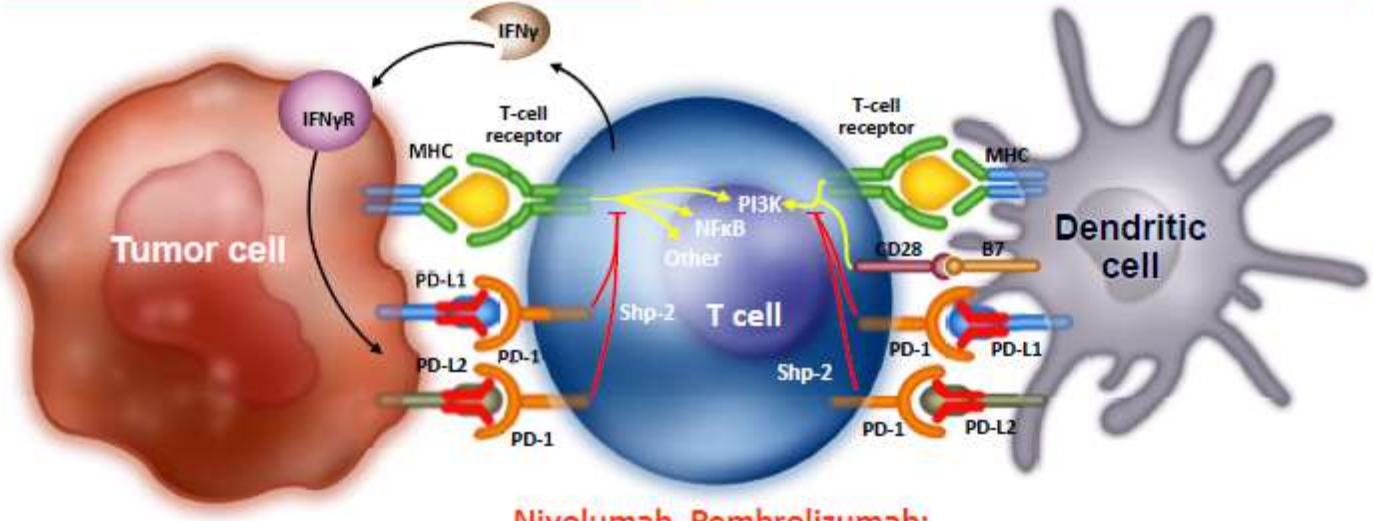


Adapted from O'Day et al. Plenary session presentation, abstract #4, ASCO 2010.

Role of PD-1 Pathway in Tumor Immunity

Recognition of tumor by T cell through MHC/antigen interaction mediates IFNγ release and PD-L1/2 up-regulation on tumor

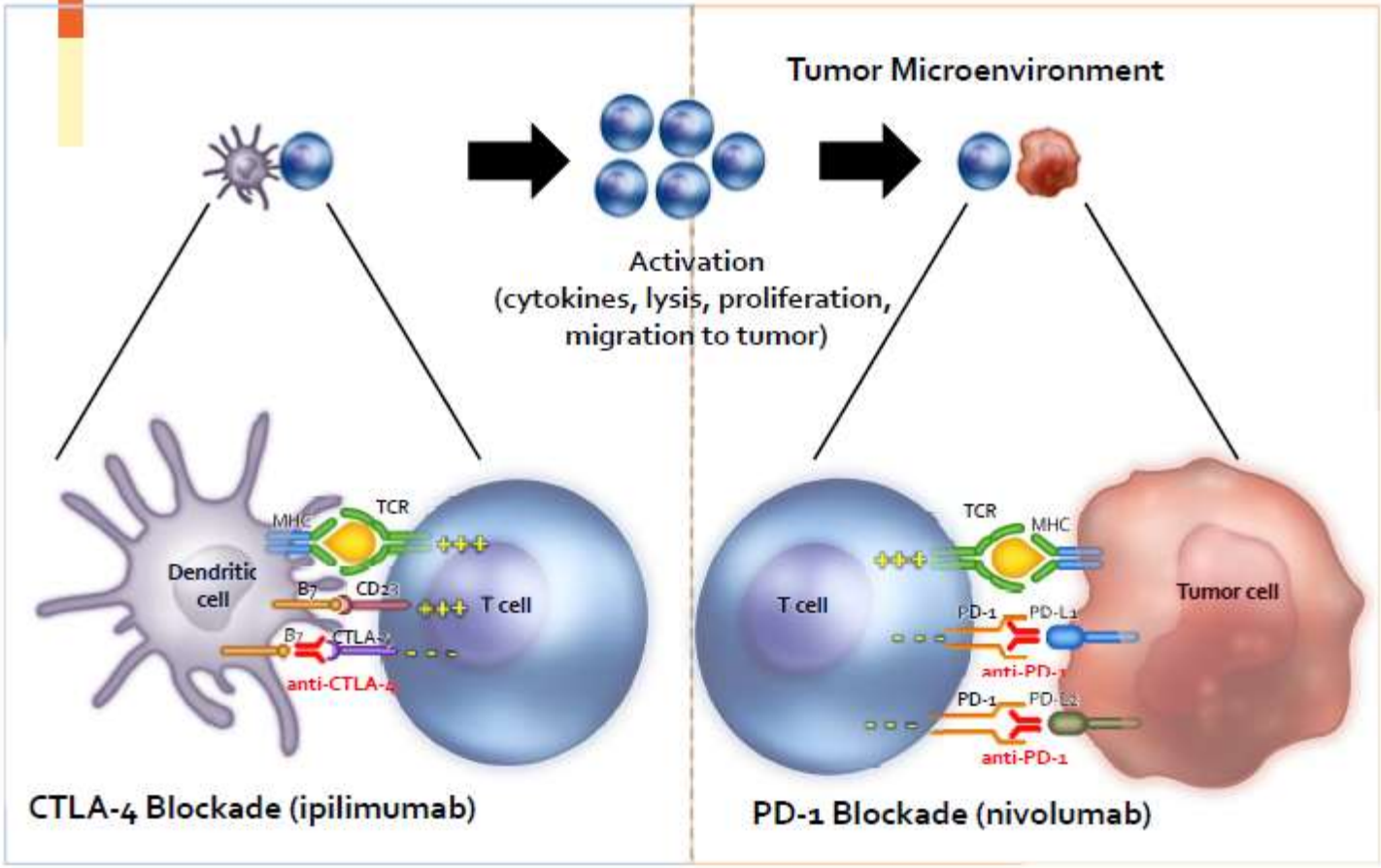
Priming and activation of T cells through MHC/antigen & CD28/B7 interactions with antigen-presenting cells

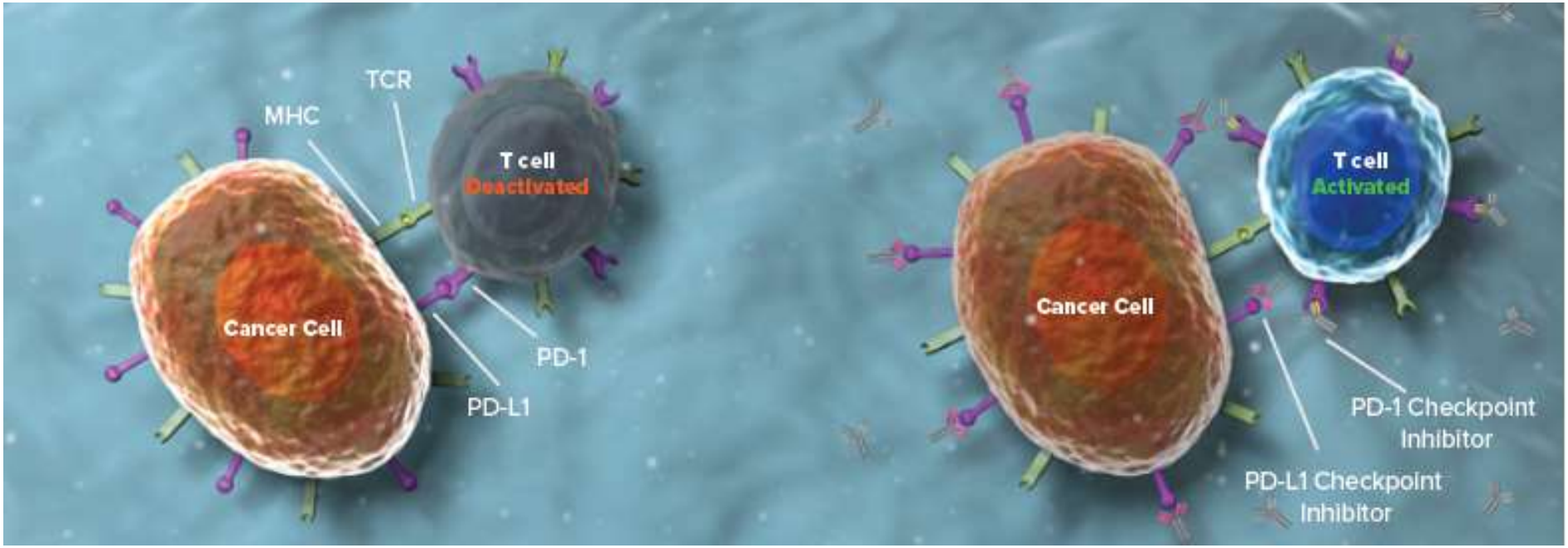


Nivolumab, Pembrolizumab:
PD-1 Receptor Blocking Abs
Atezolizumab: PD-L1 Blocking Ab

Sznol et al., ASCO, 2013

Blocking CTLA-4 and PD-1




MHC
TCR
T cell
Deactivated
Cancer Cell
PD-1
PD-L1
T cell
Activated
Cancer Cell
PD-1 Checkpoint
Inhibitor
PD-L1 Checkpoint
Inhibitor

ADVANCE OF THE YEAR: IMMUNOTHERAPY 2.0

EXPANDING USE AND REFINING PATIENT SELECTION

2017 CLINICAL CANCER ADVANCES

ASCO's 12th Annual Report on Progress Against Cancer

ASCO

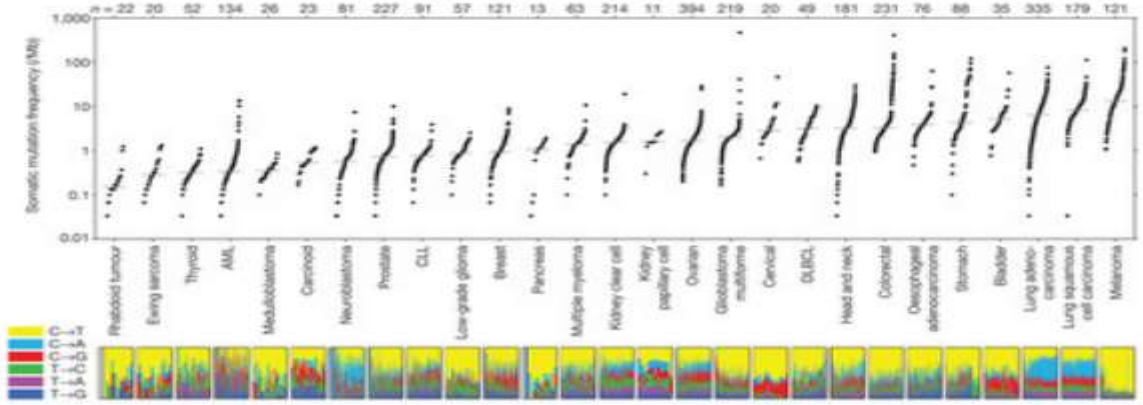
Inmunoterapia, una corta historia

- **1990**. Bacilo de Calmette-Guérin (BCG) en tratamiento de cáncer urotelial

- **2013**. Ipilimumab, (anti-CTLA-4) en melanoma
- **2014**. Pembrolizumab (anti-PD-1) y Nivolumab (anti-PD-1) en melanoma
- **2015**. Nivolumab y Pembrolizumab en CPCNP
- **2016**. Nivolumab en Carcinomas de Cabeza y Cuello.
- **2016**. Atezolizumab (anti-PD-L1) en cáncer de vejiga.
- **2016**. Atezolizumab en CPCNP
- **2016**. Nivolumab y Pembrolizumab en Linfoma de Hodgkin clásico

Inmunoterapia, una corta historia

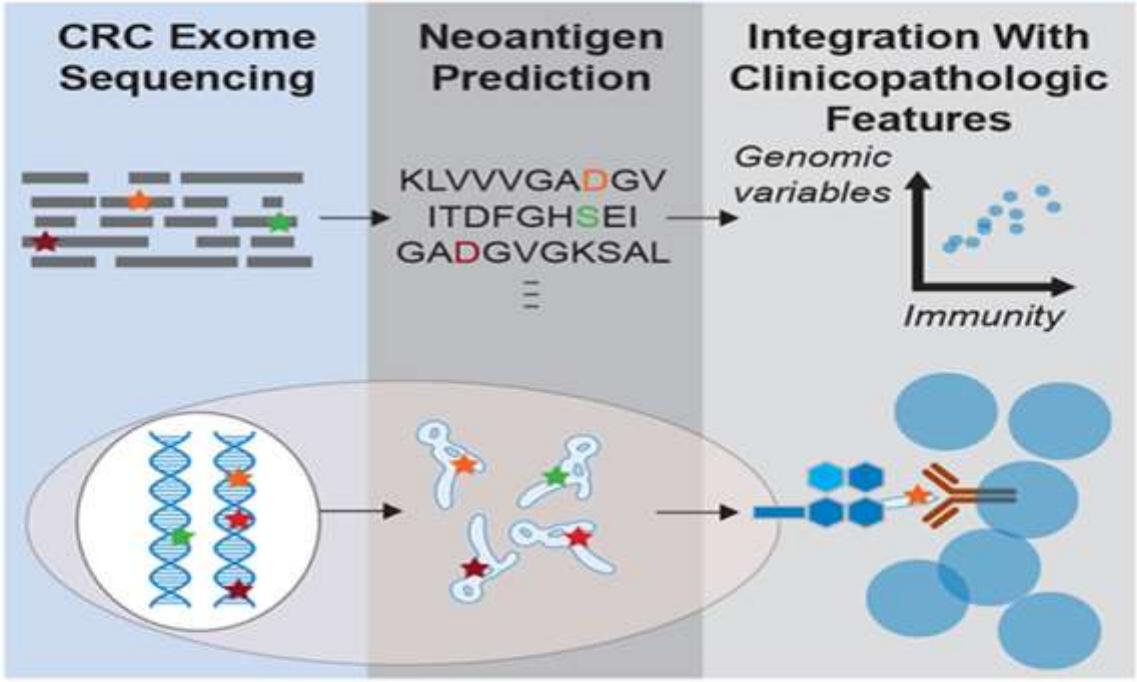
- Actualmente: Cánceres Hipermutados (*Carga Mutacional*)
  - Melanoma, Pulmón



Lawrence MS et al
Nature 499: 214-218 (Jul 2013)

- MMR (mismatch repair deficiency): colorrectal, endometrio, cerebral
  - Mutaciones germinales (Lynch) o esporádicas (MLH1, MSH2, MSH6 y PMS2)
  - Silenciamiento epigenético (hipermutación de MLH1)

dMMR and Immunotherapy: What Is the Connection?[1]



