Carcinoma del polmone non microcitoma: quali novità per il 2016 ?

Immunoterapia: biomarcatori – Quale impatto sulla pratica clinica ?

Giulio Rossi, Anatomia Patologica, Azienda Policlinico di Modena / giurossi68@gmail.com

Hotel Leon D'Oro, Verona 8-9 aprile 2016

PD-1/PD-L1 pathway

-The normal funtion of PD-L1 (CD274, a 40kDa type I transmembrane protein) is to regulate the balance bewteen T-cell activation and tolerance through interaction with its 2 receptors (PD-1, CD279) and CD80

-In cancer, PD-L1 is expressed by tumor cells and binding to PD-1 on activated T cells helps tumors to evade detection and elimination by the host immune system

Programmed Death-Ligand 1 Immunohistochemistry in Lung Cancer

In what state is this art?

Keith M. Kerr, MBChB, FRCPath, Ming-Sound Tsao, MD, PhD,† Andrew G. Nicholson, DM, FRCPath,‡ Yasushi Yatabe, MD, PhD,§ Ignacio I. Wistuba, MD, PhD,‖ and Fred R. Hirsch, MD, PhD,¶ On behalf of the IASLC Pathology Committee*

Journal of Thoracic Oncology® • Volume 10, Number 7, July 2015

Main problematic issues based on published data

- **The choice of the Ab clone (DAKO 28-8; DAKO 22C3; Ventana SP142; Ventana SP263; CST E1LN3N)**

- **Scoring system**
  - **>1%**
  - **>5%**
  - **>50%**

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On behalf of the IASLC Pathology Committee

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Drug	Biomarker Antibody	Rx Line	Definition of "Positive"[a] (%)	N Positive (%)	Positive Predictive Outcome	ORR % IHC pos. Cases	ORR % IHC neg. Cases
Nivolumab	Dako 28-8	1st	≥5 in >100 cells	59	Yes	31[b]	10
Nivolumab	Dako 28-8	≥2nd	≥5, ≥1	49, 56	No	15, 13	14, 17
Nivolumab + Ipilimumab	Dako 28-8	1st	≥5 in >100 cells	42	No	19	14
Nivolumab	Dako 28-8	≥2nd	≥5	33[c]	Yes	24	14
Nivolumab	5H1[d]	≥2nd	≥5, also studied TIICs	67	Yes	No data for lung	No data for lung
Pembrolizumab	Dako 22C3	Any	"Strong" ≥50, "Weak" 1–49	25, 70	Yes, Yes	37, 17	9
Pembrolizumab	Dako 22C3	1st	≥50, ≥1	?	Yes	47, 26	?
MPDL3280A	Roche Ventana, SP142	≥2nd	≥10,[e] ≥5, ≥1 TIICs	13, 28, 56	Yes	83, 46, 31	18, 18, 20
MEDI-4736	Roche Ventana, SP263	≥2nd	Data not available	41	Yes	25	3

Ventana Medical Systems, Inc. and MedImmune collaborate to develop a custom PD-L1 Assay for immunotherapy clinical trials

VENTANA

Media Release

Spring Bioscience launches highly sensitive PD-L1 (SP142) antibody for immunotherapy research

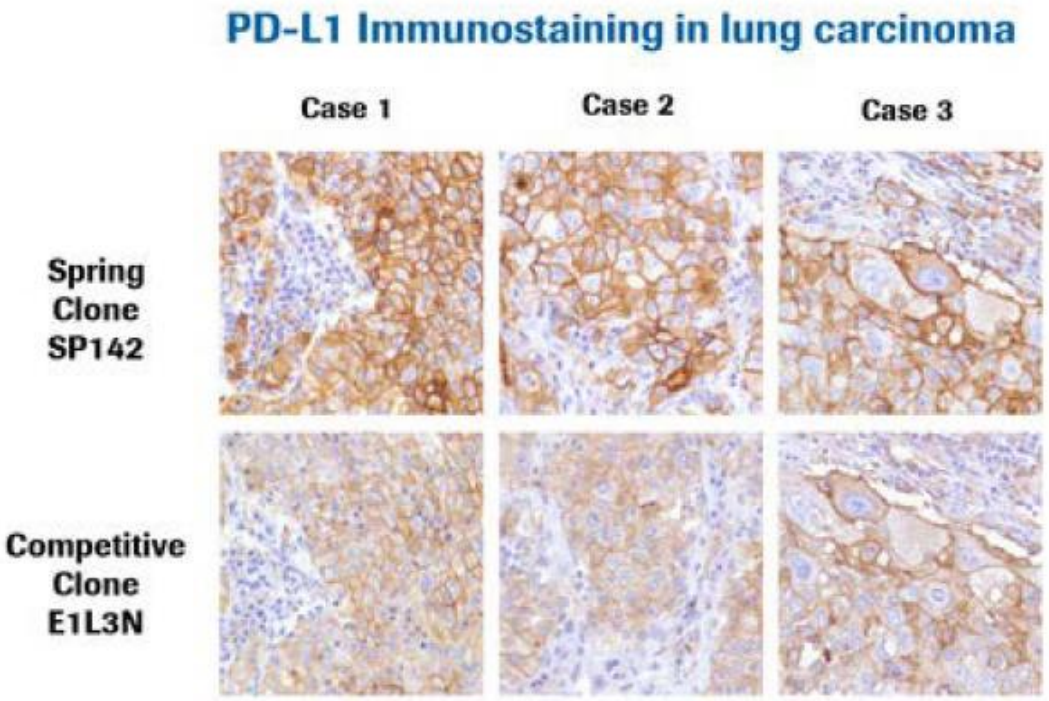
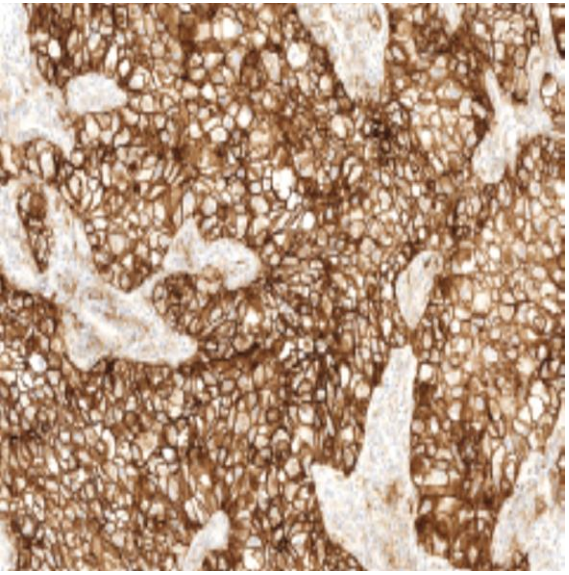