Inmunoterapia, una corta historia

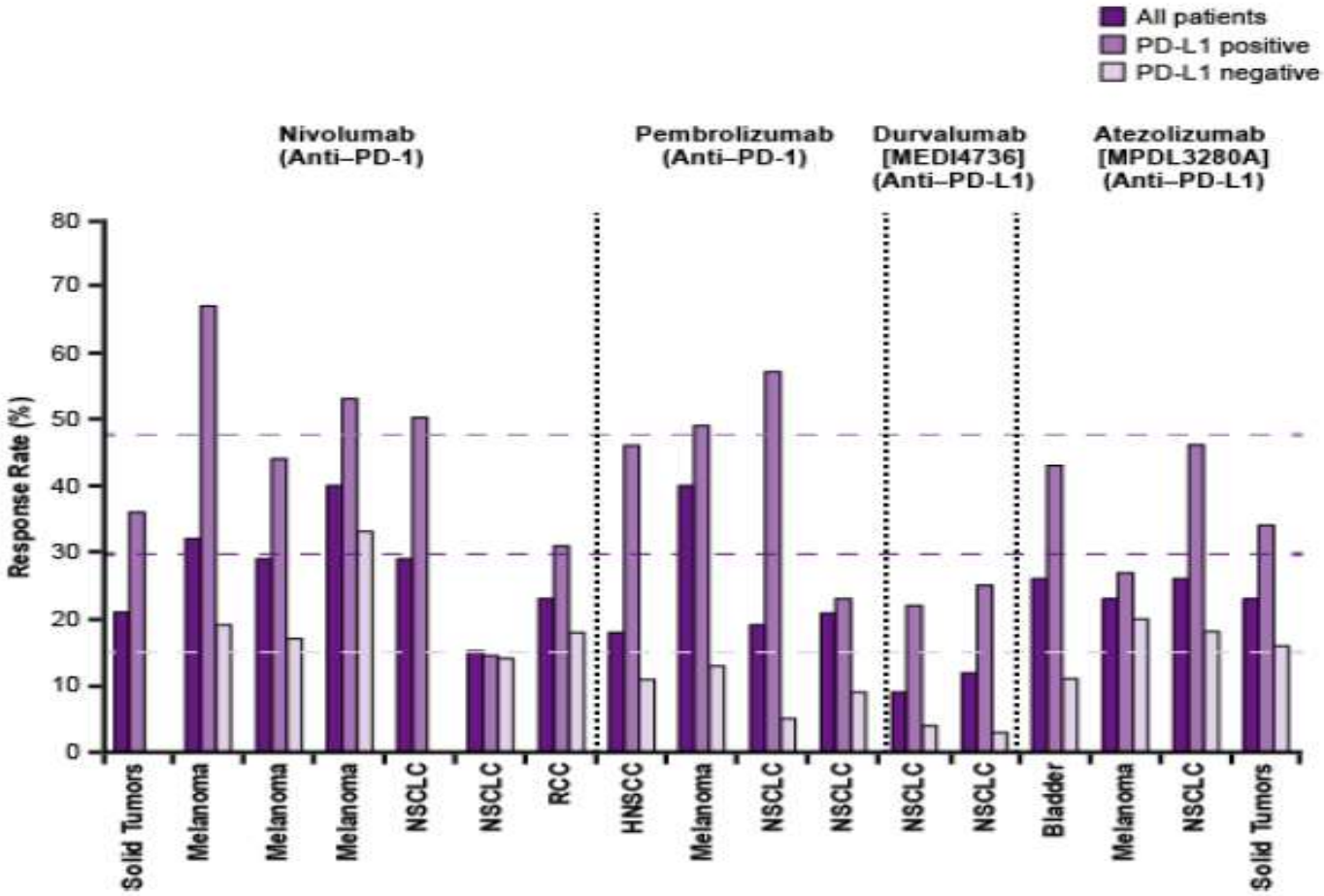
- Próximamente:
  - Nivolumab en cáncer de ovario
  - Nivolumab en carcinoma de células renales
  - Combinaciones de inmunoterapia / QT

Respuesta al tratamiento anti-PD1/PD-L1 en pacientes no seleccionados

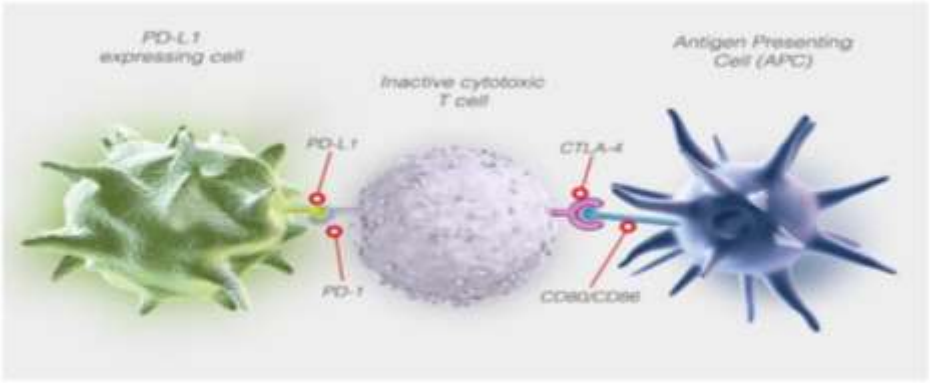
Neoplasia	Ratio de Respuesta
Pulmón	20%
CCR	25%
Vejiga	18%
Ovario	15-18%
Mama	18%

Dr R. Aggarwal
ASCO Chicago, 2016

Figure 2. Correlation of pretreatment PD-L1 expression and response rate.



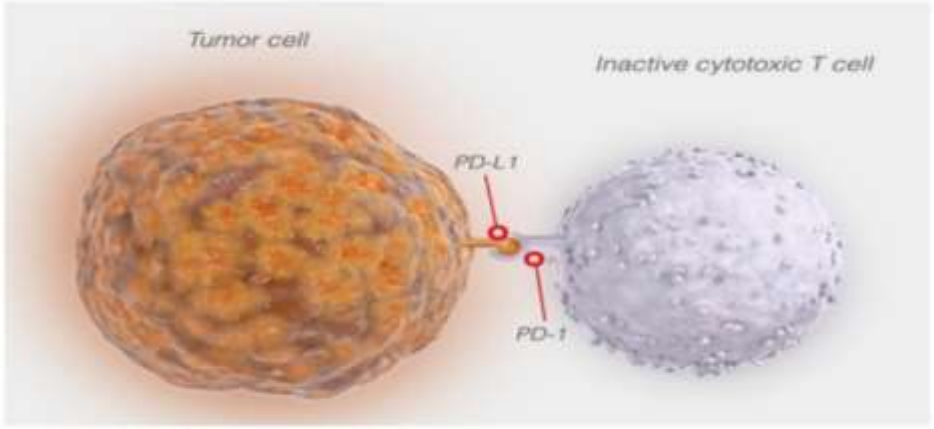
Normal cells limit damage to healthy tissue



Inactivation of T cells limits damage to healthy tissue.

PD-L1 se expresa de manera amplia y dinámica en células presentadoras de antígenos y otras células inmunes

Tumor cell escapes detection



Inactivation of T cells reduces tumor cell killing

PD-L1 se expresa en múltiples tipos de cáncer

Expresión Intratumoral de PD-L1 mediante Inmunohistoquímica

Permite identificar grupos de pacientes con tasas de respuesta importantes

Limitaciones:

1. Variabilidad de técnicas utilizadas (hasta un 25% de diferencia según el anticuerpo empleado),
2. Heterogeneidad de estudios, con marcaje de células tumorales, de células inmunitarias o de ambas e incertidumbre sobre el significado de estos diferentes tipos de marcaje,
3. Heterogeneidad/Variabilidad de expresión de PD-L1 a nivel espacial y temporal.

Inhibidores de Checkpoints inmunes y Tests Inmunohistoquímicos asociados

Farmaco	Diana	Test IHQ PD-L1	Epítopo del Ac PD-L1	Inmuno-teñidor	Sistema de Detección
Nivolumab	PD1	28-8	Extracelular	Dako Link 48	Envision Flex
Pembrolizumab	PD1	22C3	Extracelular		
Atezolizumab	PD-L1	SP142	Citoplasmático	Ventana Benchmark	Optiview + Amplificación
Durvalumab	PD-L1	SP263	Citoplasmático		Optiview
Avelumab	PD-L1	73-10	Citoplasmático	Dako Link 48	Envision Flex

Tests de Inmunohistoquímica

Farmaco	Test IHQ PD-L1	PD-L1 scoring	Puntos de corte en ensayos clínicos	FDA Diagnostic Status
Nivolumab	28-8	Cel. Tumorales	1%, 5%, 10%	Complementary
Pembrolizumab	22C3	Cel. Tumorales	1%, 50%	Companion
Atezolizumab	SP142	Cel. Tumorales(TC)	1%, 5%, 50%	Complementary
		Cel. Inmunes (IC)	1%, 5%, 10%	
Durvalumab	SP263	Cel. Tumorales	25%	Unknown
Avelumab	73-10	Cel. Tumorales	1%, 50%, 80%	Unknown
	E1L3N	Cel. Tumorales		Unknown

Companion Diagnostic: El test es esencial para el uso seguro y efectivo del fármaco.
Complementary Diagnostic: El test da información que ayuda en la decisión terapéutica sobre el riesgo/beneficio, sin restringir el acceso al fármaco

Intratumoral PD-L1 as a Predictive Marker of Tumor Response: Current Evidence from Select Studies[8,10-19]

Setting	Immune Checkpoint Inhibitor	Objective Response Rate, %		
		Unselected	PD-L1 Positive	PD-L1 Negative
Melanoma				
CheckMate-067	Nivolumab (n = 316) Nivolumab + ipilimumab (n = 314)	43.7 57.6	57.5 72.1	41.3 54.8
CheckMate-066	Nivolumab (n = 210)	40.0	52.7 (≥ 5% tumor cell)	33.1
KEYNOTE-006	Pembrolizumab (n = 556)	33.0	NR (≥ 1% tumor cell)	NR
Non-Small-Cell Lung Cancer				
CheckMate-017 (squamous)	Nivolumab (n = 135)	20.0	19.0 (≥ 10% tumor cell)	16.0
CheckMate-057 (nonsquamous)	Nivolumab (n = 292)	19.0	37 (≥ 10% tumor cell)	11.0
KEYNOTE-001	Pembrolizumab (n = 495)	19.4	45.2 (≥ 50% tumor cell)	10.7
POPLAR	Atezolizumab (n = 144)	15.0	38 (≥ 50% tumor cell or ≥ 10% immune cell)	8.0
Renal Cell Carcinoma				
	Nivolumab (n = 168)	21.0	31 (≥ 5% tumor cell)	18.0
Bladder Cancer				
	Atezolizumab (n = 92)	NR	50 (≥ 5% immune cell)	17.0
	Pembrolizumab (n = 33)	NA	27.6 (≥ 1% tumor cell or any immune cell)	NA
Squamous Cell Carcinoma of the Head and Neck				
	Durvalumab (n = 62)	11	18 (≥ 25% tumor cell)	8

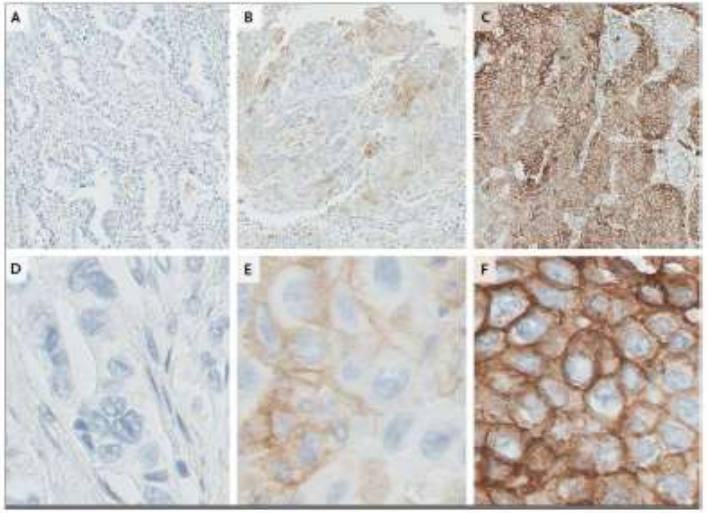
NA, not applicable; NR, not reported

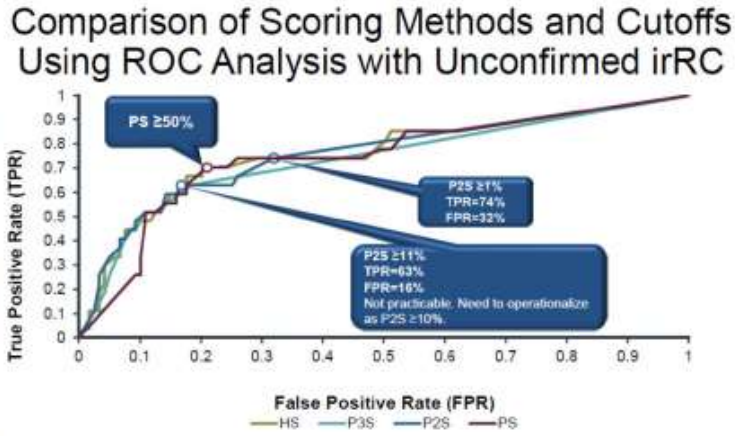
La tasa de PD-L1-positivos varía con los diferentes tests diagnósticos

Cancer	Antibody	PD-L1 cutoff	PD-L1 positive rate
NSCLC	SP142	Tumor Cell 1/2/3 or Immune Cell 1/2/3	65% J Clin Oncol 33, 2015 (suppl; abstr 3015)
NSCLC	22C3	TPS≥50%	23% Garon et al. NEJM April 2015
NSCLC	SP263	Tumor cells >25%	23% J Clin Oncol 33, 2015 (suppl; abstr 8032)
Urothelial	22C3	Tumor cells ≥1%	64% Presented By Elizabeth Plimack at 2015 ASCO Annual Meeting
Urothelial	SP142	Immune Cell 2/3	26% http://www.roche.com/investors/updates/inv-update-2016-01-08.htm

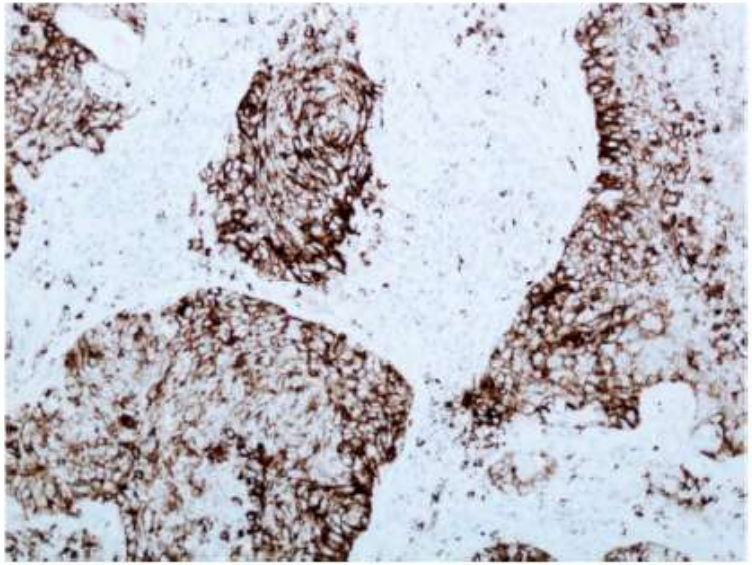
TC: tumor cells, IC: immune cells, TPS: tumor proportion score

22C3 assay and Pembrolizumab

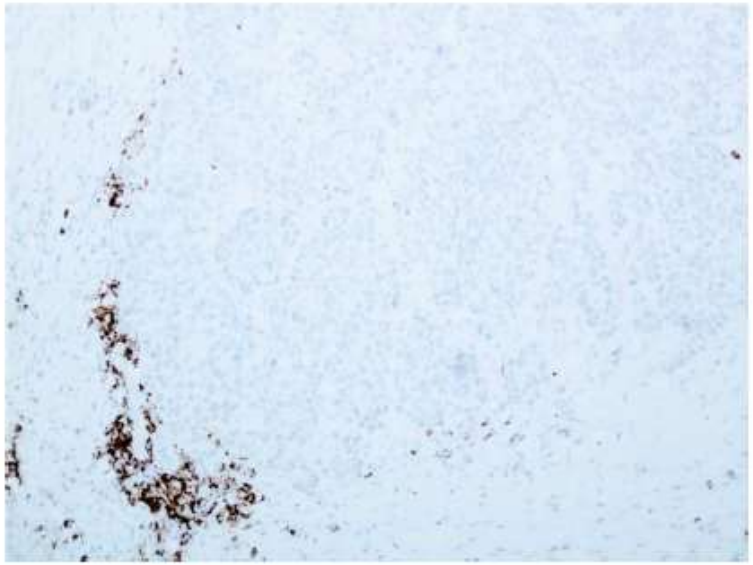




Atezolizumab & anti-PD-L1 SP142 clone based assay: Optiview detection and amplification