Image 1: Comparative IHC staining of representative lung carcinoma samples. Strong staining is observed in tissues stained with the Spring clone SP142, while weak to moderate staining is observed in tissues stained with the competitive clone E1L3N.

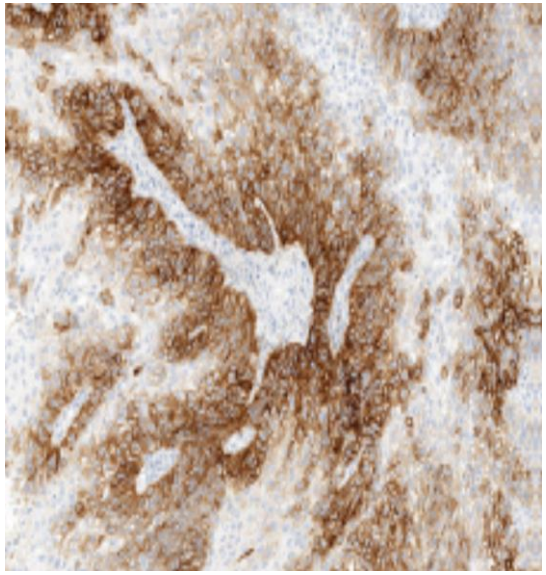
PD-L1 (SP263) IHC assay to enroll patients in clinical trials for MEDI4736 anti-PD-L1

SP142 was developed for Roche/Genentech's anti-PD-L1 (MPDL3280A) immunotherapy

PD-L1 immunoscore

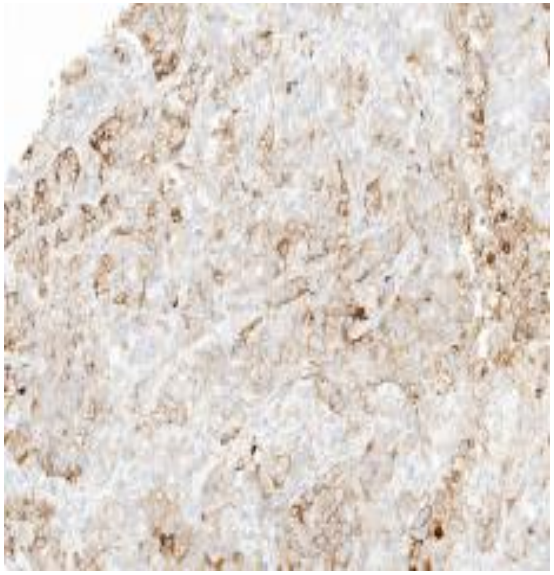


Score 3+



Score 2+in 60% ?

Score 3+in 30% ?

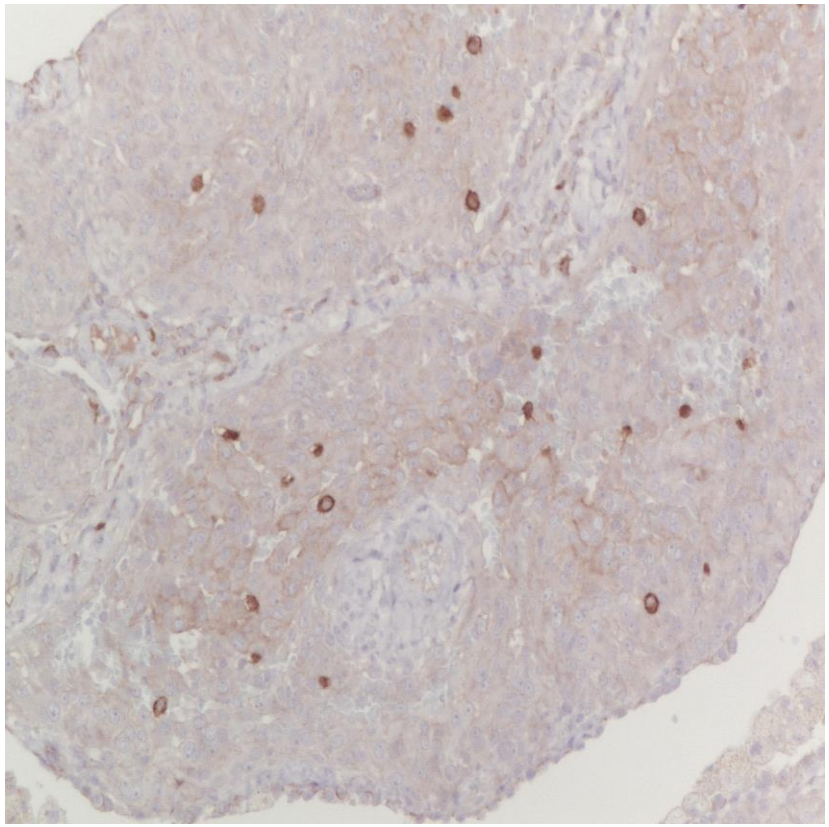


Score 1+ in 80% ?