Tumour cells: % TC positive



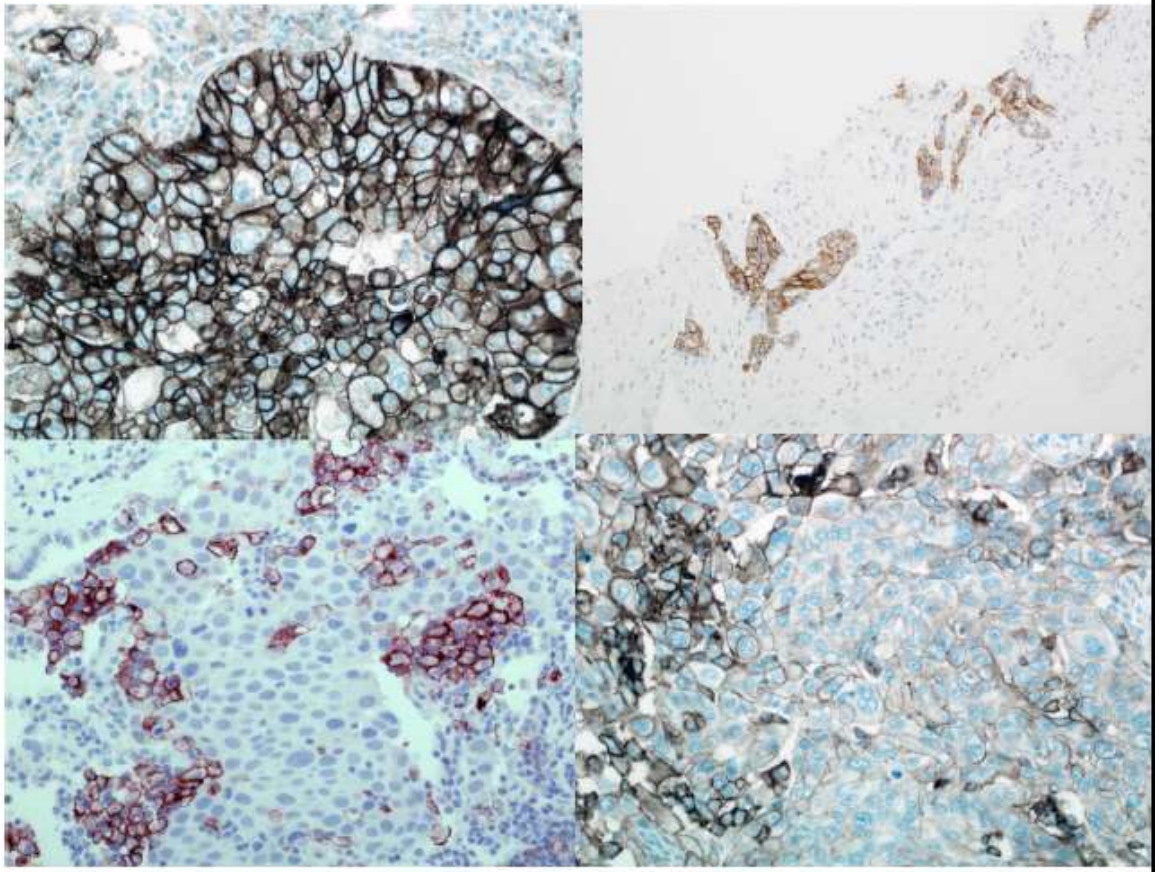
Immune cells: % area of tumour infiltrated

Nivolumab

28-8 clone assay

Complimentary or Companion diagnostic Assay?

1% threshold is 'positive'



- Los test se han desarrollado con el principio “un test-un fármaco”
- Criterios de cuantificación e interpretación específicos
- Desarrollo del test diagnóstico ligado a la respuesta clínica al fármaco

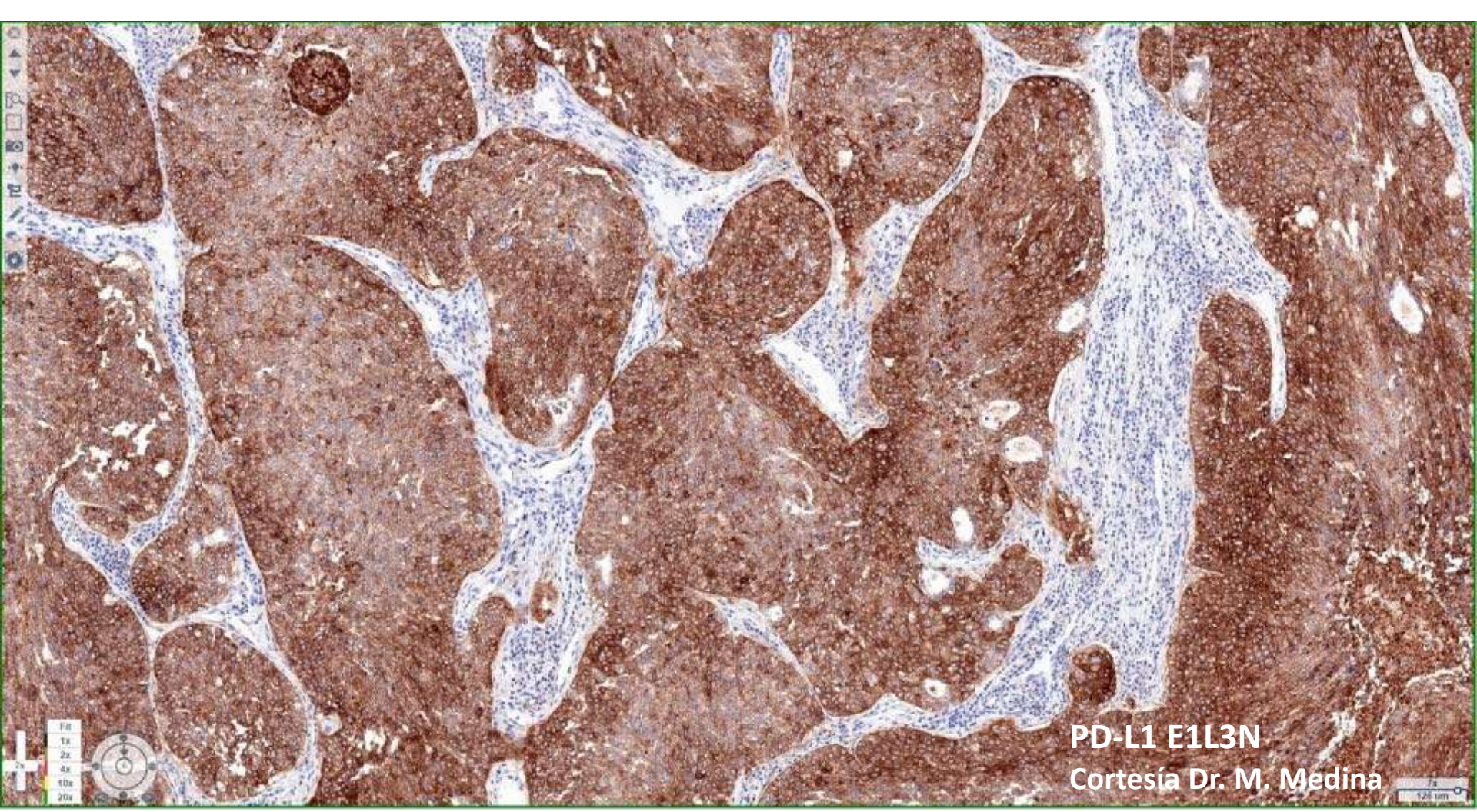
Original Investigation

Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non–Small-Cell Lung Cancer

Joseph McLaughlin, MD; Gang Han, PhD; Kurt A. Schalper, MD, PhD; Daniel Carvajal-Hausdorf, MD; Vasiliki Pelakanou, MD, PhD; Jamaal Rehman, MD; Vamsidhar Velcheti, MD; Roy Herbst, MD, PhD; Patricia LoRusso, DO; David L. Rimm, MD, PhD

JAMA Oncology Published online November 12, 2015

- Se compararon dos anticuerpos monoclonales anti-PD-L1 comerciales que reconocen el dominio intracelular:
  - SP142 Ventana
  - E1L3N Cell Signaling Technology
- 49 casos de CPCNP de archivo
- Mediante IHQ e IFC


PD-L1 E1L3N
Cortesía Dr. M. Medina

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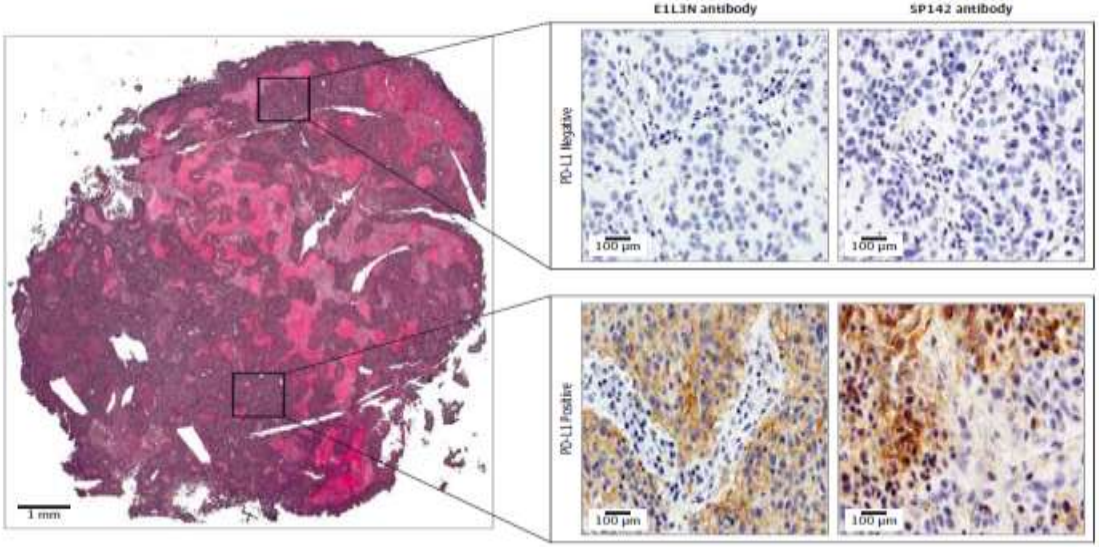
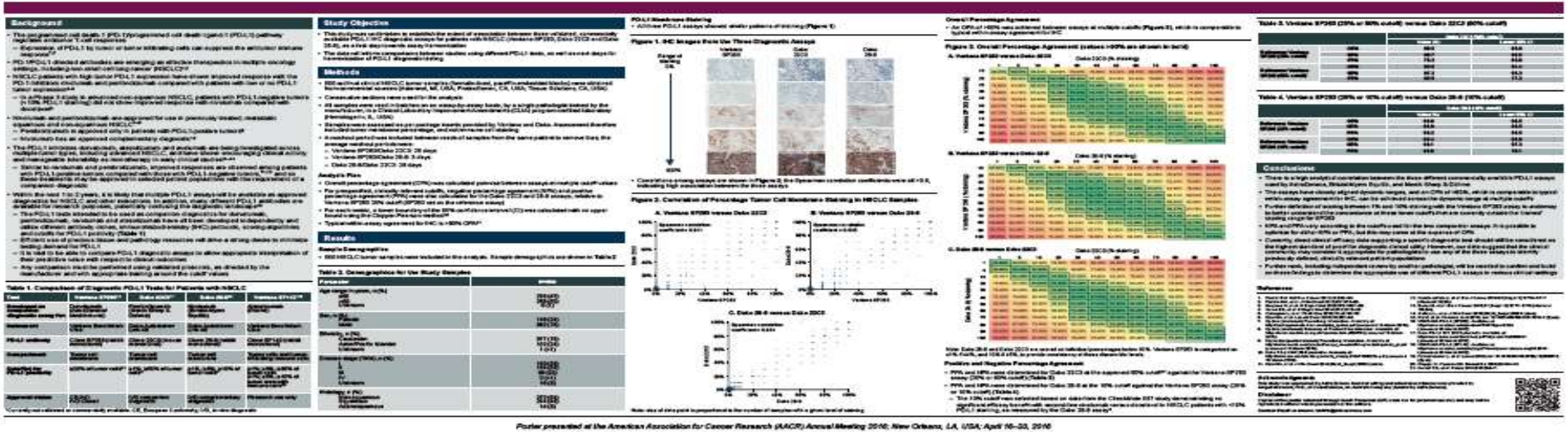
JAMA Oncology Published online November 12, 2015

Resultados:

- Gran heterogeneidad intratumoral
- No concordancia en IHQ

Figure 1. PD-L1 Protein Heterogeneity Using Diaminobenzidine





M. Ratcliffe. 2016 American Association for Cancer Research Annual Meeting. Abstract LB-094

Radcliffe's study

- Se testaron tres métodos disponibles comercialmente:
  - Ventana SP263
  - Dako 28-8
  - Dako 22C3
- 500 NSCLC de archivo (54% no escamosos y 43% escamosos)
- Evaluados por un único patólogo

Radcliffe's study

	Reference: Ventana SP-263 (≥25% tumour membrane staining)		
Dako 28-8 assay cut off	PPA (%)	NPA (%)	OPA (%)
>1%	58	100	81
>5%	72	100	90
>10%	91	98	96

Conclusions: This study indicates that the patient population defined by Ventana **SP263** at the 25% cut off is similar to that identified by the Dako-**28-8** assay at the 10% tumour membrane cut off.

This, together with data on the **22C3** assay, will enable cross comparison of studies using different PD-L1 tests, and widen options for harmonization of PD-L1 diagnostic testing.

A “Blueprint Proposal” for Companion Diagnostic Comparability

Blueprint Working Group Members:

Steven Averbuch, MD. Vice President, Development, Oncology & Pharmacodiagnostics, **Bristol-Myers Squibb**

Kenneth Emancipator, MD. Executive Medical Director, **Merck Research Laboratories**

Ian McCaffery, PhD. Head Companion Diagnostic Development, Oncology Biomarker Development, **Genentech**

Abigail McElhinny, PhD. Vice President, Assay and Reagent Development, **Ventana Medical Systems Inc**.

Dave Stanforth. Director, Head of R&D, Companion Diagnostics, **Agilent Technologies**

Jill Walker, PhD. Executive Director, Companion Diagnostic Development, Immuno-Oncology, **AstraZeneca**

Doug Ward. Vice President & General Manager, Companion Diagnostics, **Ventana Medical Systems Inc**.

March 2015

The Blueprint PD-L1 IHC Assay Comparison Project

Retos

- Realizar un test para cada fármaco no es práctico:
  Falta de muestra, retraso en tiempo, costes

- Emplear un único test para todos los fármacos tampoco lo es
  Equipamiento, realización del test y criterios de gradación e interpretación son específicos para cada test desarrollado

- Podría ser perjudicial para los pacientes si se cruzaran test-fármacos de forma indebida sin una revisión de validez clínica adecuada

A Blueprint Proposal for Companion Diagnostic Comparability

PD-L1 Assay Analytical Performance Comparison

Objetivo: Comparar los sistemas Dako y Ventana de detección inmunohistoquímica de PD-L1 para establecer el nivel de similaridad analítica.

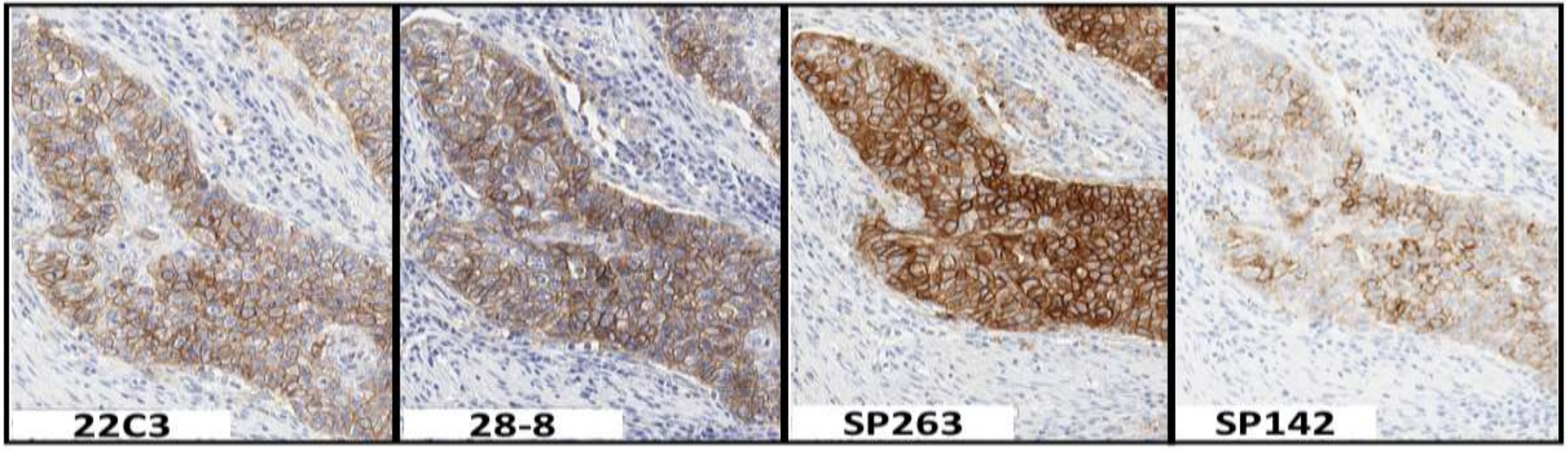
PD-L1 Assay Systems used in the Blueprint Project

	Nivolumab	Pembrolizumab	Atezolizumab	Durvalumab
Primary antibody clone used in the assay system	28-8 (Dako)	22C3(Dako)	SP142(Ventana)	SP263(Ventana)
Interpretative Scoring	Tumor cell membrane	Tumor cell membrane	-Tumor cell membrane - Infiltrating immune cells	Tumor cell membrane
Instrument and Detection Systems Required	EnVision Flex on Autostainer Link 48	EnVision Flex on Autostainer Link 48	OptiView Detection and Amplification on Benchmark ULTRA	OptiView Detection on Benchmark ULTRA
Therapeutic Developer	Bristol-Myers Squibb	Merck	Genentech	AstraZeneca

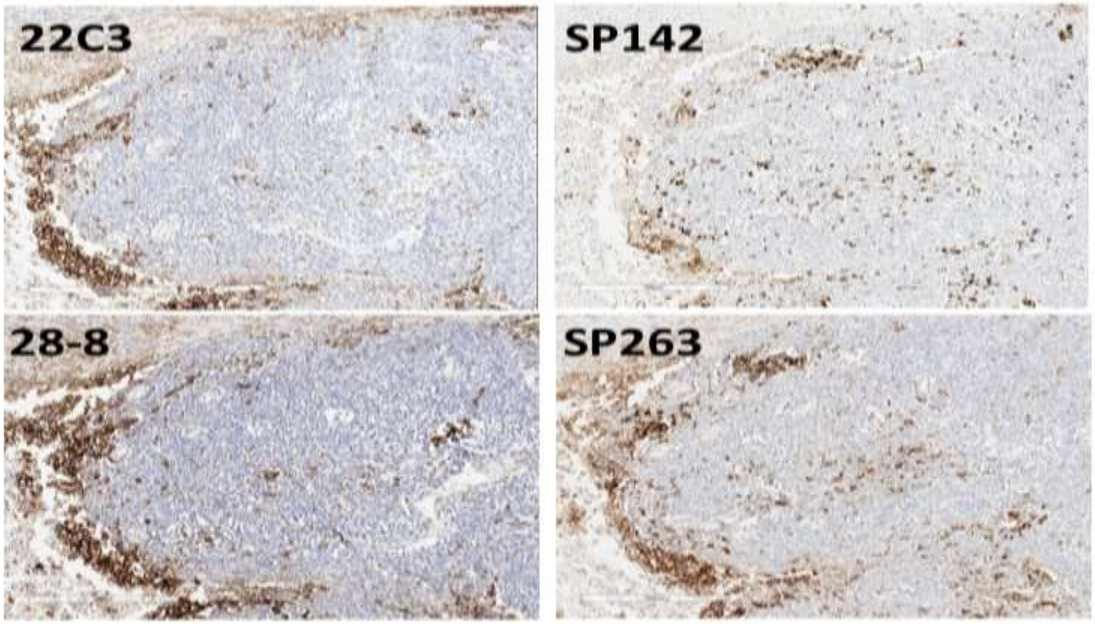
Blueprint Project

- **Fase 1:**
  - 39 casos de resección de CPCNP y 4 controles
  - Evaluados por tres patólogos
  - Análisis de
    - % de células tumorales teñidas y
    - % de células inflamatorias teñidas

Expresión en células tumorales



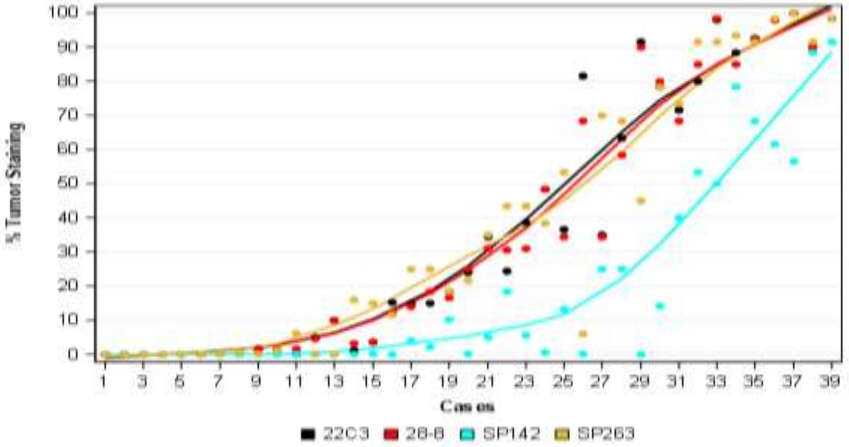
Expresión en células inmunes



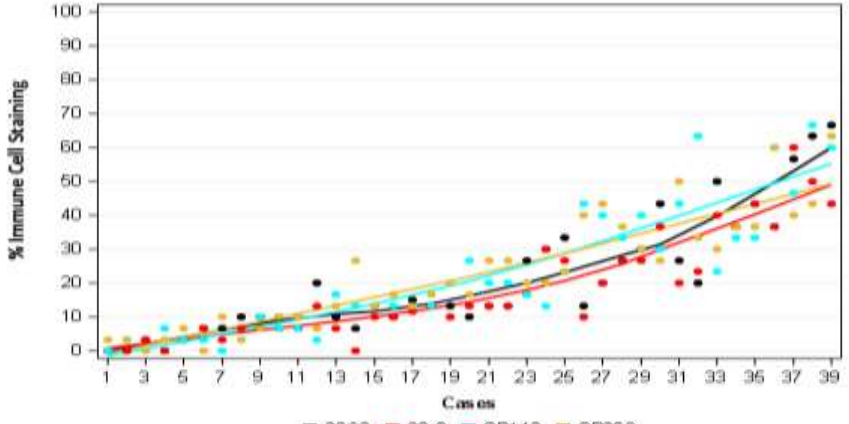
Blueprint Project

Resultados:

- 3 de los 4 anticuerpos evaluados proporcionaron datos muy similares en tinción de células tumorales



- Gran variabilidad en cuantificación de células inmunes (no training)



FR Hirsch. AACR 2016

JAMA Oncology | Original Investigation

A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non–Small Cell Lung Cancer

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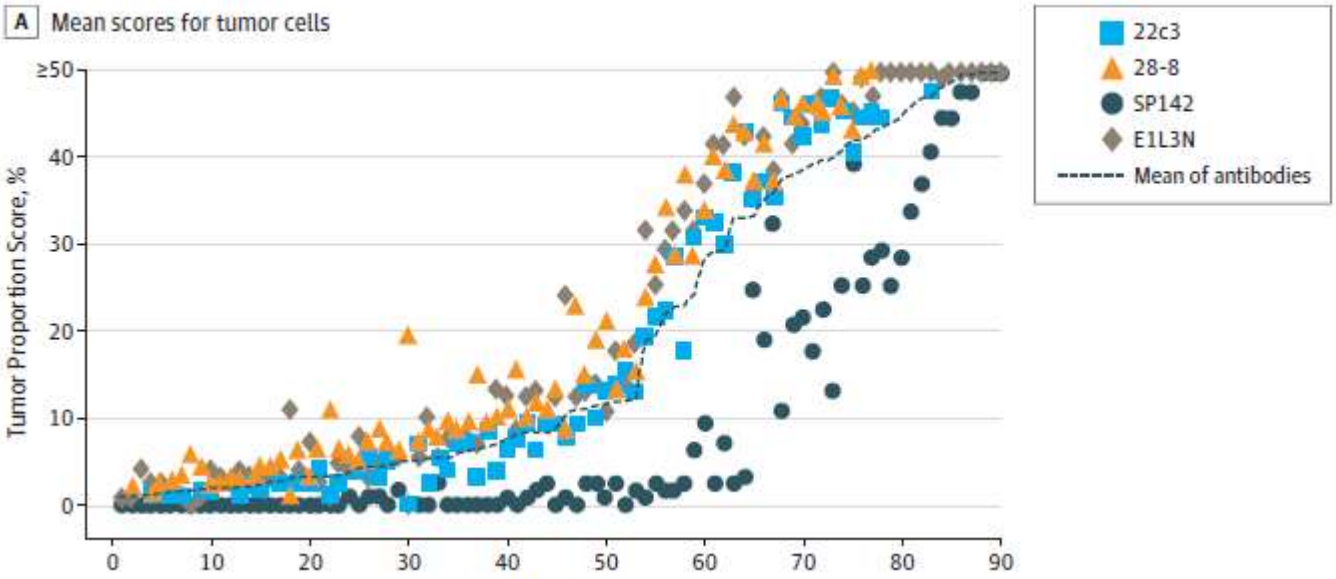
Figure 2. Tumor Proportion Scores and Immune Cell Proportion Scores



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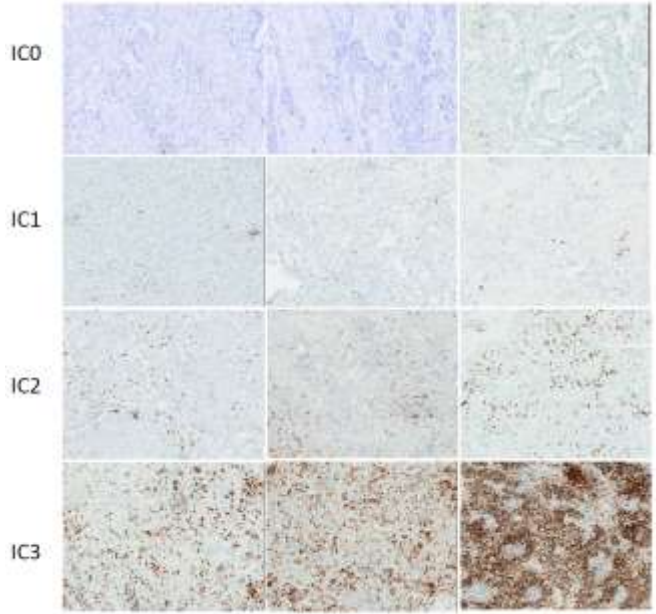
Blueprint Project

- **Fase 2:**
  - 81 casos de bloques celulares, biopsia y resección de CPCNP
  - Evaluados por 25 patólogos
  - Ac: **22C3**(Dako), **28-8**(Dako), **SP263**(Ventana), **SP142**(Ventana), **73-10**(Dako)
  - Análisis de
    - % de células tumorales teñidas y
    - % de células inflamatorias teñidas

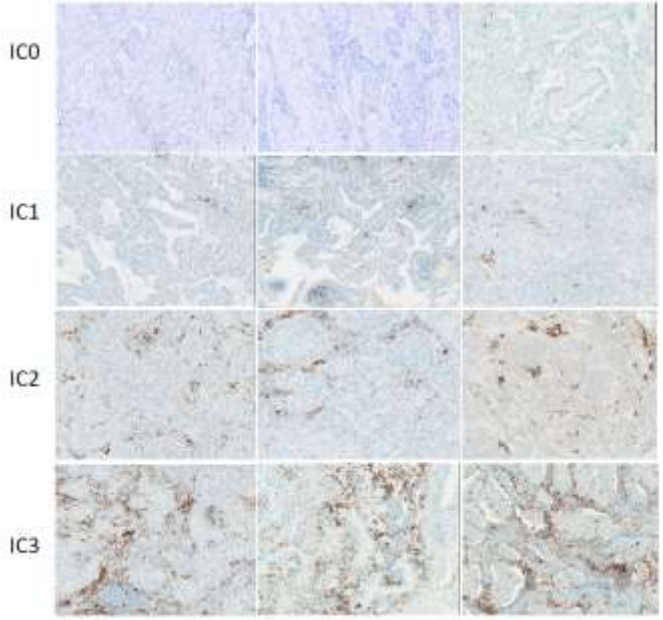
M.S. Tsao, IASLC 18th World Conference on Lung Cancer, Octubre 2017

Immune cell scoring (SP142)

Single cell spread



Aggregates



Buena reproducibilidad entre todos los patólogos en el análisis de células tumorales

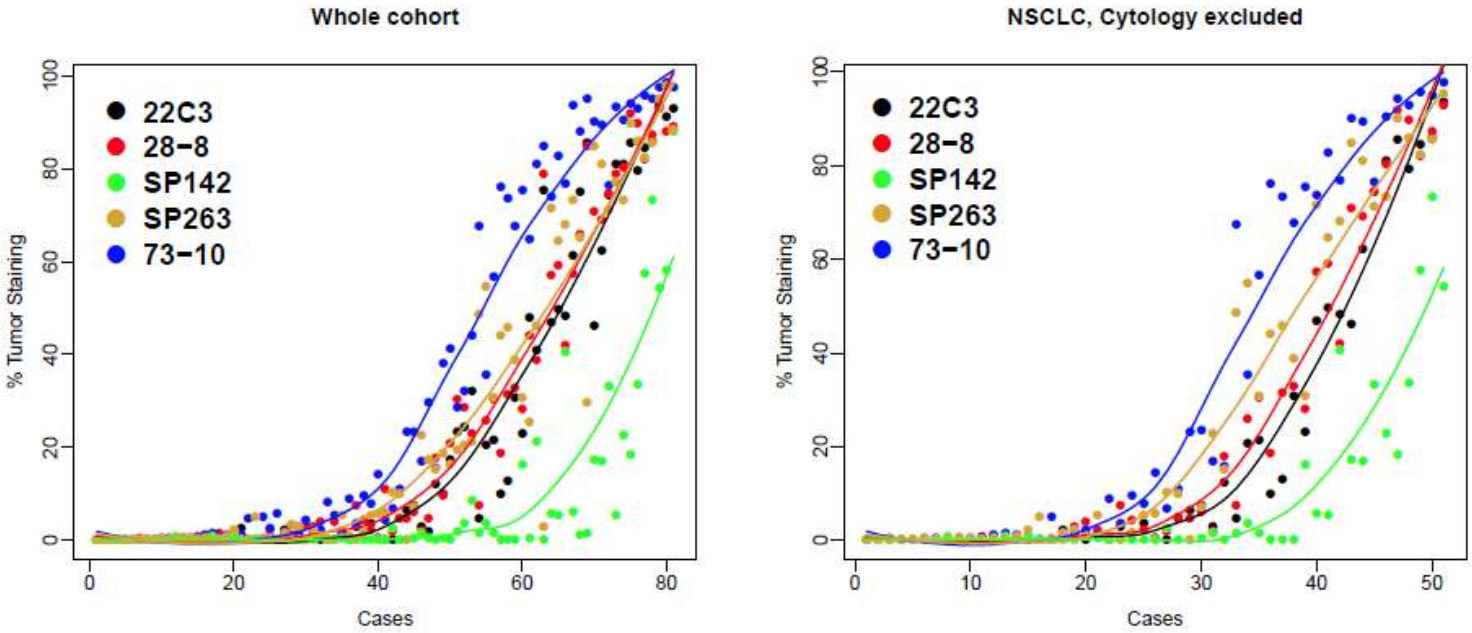
DIGITAL	All cases	NSCLC tissue only	Cytology only
22C3	0.91	0.91	0.91
28-8	0.86	0.88	0.77
SP-142	0.81	0.85	0.76
SP-263	0.90	0.93	0.82
73-10	0.89	0.91	0.82
All assays	0.91	0.93	0.84

GLASS SLIDE	All cases	NSCLC tissue only	Cytology only
22C3	0.89	0.87	0.88
28-8	0.92	0.94	0.87
SP-142	0.86	0.84	0.90
SP-263	0.86	0.89	0.79
73-10	0.93	0.93	0.84
All assays	0.86	0.89	0.77

ICC: **>0.90 excellent** **0.75-0.9: good**

Koo TK & Li MY, J Chiropr Med 2016;15:155-63

Comparability among five assays on tumor cell staining



Each circle represents the mean of all scores (glass slide & digital combined)

Escasa reproducibilidad entre patólogos en análisis de células inmunes

DIGITAL		
	All cases	NSCLC tissue only
22C3	0.28	0.23
28-8	0.19	0.14
SP-142	0.36	0.28
SP-263	0.25	0.13
73-10	0.17	0.10
All assays	0.19	0.11

GLASS SLIDE		
	All cases	NSCLC tissue only
22C3	0.27	0.19
28-8	0.29	0.19
SP-142	0.33	0.25
SP-263	0.17	0.10
73-10	0.17	0.11
All assays	0.21	0.13

Fleiss Kappa statistics: **0.40-0.59: weak** **0.20-0.39: minimal** **<0.01-0.20: slight/none**

Conclusiones de BluePrint 2

1. **22C3**, **28-8** y **SP263** son comparables, **SP142** detecta menos y **73-10** tiñe mas células tumorales
2. Análisis de PD-L1 en imágenes digitales y laminillas muestra resultados comparables
3. La cuantificación de PD-L1 en células tumorales por patólogos en muestras de tejido es muy reproducible
4. La cuantificación de expresión de PD-L1 en células inmunes es poco reproducible
5. El análisis de expresión de PD-L1 expresion en bloques celulares puede tener cierta reproducibilidad. Requiere confirmación.