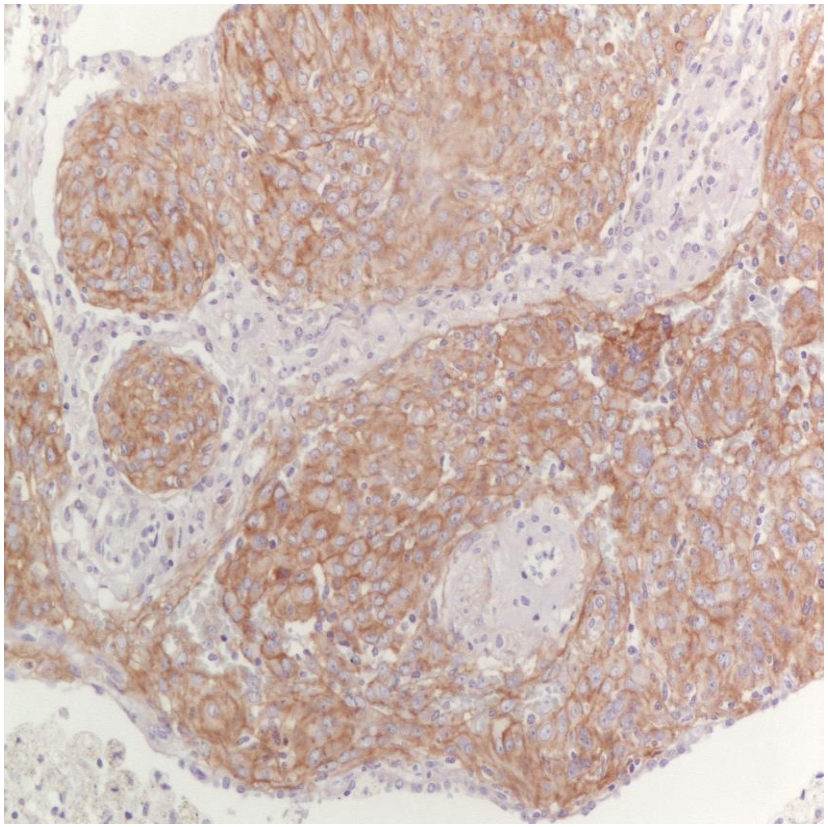
Score 2+ in 30% ?

NSCLC positive with PD-L1 (SP263) IHC with OptiView DAB detection

PD-L1 immunoscore



Clone CST E1LN3N



Clone Ventana SP142

Same case, different score with different clones !

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- **Our understading of the significance of PD-L1 expression is still unclear**

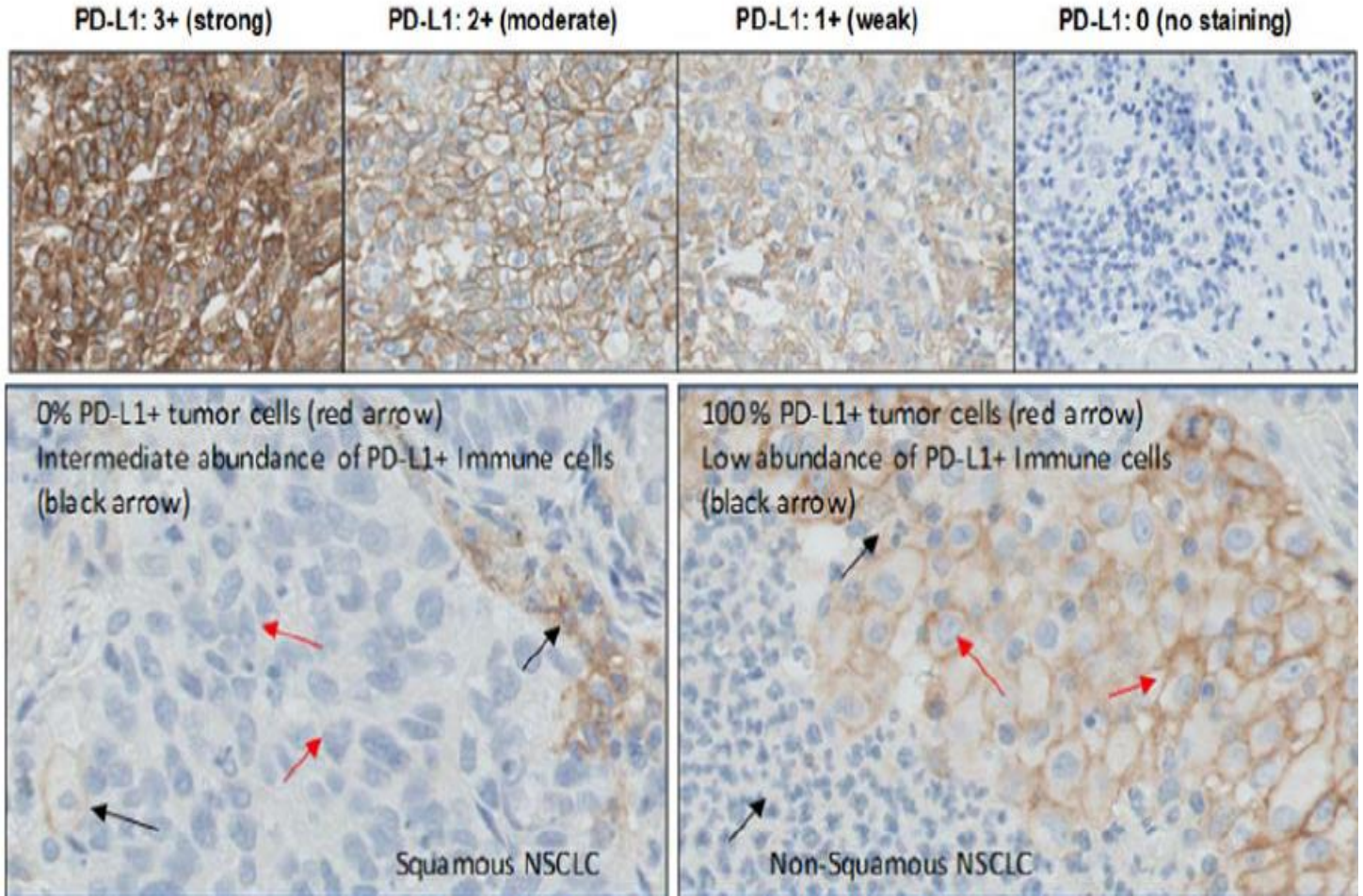
- **Patients without evidence of PD-L1 expression may also respond to PD-1– or PD-L1–targeting agents**

- **Multicenter, International standardization effort could address many questions and develop 1 standardized test (Blueprint project ?)**

Development of an Automated PD-L1 Immunohistochemistry (IHC) Assay for Non–Small Cell Lung Cancer

Therese Phillips, MA, Pauline Simmons, BS,* Hector D. Inzunza, MD, PhD,† John Cogswell, PhD,† James Novotny, Jr, PhD,† Clive Taylor, MD, PhD,‡ and Xiaoling Zhang, PhD**

Appl Immunohistochem Mol Morphol • Volume 23, Number 8, September 2015



DAKO 28-8

Assays for predicting and monitoring responses to lung cancer immunotherapy

Cancer Biol Med 2015;12:87-95.

Cristina Teixidó[1], Niki Karachaliou[2], Maria González-Cao[2], Daniela Morales-Espinosa[2], Rafael Rosell[1,2,3]

Clone N (mAb)/Catalog N (pAb)	Provider	Host organism
MIH1	eBioscience, San Diego, CA	Mouse
5-496	O. Majdic, University of Vienna Medical School, Vienna	Mouse
2-272	O. Majdic, University of Vienna Medical School, Vienna	Mouse
27A2	MBL International, Wobum, MA	Mouse
16E11	Medarex, Princeton, NJ	Mouse
9A6	Medarex, Princeton, NJ	Mouse
16A4	Medarex, Princeton, NJ	Mouse
6H3	Medarex, Princeton, NJ	Mouse
ETM-79	Medarex, Princeton, NJ	Rabbit
ETM-80	Medarex, Princeton, NJ	Rabbit
1105	Medarex, Princeton, NJ	Human
25C8E8.F8	Medarex, Princeton, NJ	Human
24B10.G6.D7	Medarex, Princeton, NJ	Human
5H1	L. Chen, Johns Hopkins University, Baltimore, MD	Mouse
4059	ProSci/Sigma, Poway, CA	Rabbit
AF-156	R&D Systems, Minneapolis, MN;	Goat
22	US biological, Salem, MA	Rabbit
22E	US biological, Salem, MA	Mouse
20C3	Merck, Whitehouse Station, NJ	Mouse
22C3	Merck, Whitehouse Station, NJ	Mouse
SP142	Roche, Basel	Rabbit
SP263	Roche, Basel	Rabbit
58810	Abcam, Cambridge, UK	Rabbit
28-8	Bristol-Myers Squibb, New York, NY	Rabbit

More evidence is required to support the use of PD-L1 as a potential predictive biomarker

- Different antibodies
- Pre-analytical phase
- Scoring system
- Lack of standardization & reproducibility

PD-L1 in NSCLC: much more shadows than lights

- **All lung cancer histotype do express PDL1 expression**
- Is the cutoff value of ≥ 1% the appropriate choice?
- IHC methodology (different antibodies; no validated cutoff value; the cutoff value may be differ by histology, PD-L1 is a dynamic marker).
- **Are there better predictors of responsiveness?**

• immune infiltrate (CD8+ cells; TILs; PD-L1+; MDSC)
• mutational burden
• cytokine gene signature
• microsatellite instability & MMR system alterations

Programmed Death Ligand 1 Immunohistochemistry—A New Challenge for Pathologists

A Perspective From Members of the Pulmonary Pathology Society

Lynette M. Sholl, MD; Dara L. Aisner, MD; Timothy Craig Allen, MD, JD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Philip T. Cagle, MD; Vera Capelozzi, MD, PhD; Sanja Dacic, MD, PhD; Lida Hariri, MD, PhD; Keith M. Kerr, BSc, MB, ChB, FRCPath, FRCPE; Sylvie Lantuejoul, MD, PhD; Mari Mino-Kenudson, MD; Kirtee Raparia, MD; Natasha Rekhtman, MD, PhD; Sinchita Roy-Chowdhuri, MD, PhD; Eric Thunnissen, MD, PhD; Ming Sound Tsao, MD; Yasushi Yatabe, MD, PhD; for the members of the Pulmonary Pathology Society

- Antibody clones targeting the intracellular domain (E1L3N and SP142) versus the extracellular domain (SP263, 22C3, and 28-8) of 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

Arch Pathol Lab Med 2016

Programmed Death Ligand 1 Immunohistochemistry

Friend or Foe?