¿Podemos emplear un único test para todos los fármacos?

El CAP no se compromete…. Aconseja seguir las indicaciones

Las farmaceuticas se van adaptando….

Pembrolizumab ya puede prescribirse con IHQ positiva con 22C3 o SP263

Acuerdos locales con Oncología en función de disponibilidad, fármacos a emplear, etc.

Aspectos Prácticos

Emplear controles de inmunotinción (líneas celulares/ amígdala)

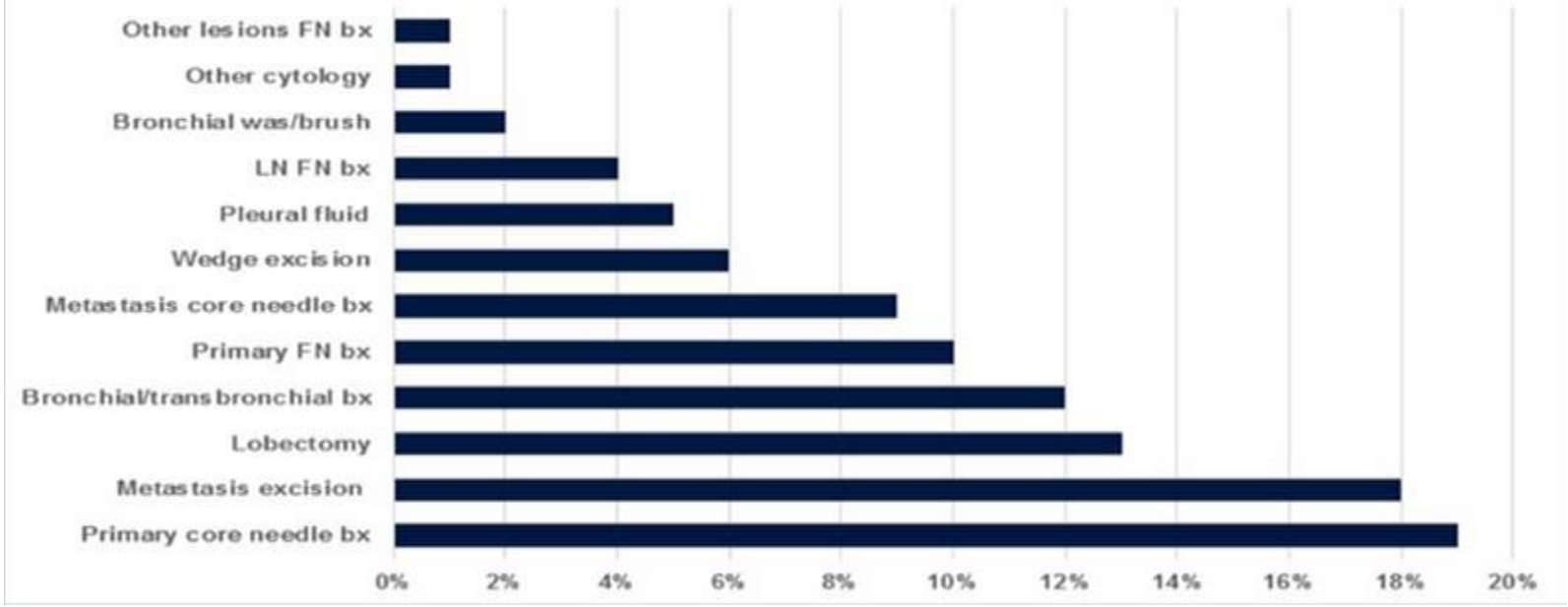
La muestra debe contener al menos 100 células tumorales

Evaluar tinción de membrana, parcial o total, de cualquier intensidad

Indicar el porcentaje final de células tumorales teñidas

Types of Specimens Submitted for Analysis

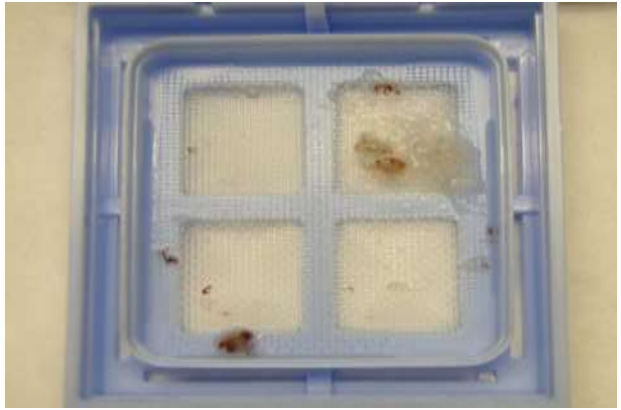
Shiau CJ et al. J Thorac Onc 2014;9:947-956



Primary Tumor: 62%
- FNA/core needle biopsy 29%
- Resection (lobectomy/wedge) 19%
- Bronchial biopsy/washing-brushing 14%

Non-primary Tumor: 38%
- Metastasis (excision/core biopsy) 28%
- Pleural fluid 5%
- Lymph node biopsy 4%
- Others 1%

Muestras limitadas



Bronchial biopsy

Core needle biopsy 19G

Testing algorithm

2 x 1mm tissue Fragments

On average only 20% is tumour

Diagnose & subtype lung cancer

Squamous
Adeno
etc

Immuno-Histochemistry IHC If required

Biomarker testing dictated by histology and protocol

Sections for DNA extraction

'Test tube' tests

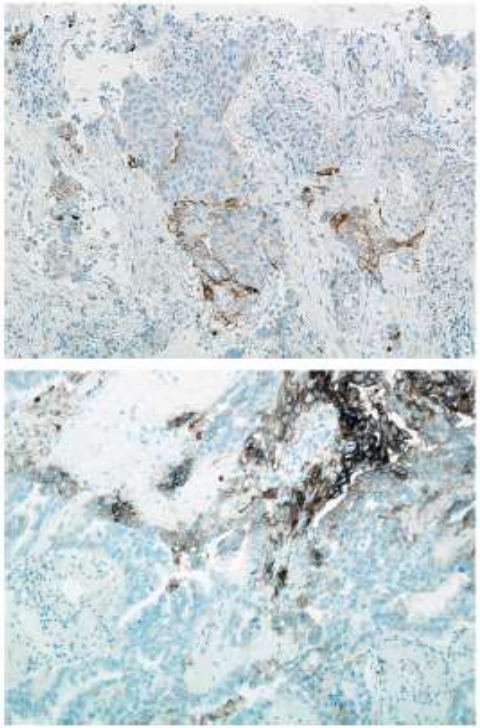
EGFR, KRAS, BRAF mutation (NGS panels)

Sections for Biomarker IHC & FISH

Morphology-based tests

ALK, ROS1
EGFR
PD-L1

Heterogeneity and PD-L1



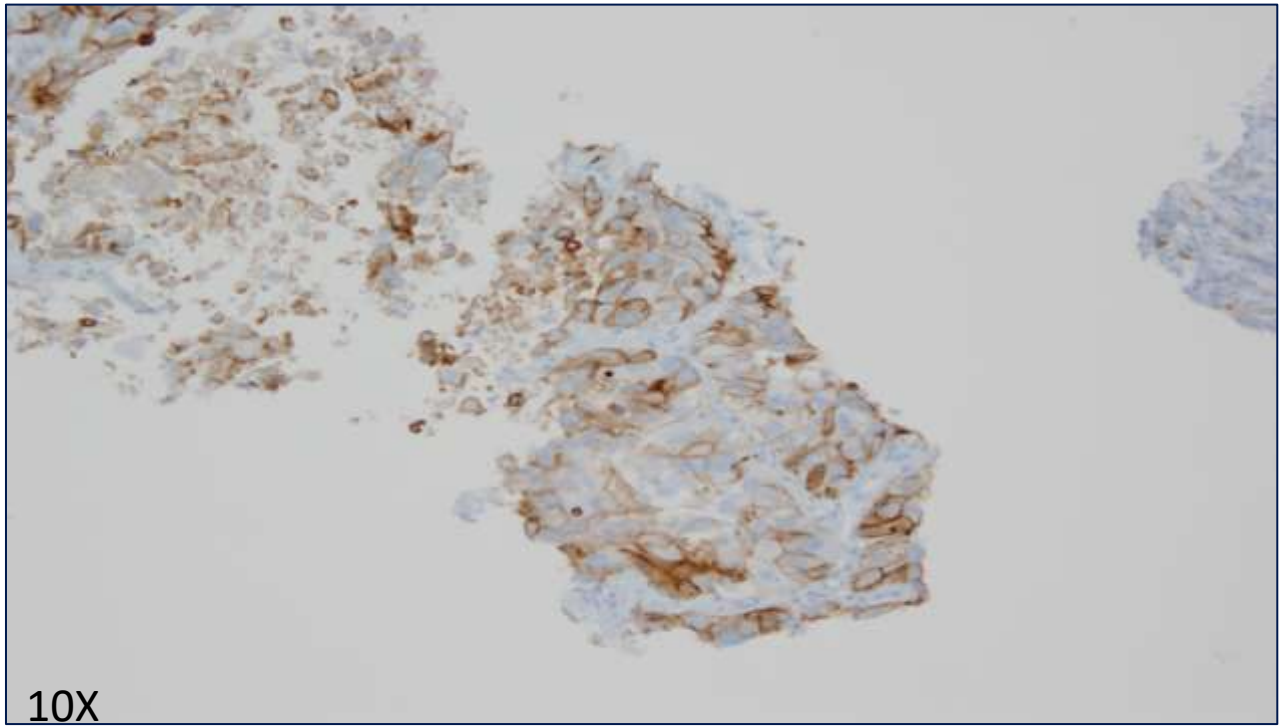
- Expression is dynamic
- Expression is heterogeneous
- Sampling ‘error’ must occur
- Greater impact at lower thresholds

- Part of the reason why the biomarker appears worse than it is
- Trials ‘control’ for heterogeneity to some extent

PD-L1 Immunohistochemistry: Expression Heterogeneity and Potential for Sampling Error

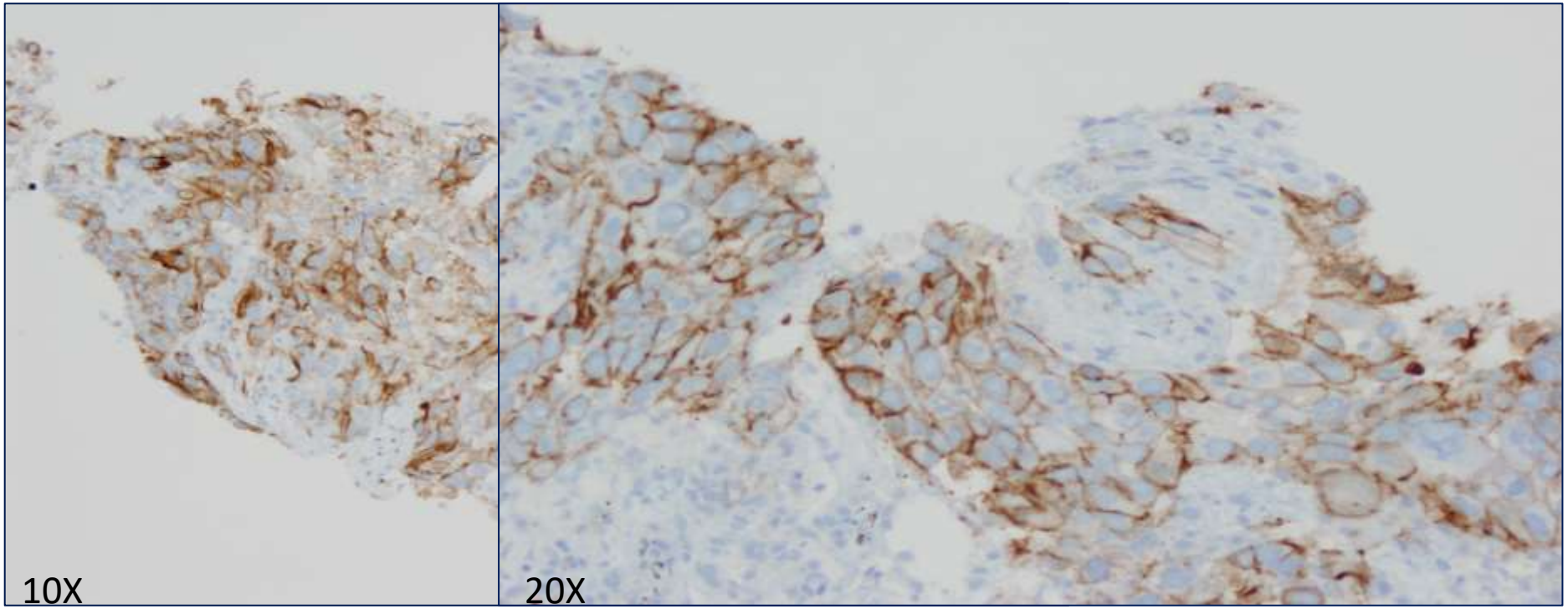
Hirsch FR, 2017.

Case #1



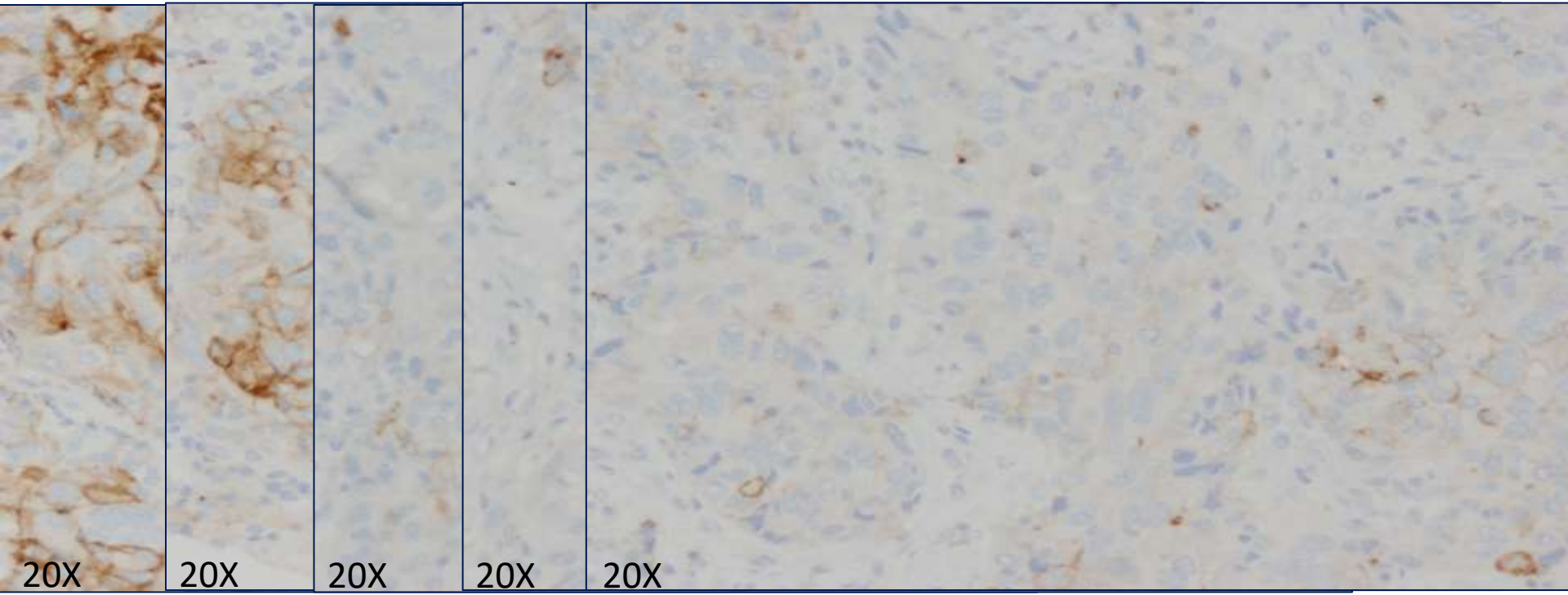
PD-L1 22C3: 95% tumor cells

Case #1



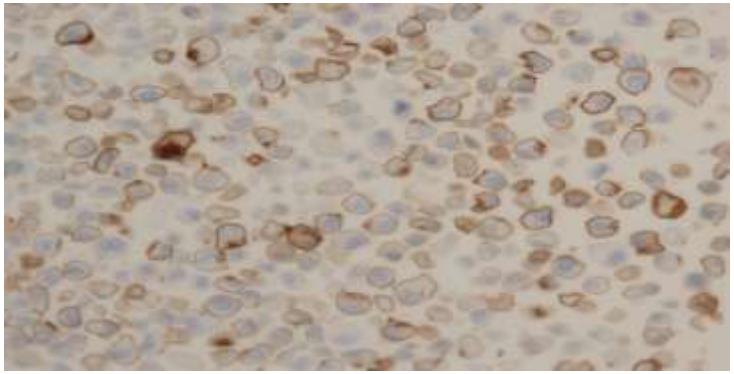
PD-L1 22C3: 95% tumor cells

Case #2

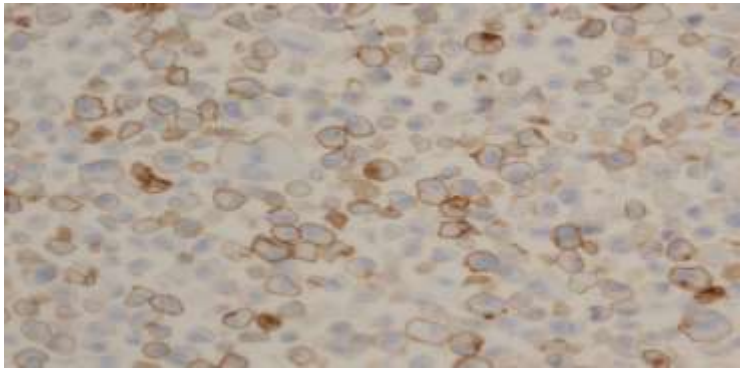


PD-L1 20% tumor cells

Control positivo células



Control positivo 1



Control positivo 2

Control positivo 3

Control positivo (Amígdala teñida con PD-L1)

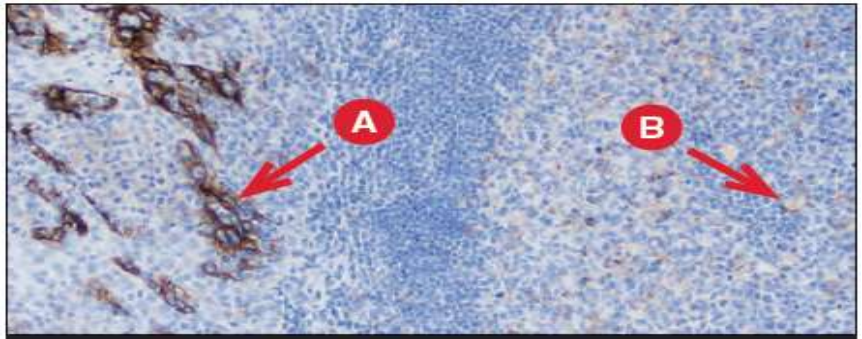
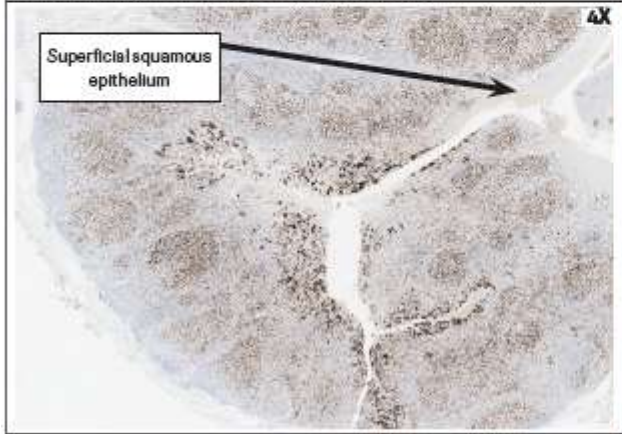


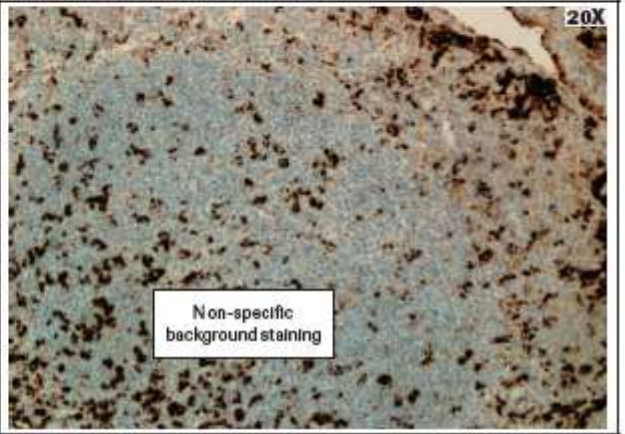
Figure 11: Tonsil stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak to moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

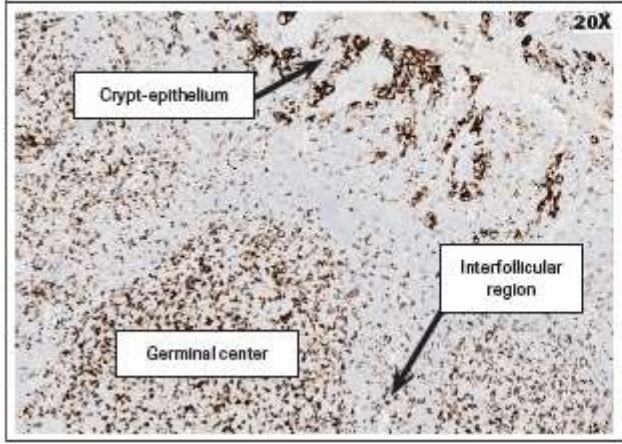
1. Dako, An Agilent Technologies Company. *PD-L1 Interpretation manual. http://www.dako.com/us/29109_pd-l1-ihc-22c3-interpretation-manual.pdf*. Accessed 28 September 2016.

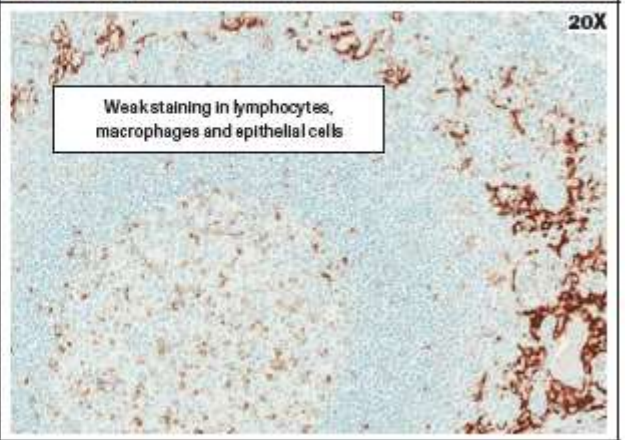
Table 2: Tonsil Qualification and Acceptability Criteria

Acceptable	Unacceptable
Positive tissue elements: Moderate to strong PD-L1 staining noted in lymphocytes and macrophages in germinal centers, with diffuse staining in reticulated crypt epithelial cells.	Excessive non-specific background staining obscuring the identification of PD-L1 positive cells.
Negative tissue elements: PD-L1 negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Weak to no PD-L1 staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.

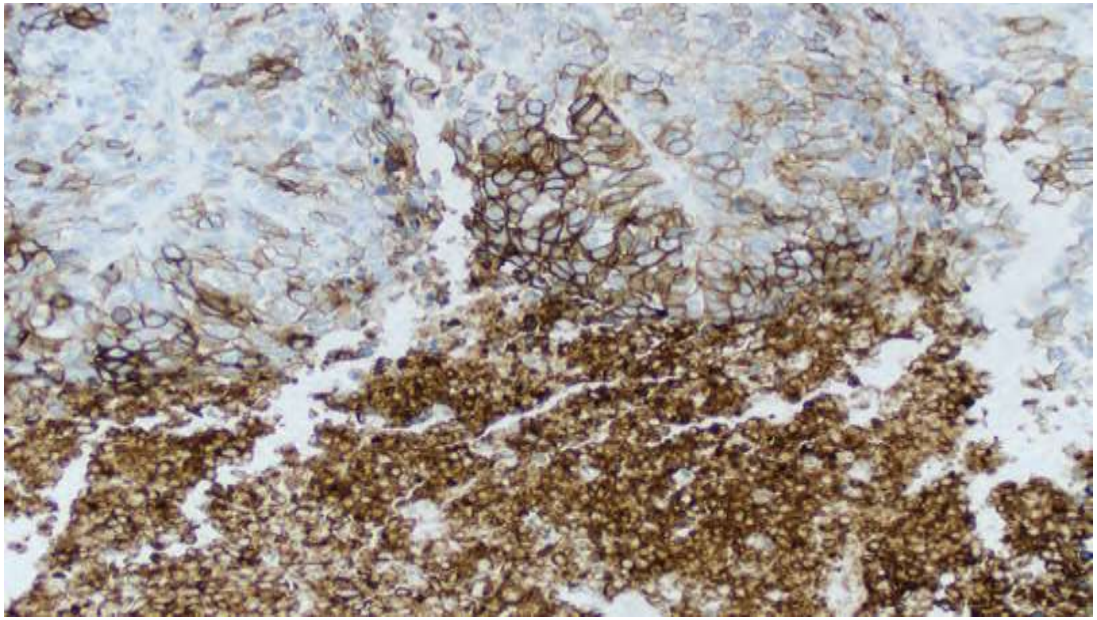








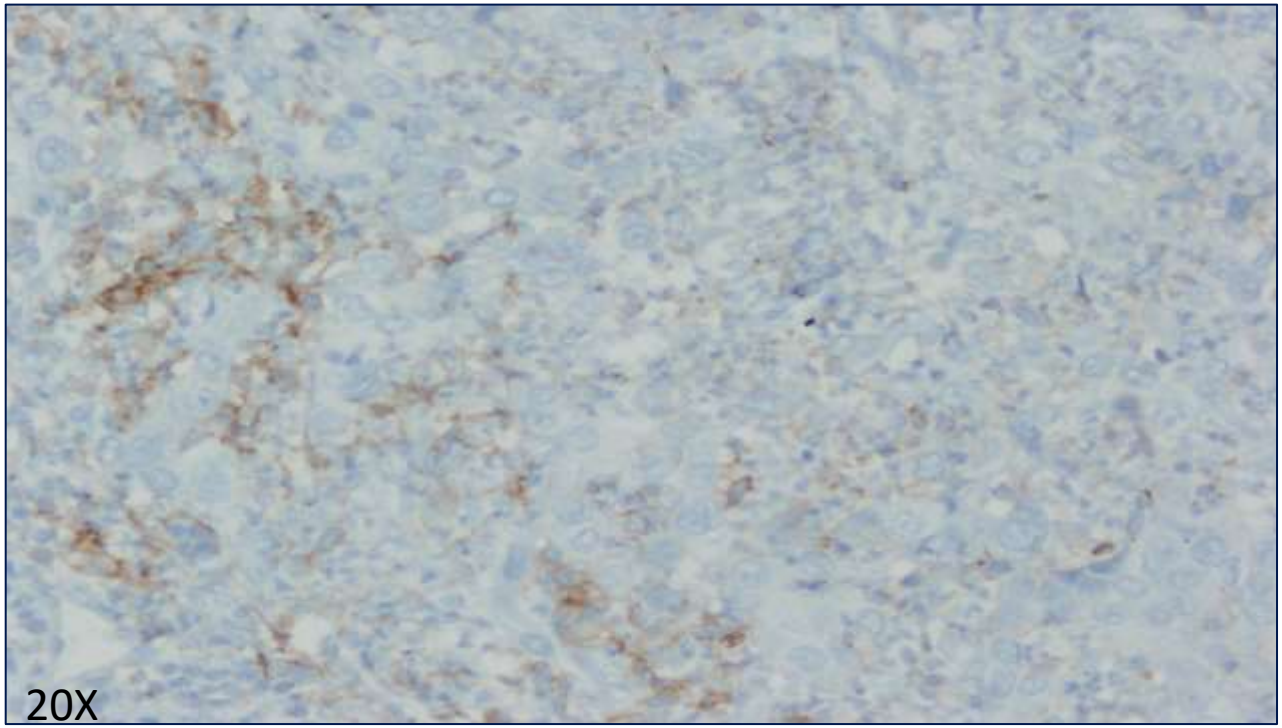
Consideraciones generales-Tejido necrótico



NSCLC stained with PD-L1 primary antibody exhibiting strong staining of necrosis and viable tumor cells; necrosis staining should be excluded from the scoring (20× magnification).

1. Dako, An Agilent Technologies Company. *PD-L1 Interpretation manual. http://www.dako.com/us/29109_pd-l1-ihc-22c3-interpretation-manual.pdf*. Accessed 28 September 2016.

Tinción de células inmunes intratumorales



PD-L1 SP142 0% TC ; 20% IC (TC0/IC3)

Informe de tinción de PD-L1

	Nivolumab: BMS		Pembrolizumab: Merck	Atezolizumab: Roche	Durvalumab: AstraZeneca	Avelumab: Pfizer
Ab Clone	28-8	SP263	22C3	SP142	SP263	73-10
Diagnostic Partner	Dako	Ventana	Dako	Ventana	Ventana	Dako
Scoring Method	% of PD-L1–expressing **tumour cells**		% of PD-L1– expressing **tumour cells**	% of PD-L1– expressing **tumour cells or immune cells**	% of PD-L1–expressing **tumour cells**	% of PD-L1–expressing **tumour cells**
PD-L1 Thresholds	TC ≥1% (pos), ≥5% (strong), or ≥10%		TC ≥1% (pos) TC ≥50% (strong)	TC / **IC 3(+)** TC / **IC 2(+)** TC / **IC 1(+)** TC / **IC 0(–)**	TC PD-L1(+): ≥ 25%	TBC, TC between all >1% and 25% with moderate or high intensity

¿Cómo informar la Positividad?

Puntos de corte diferentes para anticuerpos, tumores y fármacos….