Keith M. Kerr, BSc, MB, ChB, FRCPath, FRCPE; Fred R. Hirsch, MD, PhD

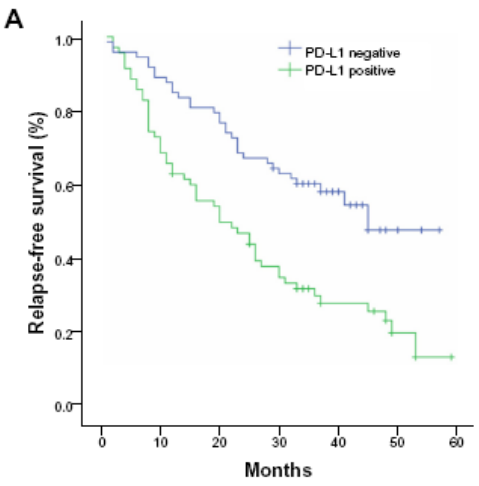
	ORR %		Median PFS, mo		Median OS, mo	
Definition of PD-L1 Positivity[a]	Positive	Negative	Positive	Negative	Positive	Negative
≥5% in ≥100 TCs	24	14	NA	NA	NA	NA
≥1% in ≥100 TCs	17	17	3.3	3.1	9.3	8.7
≥5% in ≥100 TCs	21	15	4.8	2.2	10.0	8.5
≥10% in ≥100 TCs	19	16	3.7	2.3	11.0	8.2
≥1% in ≥100 TCs	31	9	4.2	2.1	17.7	10.5
≥5% in ≥100 TCs	36	10	5.0	2.1	19.4	9.8
≥10% in ≥100 TCs	37	11	5.0	2.1	19.9	9.9
≥50% (strong)	45	11	6.4	4.0	NR	10.4
1%–49% (weak)	17		4.1		10.6	
TCs						
TC staining: ≥50%; ≥5%; ≥1%	TC data: 38; 22; 18	TC or IC: 8	7.8; 4.0; 3.3	1.9	NR; 13.0; NR	9.7
IC staining: ≥10%; ≥5%; ≥1%[d]	IC data: 13; 15; 18					
≥25% TCs staining	27	5	NA	NA	NA	NA
≥1% TCs staining	16	10	2.8	1.4	8.9	4.6

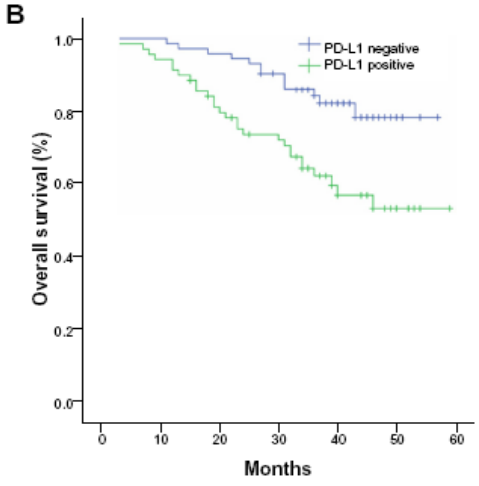
[a] All assays score PD-L1 staining on TCs. Intensity is not a factor in scoring; only the percentage of TCs showing any staining is a factor.

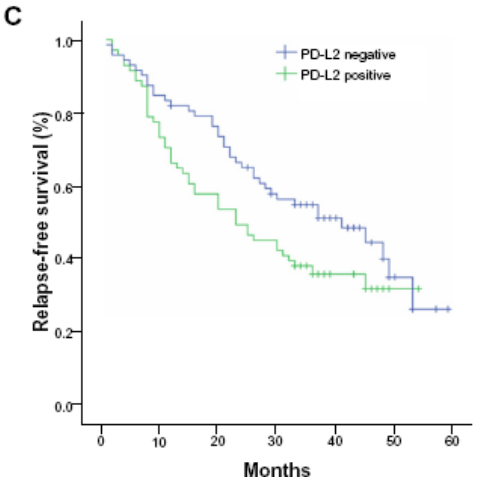
Arch Pathol Lab Med 2016

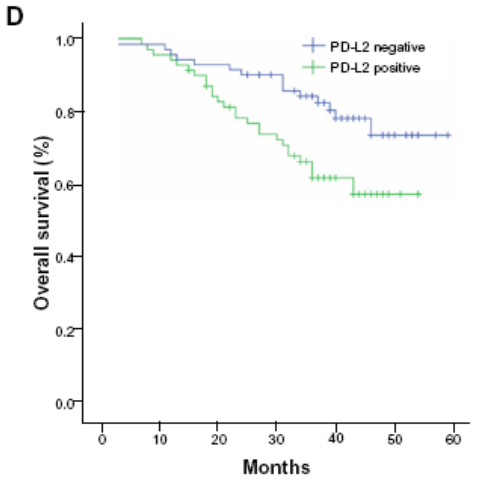
Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma

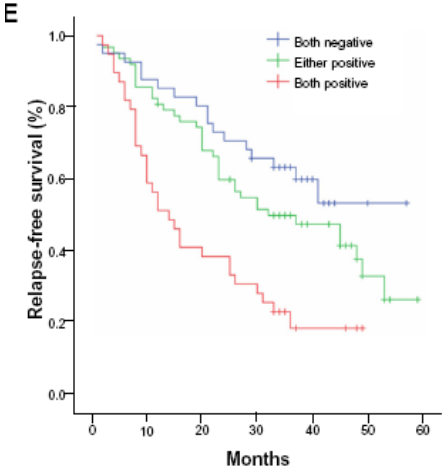
Yang Zhang[1,2,*]
Lei Wang[1,2,*]
Yuan Li[2,3]
Yunjian Pan[1,2]
Rui Wang[1,2]
Haichuan Hu[1,2]
Hang Li[1,2]
Xiaoyang Luo[1,2]
Ting Ye[1,2]
Yihua Sun[1,2]
Haiquan Chen[1,2]