Mi opinión:

- No informar como de Positividad/Negatividad
- Informar sobre el anticuerpo y técnica empleado
- Expresar el porcentaje de células neoplásicas teñidas

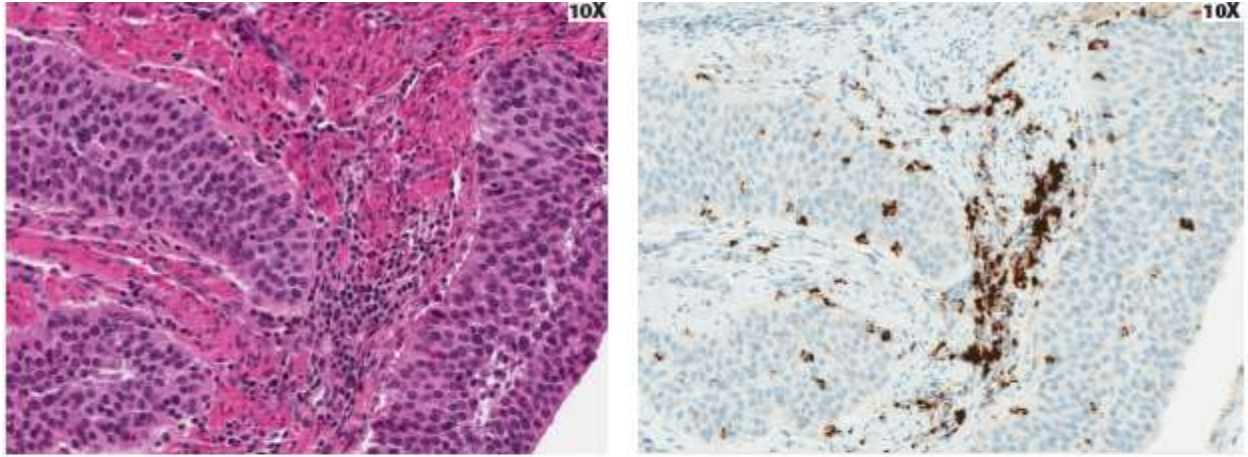


Figure 2: Urothelial carcinoma tissue showing dark brown punctate and linear IC staining

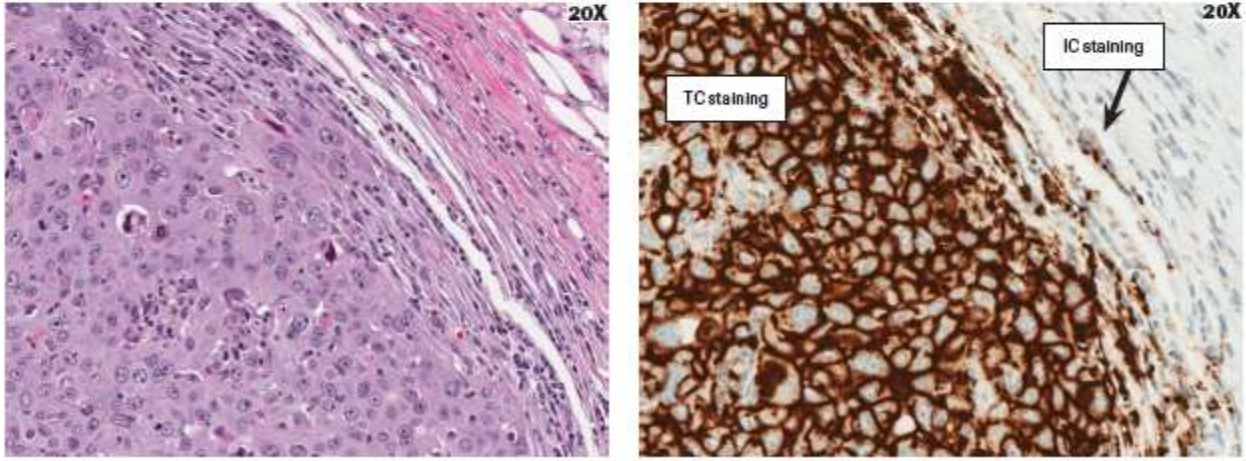
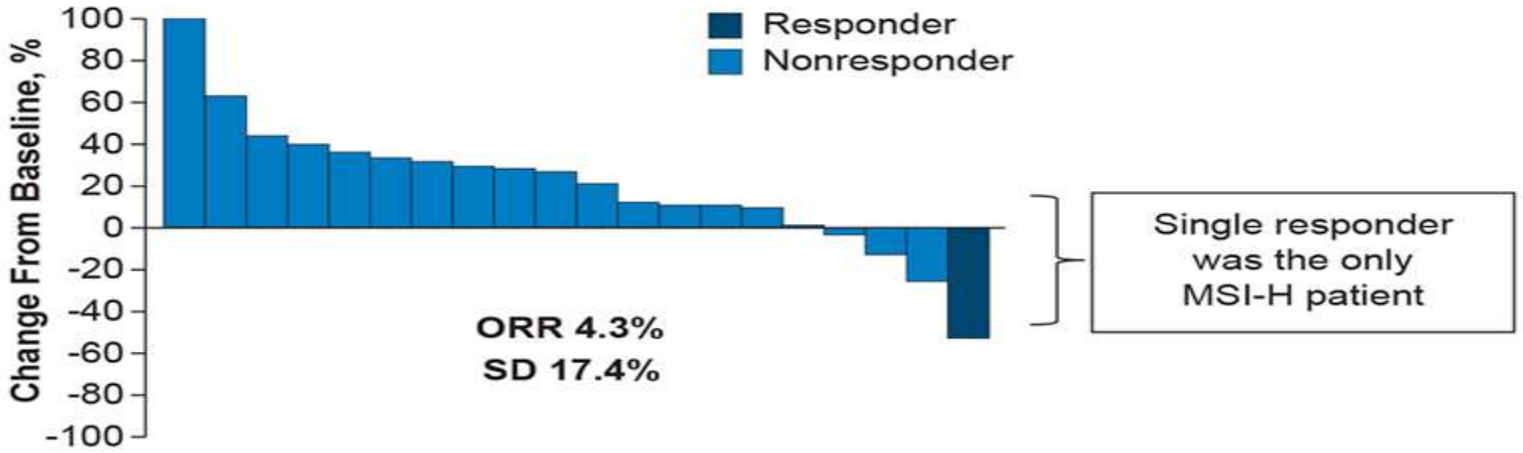
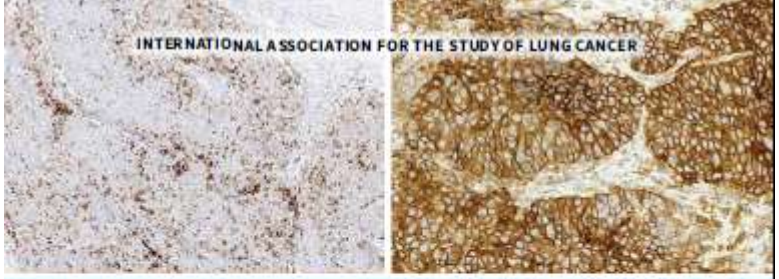


Figure 3: Urothelial carcinoma tissue showing strong circumferential TC membrane staining, as well as IC staining.

KEYNOTE-028 Study: PD-L1 Expression Is Not Predictive of PD-1 Inhibitor Activity in CRC[1]

- Pembrolizumab 10 mg/kg IV every 2 weeks
- PD-L1+: defined as membranous PD-L1 expression in ≥1% of cells in tumor and stroma
- 33/137 (24.1%) PD-L1+ with 23 enrolled





IASLC ATLAS OF PD-L1 IMMUNOHISTOCHEMISTRY TESTING IN LUNG CANCER

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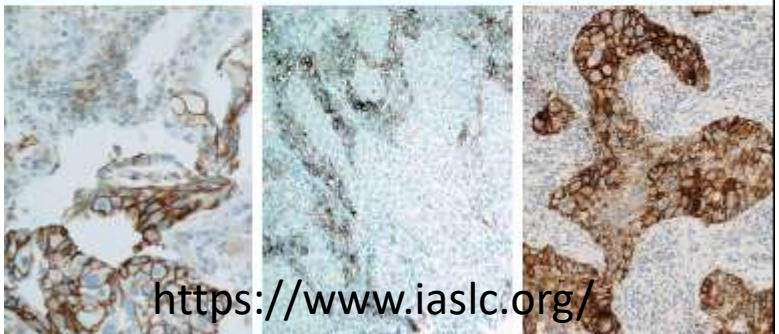
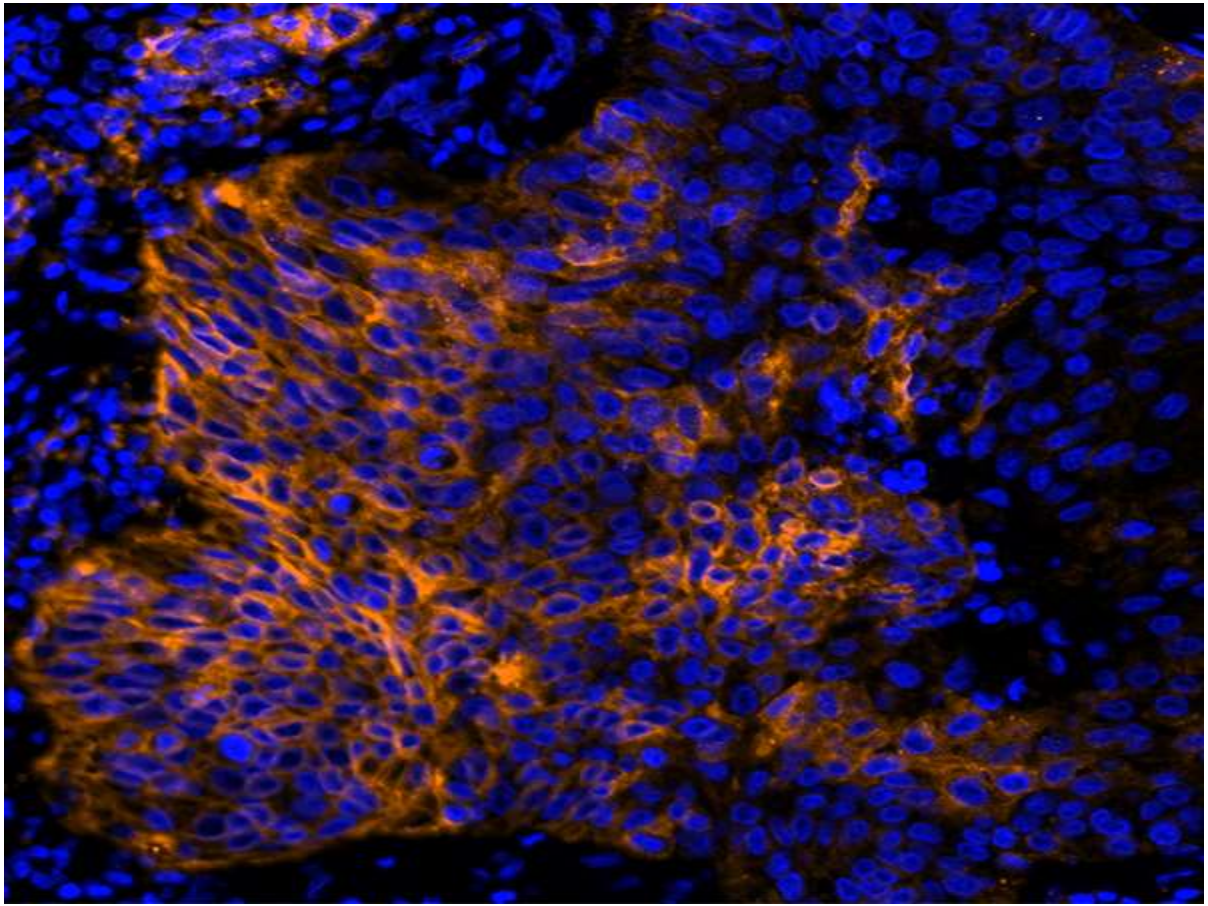
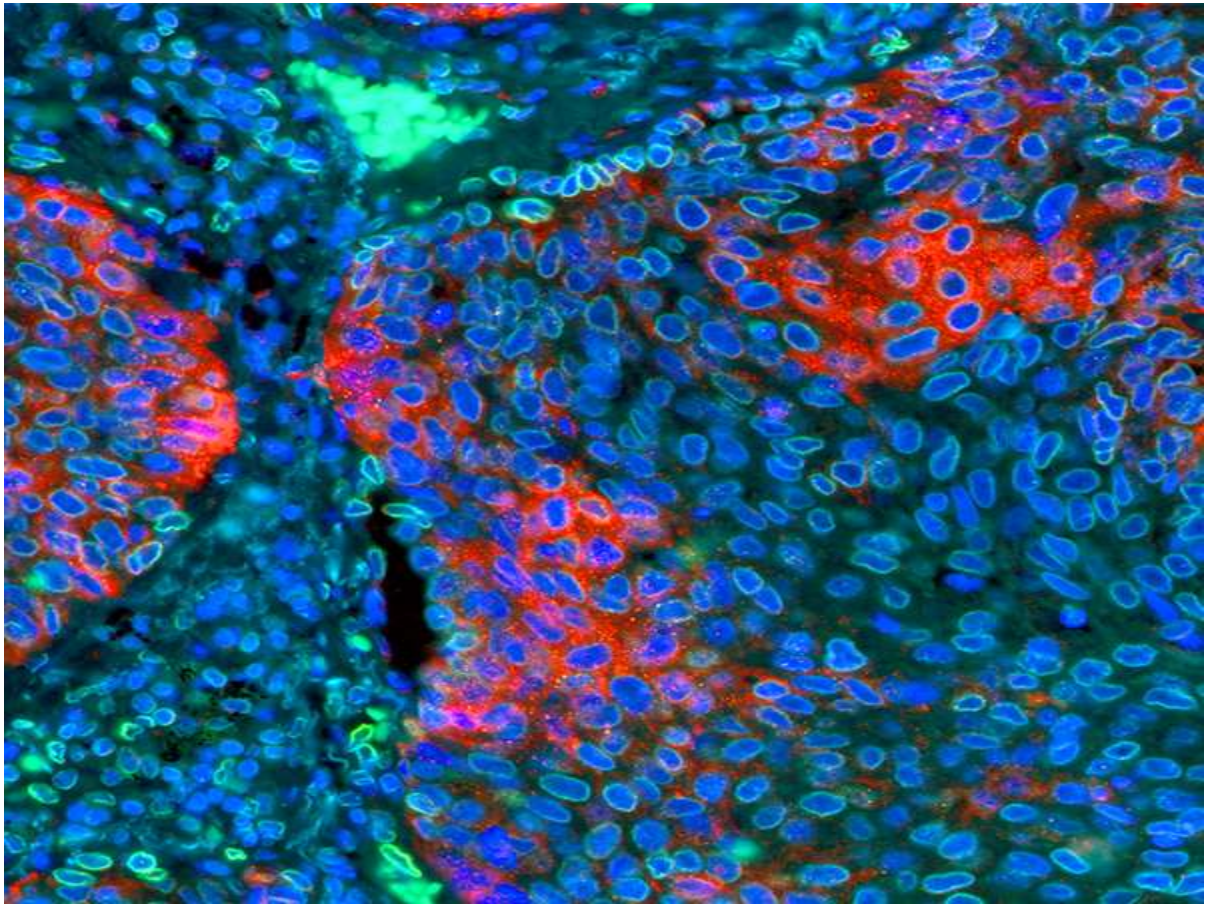