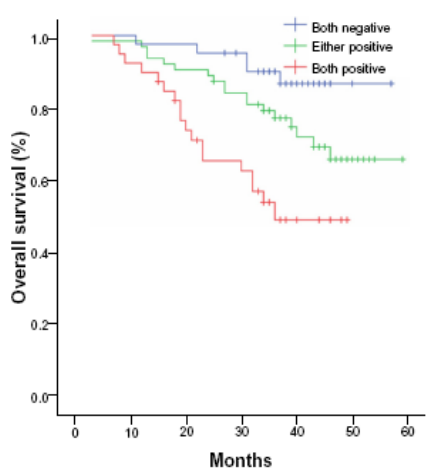












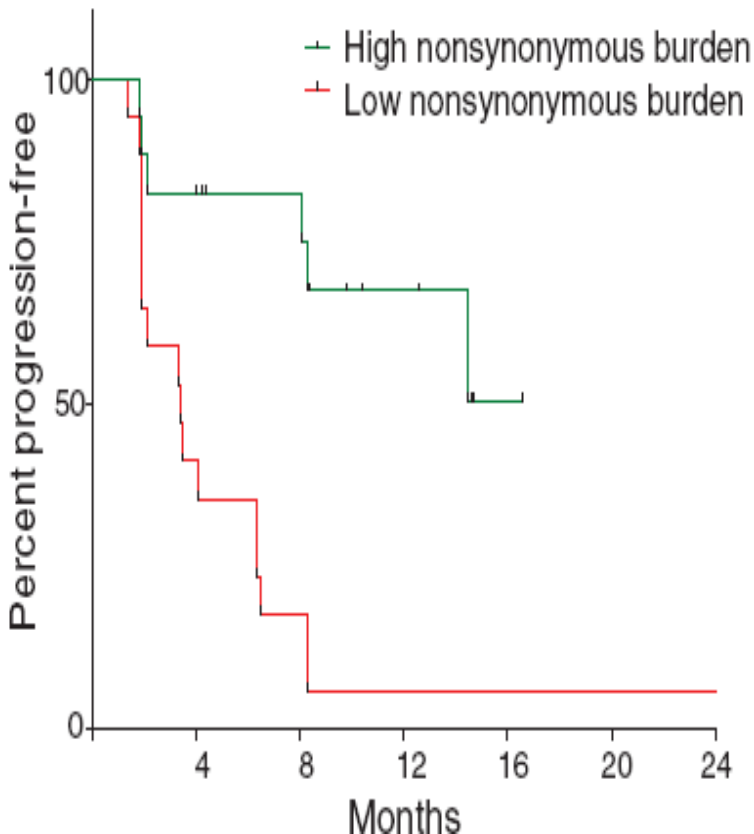
OncoTargets and Therapy 2014:7 567–573

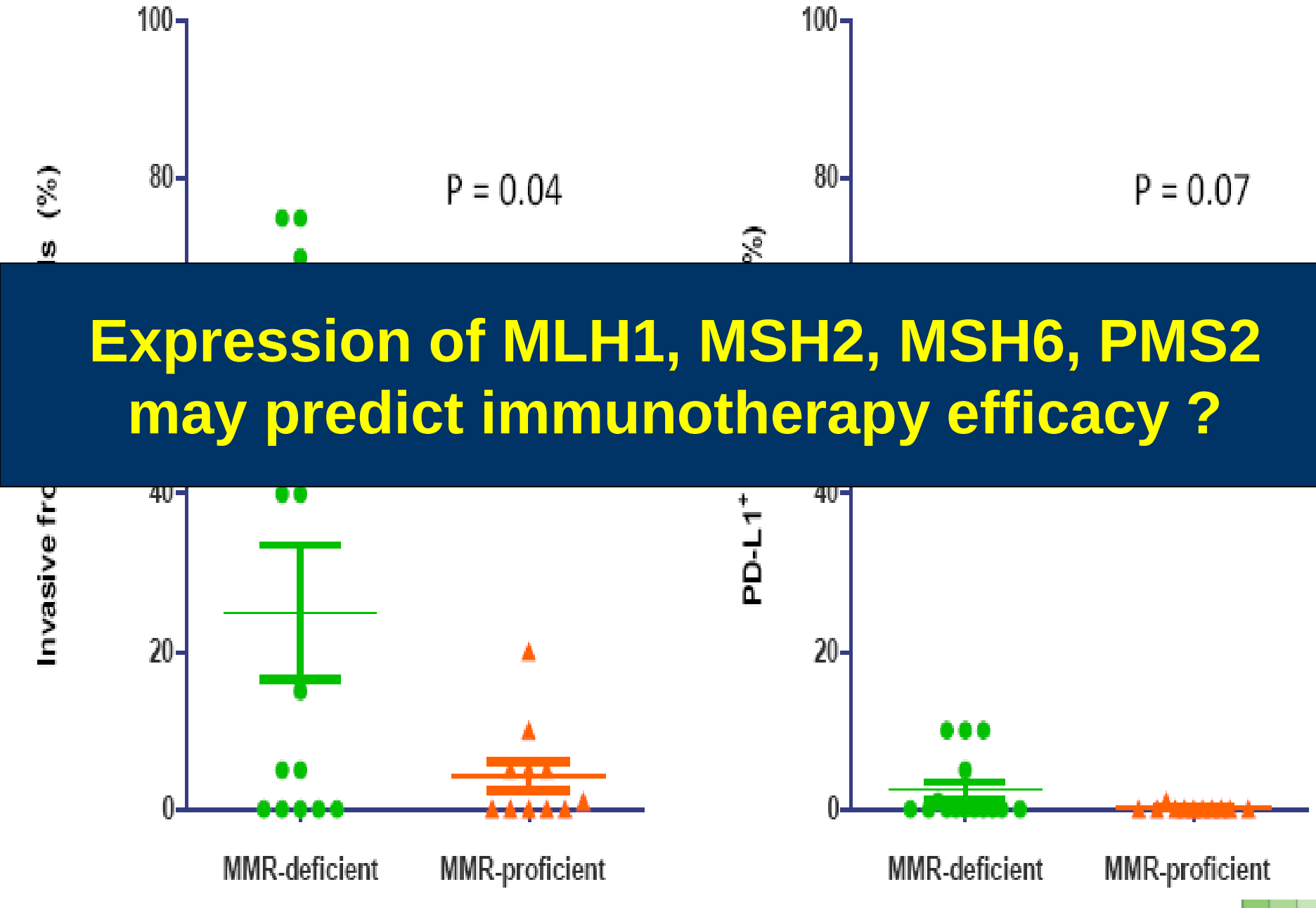
SCIENCE 2015;348:124-8

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

Naiyer A. Rizvi,[1,2*†] Matthew D. Hellmann,[1,2*] Alexandra Snyder,[1,2,3*] Pia Kvistborg,[4] Vladimir Makarov,[3] Jonathan J. Havel,[3] William Lee,[5] Jianda Yuan,[6] Phillip Wong,[6] Teresa S. Ho,[6] Martin L. Miller,[7] Natasha Rekhtman,[8] Andre L. Moreira,[8] Fawzia Ibrahim,[1] Cameron Bruggeman,[9] Billel Gasmi,[10] Roberta Zappasodi,[10] Yuka Maeda,[10] Chris Sander,[7] Edward B. Garon,[11] Taha Merghoub,[1,10] Jedd D. Wolchok,[1,2,10] Ton N. Schumacher,[4] Timothy A. Chan[2,3,5‡]

- Higher mutation burden, smoking signature in tumors, higher neoantigen burden, DNA repair pathway mutations were associated with improved objective response, durable clinical benefit, and progression-free survival





Expression of MLH1, MSH2, MSH6, PMS2 may predict immunotherapy efficacy ?

Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer

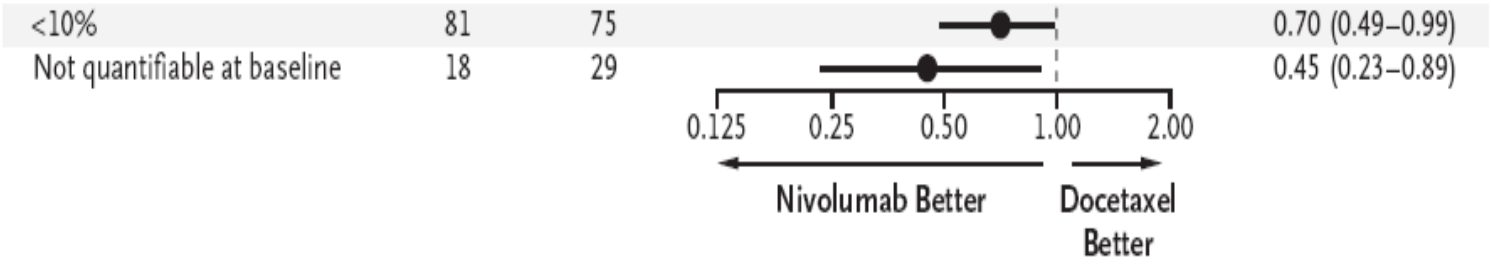
Julie Brahmer, M.D., Karen L. Reckamp, M.D., Paul Baas, M.D., Lucio Crinò, M.D., Wilfried E.E. Eberhardt, M.D., Elena Poddubskaya, M.D., Scott Antonia, M.D., Ph.D., Adam Pluzanski, M.D., Ph.D., Everett E. Vokes, M.D., Esther Holgado, M.D., Ph.D., David Waterhouse, M.D., Neal Ready, M.D., Justin Gainor, M.D., Osvaldo Arén Frontera, M.D., Libor Havel, M.D., Martin Steins, M.D., Marina C. Garassino, M.D., Joachim G. Aerts, M.D., Manuel Domine, M.D., Luis Paz-Ares, M.D., Martin Reck, M.D., Christine Baudelet, Ph.D., Christopher T. Harbison, Ph.D., Brian Lestini, M.D., Ph.D., and David R. Spigel, M.D.

N ENGL J MED 373;2 NEJM.ORG JULY 9, 2015

DAKO 28-8

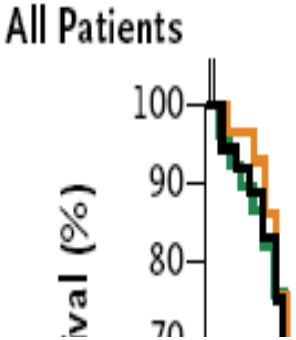
The **efficacy** of nivolumab, including a survival benefit, was observed **regardless of tumor PD-L1 expression levels**, with results showing that PD-L1 expression was neither prognostic nor predictive of efficacy in the population of patients with squamous-cell NSCLC

The lack of an association between PD-L1 expression and efficacy is probably not related to the performance of the PD-L1 assay but is rather a function of complex interactions between tumors and the immune system



Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

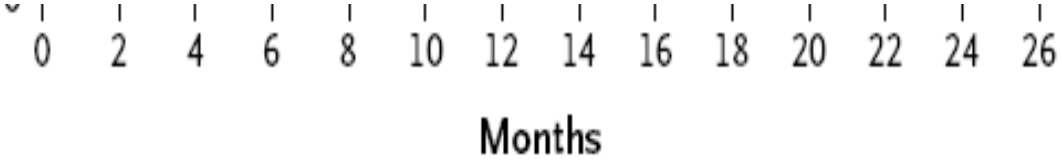
Edward B. Garon, M.D., Naiyer A. Rizvi, M.D., Rina Hui, M.B., B.S., Natasha Leighl, M.D., Ani S. Balmanoukian, M.D., Joseph Paul Eder, M.D., Amita Patnaik, M.D., Charu Aggarwal, M.D., Matthew Gubens, M.D., Leora Horn, M.D., Enric Carcereny, M.D., Myung-Ju Ahn, M.D., Enriqueta Felip, M.D., Jong-Seok Lee, M.D., Matthew D. Hellmann, M.D., Omid Hamid, M.D., Jonathan W. Goldman, M.D., Jean-Charles Soria, M.D., Marisa Dolled-Filhart, Ph.D., Ruth Z. Rutledge, M.B.A., Jin Zhang, Ph.D., Jared K. Lunceford, Ph.D., Reshma Rangwala, M.D., Gregory M. Lubiniecki, M.D., Charlotte Roach, B.S., Kenneth Emancipator, M.D., and Leena Gandhi, M.D., for the KEYNOTE-001 Investigators*