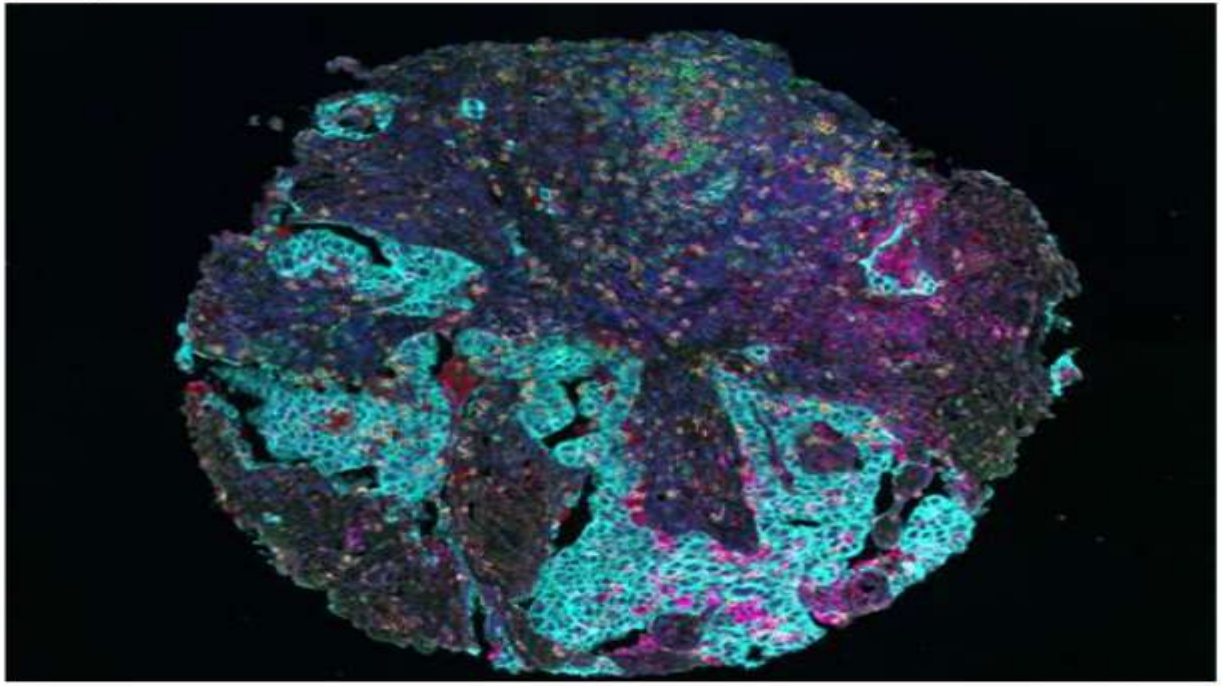
https://www.iaslc.org/





Examples of Opal Multiplexing

Lung Cancer – CD4, CD8, CD68, FoxP3, PD-L1, CK, DAPI

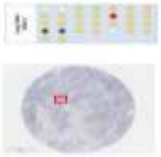


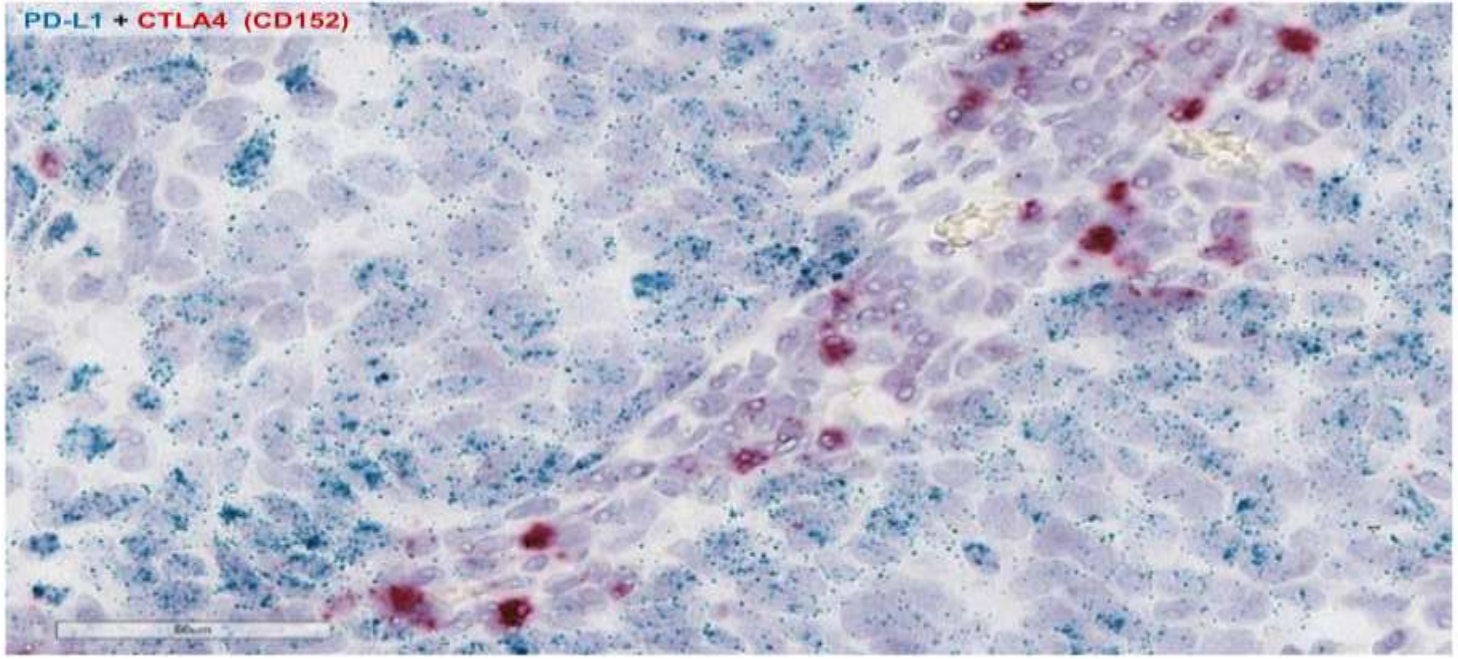
Opal Multiplexing, and imaged with Vectra

RNAscope®

Co-expression Profiles of Immune Checkpoint Markers in Selected Tumors

Core 2E2 (high PD-L1)

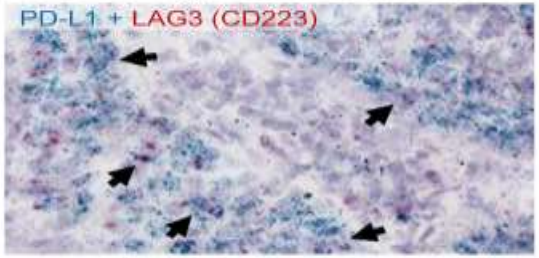




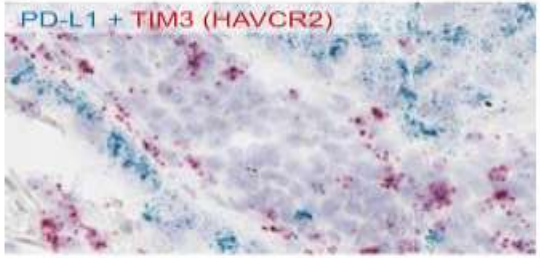
Co-expression Profiles of Immune Checkpoint Markers in Selected Tumors

Not all PD-L1 high tumors are the same!

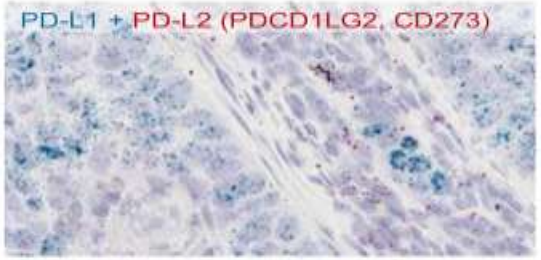
Core 1B1



LAG3 in tumor and stromal regions

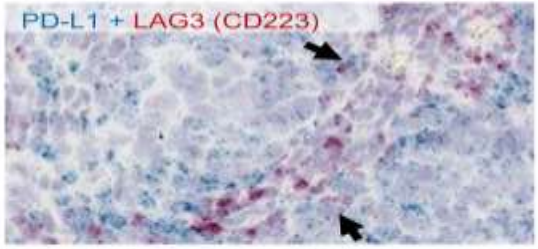


TIM3 and PD-L1 in different cells;
TIM3 primarily in stroma

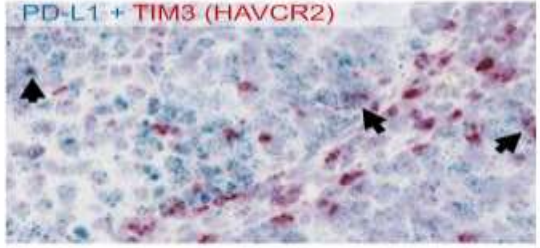


PD-L2 has little/no expression in tumor

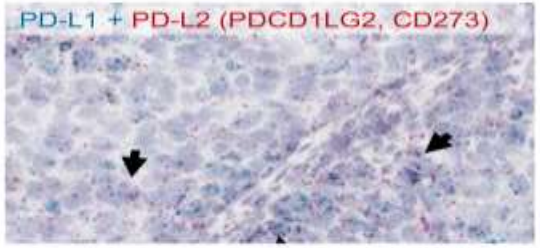
Core 2E2



LAG3 primarily in stromal regions



Co-expression of TIM3 and PD-L1 in the same cells

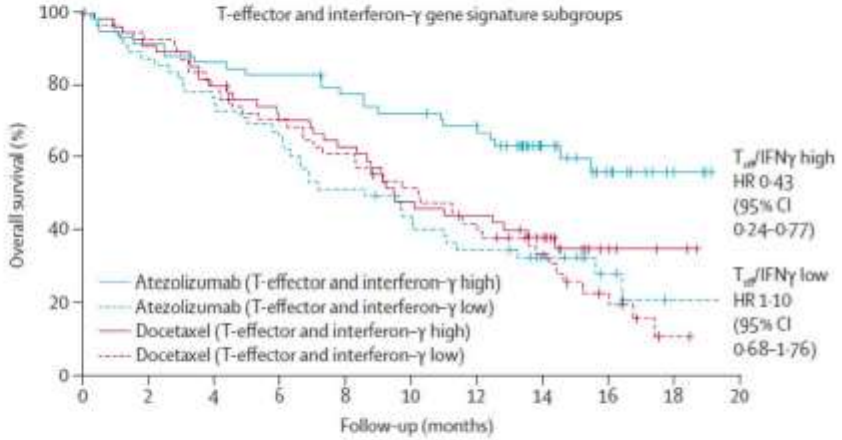


PD-L2 and PD-L1 in the same cells (high freq.) in tumor and stroma

Heterogeneous co-expression profiles of different checkpoint markers in different tumors!

Alternative Potential Biomarkers for Response?

- **Immune gene signatures**
- Immune cells
  - Overall infiltrate
  - Specific cell types
- Other Immune checkpoints
  - PD-L2, IDO, etc
- Mutational Burden



Fehrenbacher L et al. Lancet 2016; 387, 1837

Alternative Potential Biomarkers for Response?

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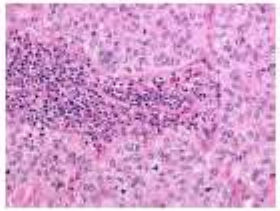
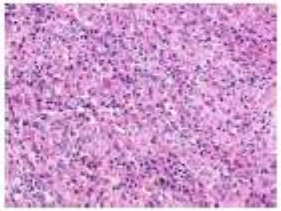
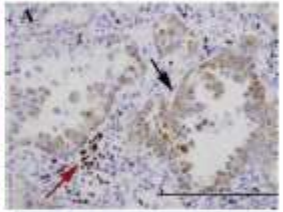
- Mutational Burden

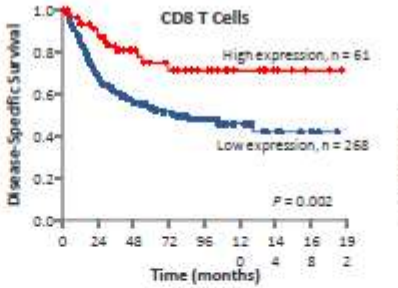




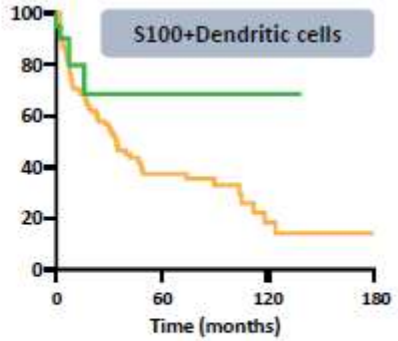


Where is the infiltrate?

FoxP3+ Tregs



Or CD4+ or CD56+ lymphoid cells



Kerr, et al Histopathol 1998; Johnson, et al. Lung Cancer 2000;
Suzuki, et al. Clin Can Res 2011; Bremnes, et al. J Thorac Oncol 2011; Tao et al. Lung cancer 2012, Al-Shibli KI, et al. *Clin Cancer Res* 2008;14:5220–7; Al-Shibli KI, et al. *Histopathol* 2009;55:301–12; Shimizu K, et al. *J Thorac Oncol* 2010;5:585–90.

Alternative Potential Biomarkers for Response?

- **Immune gene signatures**
- **Immune cells**
  - Overall infiltrate
  - Specific cell types
- **Other Immune regulators**
  - **PD-L2, IDO, LAG3,**
  - **Interferon gamma**
- **Mutational Burden**

Anti-PD-L1 therapy – Durvalumab

PD-L1 protein AND
interferon gamma mRNA expression

Higher RR (46%) in combined expression versus

Interferon gamma (33%) or PL-L1 (27%) alone

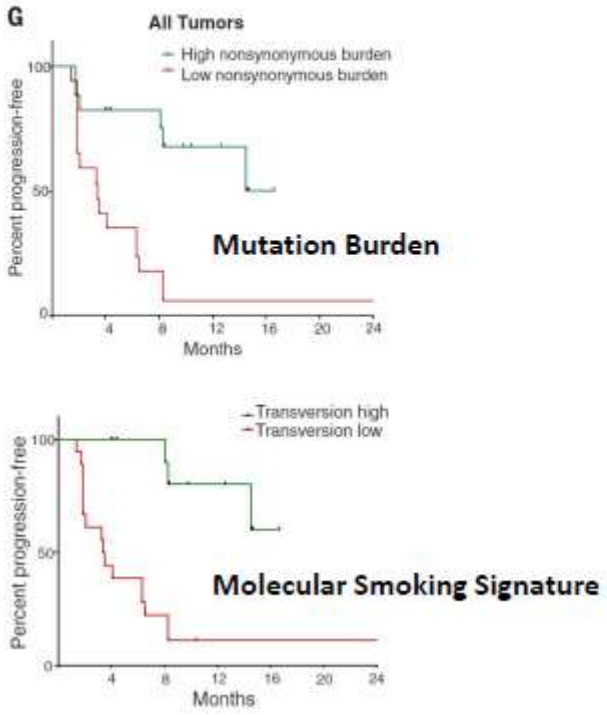
Higgs et al, 15LBA, ECCO 2015

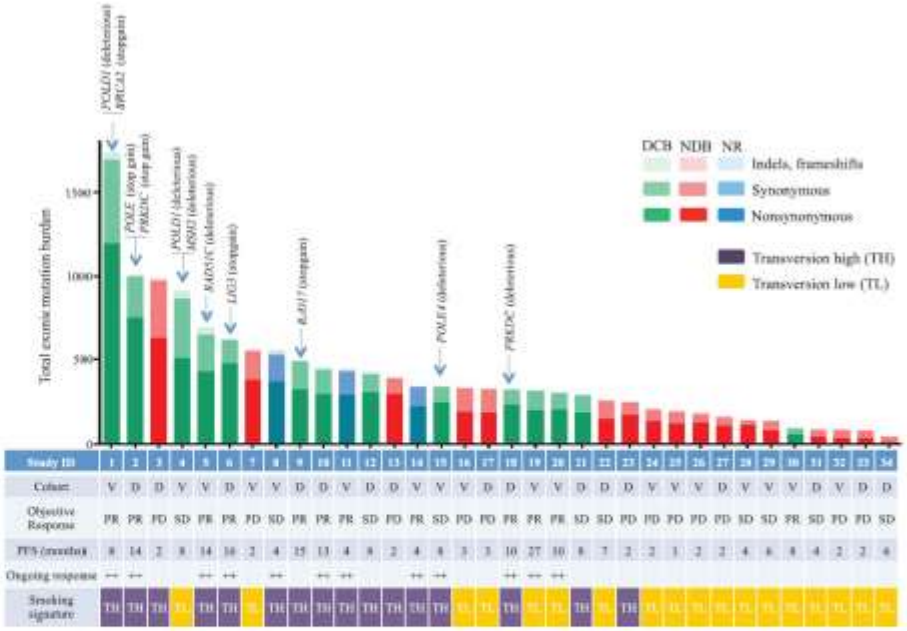
Anti-PD-1 therapy – Pembrolizumab

PD-L1 & PD-L2 IHC better than either alone

Crowley et al. LBA, ECCO 2015

Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer

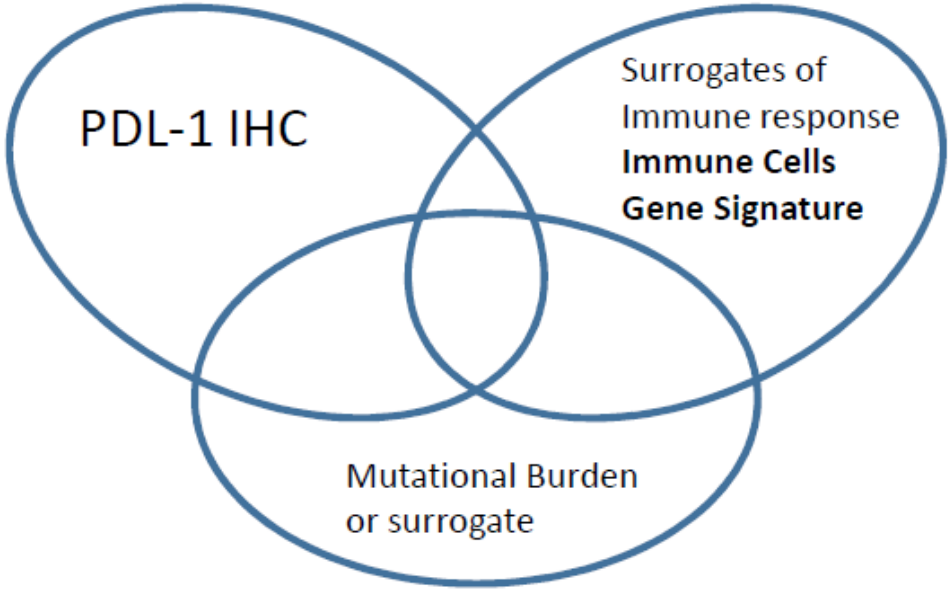




Polymerase E (POLE) mutations
Mismatch repair genes (MMR)
Microsatellite Instability (MSI)

Rizvi NA, et al. *Science*. 2015;348:124–128.

Biomarkers for Immunotherapy?



Inmunoterapia y Patólogos

La inmunoterapia es una nueva dimensión en el tratamiento del cáncer.

PD-L1 es un biomarcador real:

- En tejidos
- En un ambiente complejo, con múltiples fármacos y tests

Los patólogos debemos proporcionar esta información