DAKO 22C3

Tumor PD-L1 expression alone does not accurately assess the **dynamic immune microenvironment**

Additional diagnostic approaches, including assessment of the genomic landscape and the presence of preexisting CD8+ T cells and cytokines in tumor samples, could supplement PD-L1 expression as a means of identifying patients who might have a response to pembrolizumab



Predictive biomarkers & Drug efficacy

EGFR mutations
ALK rearrangement

Immunotherapy efficacy

Drug efficacy predicts the best biomarker

Biomarker predicts efficacy

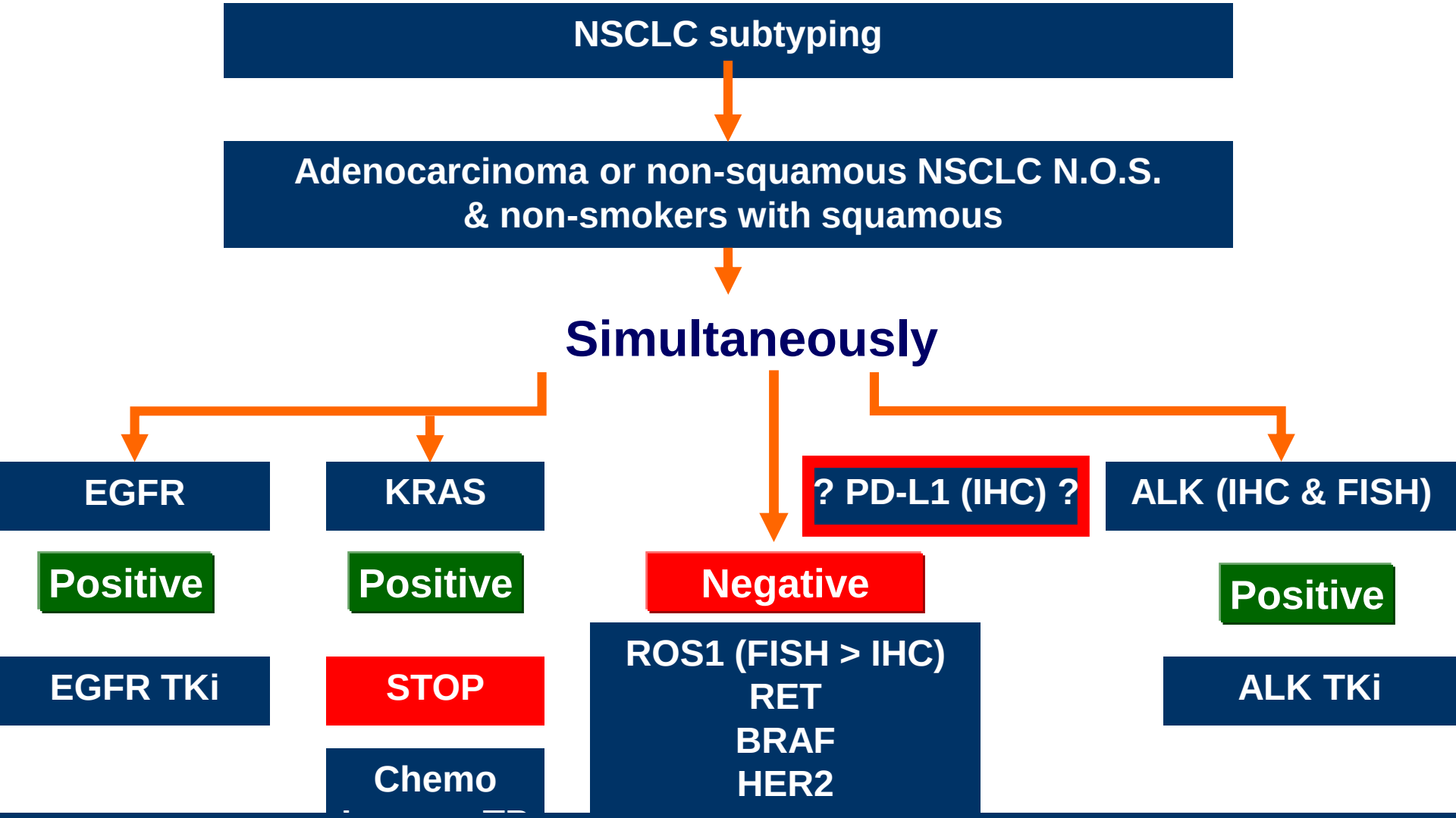
TKI efficacy

PD-L1 expression ?
PD-1 expression ?
Mutational burden ?
Cytokines ?
Lymphocytes ?

Is PD-L1 a robust biomarker in immunotherapy ?

Pembrolizumab	**DAKO, clone 22C3 (≥ 50%)**
Nivolumab	**No biomarker**
Atezolizumab	**Ventana, SP142 (>10%) ?**
and many others...	**and may other clones with various score....**

Algorithm for Predictive Genetic Testing in Advanced NSCLC Routine Practice



We face with cytology and small biopsy in about 2/3 of cases

Releasing the Brakes on Cancer Immunotherapy

Antoni Ribas, M.D., Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

- **Dynamic process rather than a fixed picture of the tumor (immunotherapy is not a TKI)**

- **NSCLC expressing PD-L1 respond better to checkpoint inhibitors**

- **No single predictive biomarker**

- **PD-L1 expression is promising as predictive marker, but several pre-analytical and analytical factors are still to be defined**