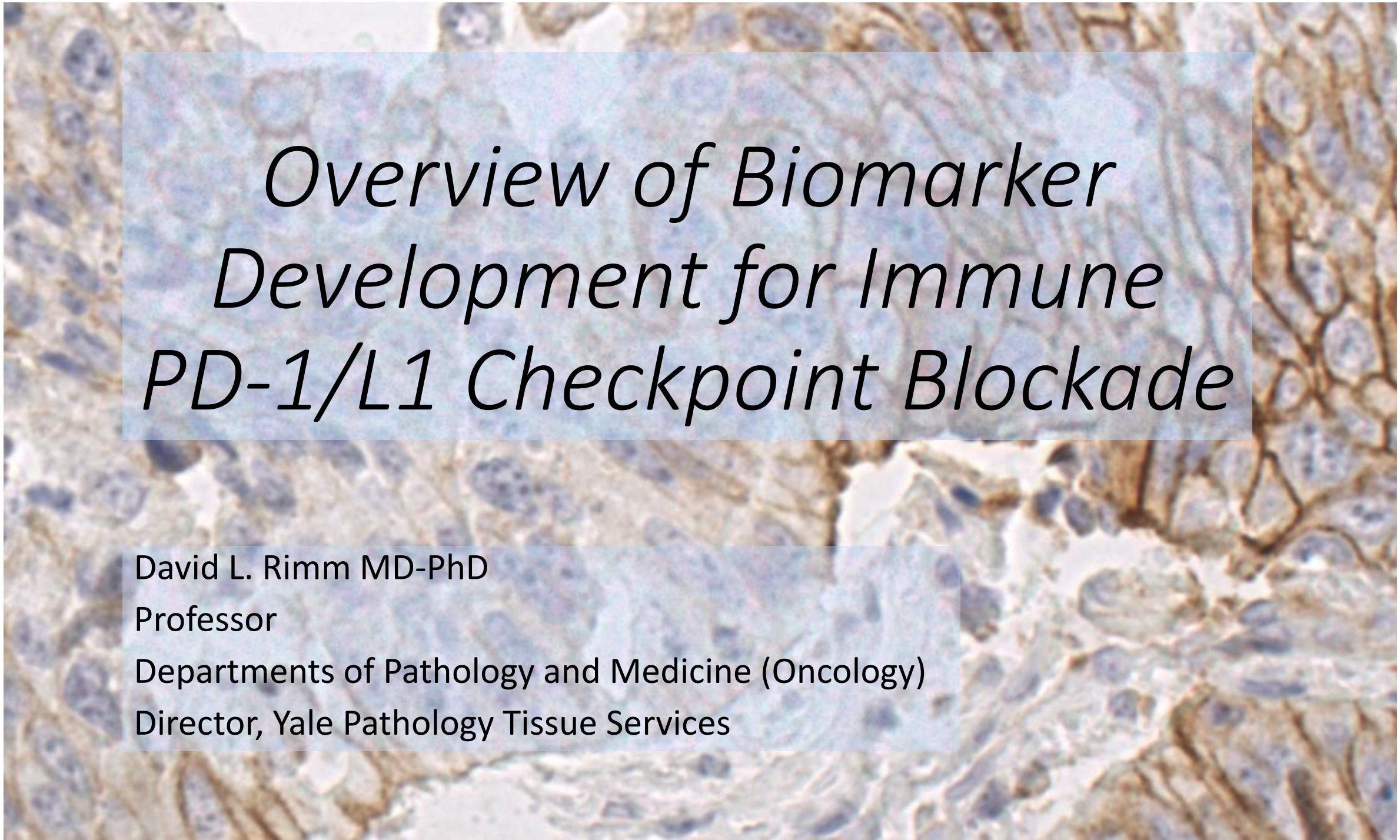


Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

David L. Rimm MD-PhD
Professor
Departments of Pathology and Medicine (Oncology)
Director, Yale Pathology Tissue Services

Disclosures for David L. Rimm MD-PhD

- In the last 12 months I have been engaged in the following relationships:
- I am a Consultant/Advisor to Astra Zeneca, Biocept, BMS, Cell Signaling Technology, Merck, Novartis, PAIGE, Perkin Elmer and Ultivue
- Astra Zeneca, Cepheid, NavigateBP, NextCure, Lilly, Perkin Elmer, and Ultivue fund research in my lab.

Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

- Companion vs Complementary Diagnostic Tests
- Immunohistochemistry
- Genomic testing (targeted and TMB)
- Expression (mRNA) signatures
- Multiplex Fluorescence
- cfDNA and other circulating markers (NLR, LIPI)

PD-L1 Assay Terminology:

- Companion Diagnostic Test (Cdx)
  - **Test result required for prescription of the drug.**
  - Specified on the Drug Label
  - Often, this category is typically used when the test is an inclusion criteria for the trial (but not always – see gastro-esophageal)
- Complementary Diagnostic Test
  - **Test result is predictive, but not required for prescription of the drug**
  - Nice to have, but not need to have – No clear message on reimbursement
  - Term attributed to Liz Mansfield when she was at the FDA
  - Mostly, this category is used when the assay is integrated into the trial, but not used for inclusion criteria (All-comers trials)

FDA Cleared or Approved Companion Dx's (protein)

Test	Scoring System	Indication	Drug	Date of Decision or Notice
PD-L1 IHC 22c3 PharmDx	TPS (0, 1-49, >50)	Lung Cancer	Pembrolizumab	10/24/2016
PD-L1 IHC 22c3 PharmDx	CPS (<1, >1)	Gastroesophageal/GEJ adenoca	Pembrolizumab	10/23/2017
PD-L1 IHC 22c3 PharmDx	CPS (<1, >1)	Cervical Cancer	Pembrolizumab	6/12/2018
Ventana ALK D5F3 CDx	+/-	Lung Cancer	Ceritinib or Crizotinib	6/12/2015
Dako EGFR PharmDx Kit	+/-	Colorectal Cancer	Cetuximab or panatumumab	9/27/2006
Dako C-Kit PharmDx	+/-	GIST	Imatinib	11/02/2012
Pathway Anti-HER2 (4B5)	0-3+	Breast Cancer	trastuzumab	4/9/2014
Bond Oracle HER2 IHC system	0-3+	Breast Cancer	trastuzumab	4/18/2012
Herceptest (Dako)	0-3+	Breast Cancer	Trastuzumab, pertuzumab and adotrastuzumab emtansine	9/25/1998

Current Companion Dx for PD-L1

Test	Scoring System	Indication	Drug	Date of Decision or Notice
PD-L1 IHC 22c3 PharmDx	TPS	Lung Cancer	Pembrolizumab	10/24/2016
PD-L1 IHC 22c3 PharmDx	CPS	Gastroesophageal/GEJ adenoca	Pembrolizumab	10/23/2017
PD-L1 IHC 22c3 PharmDx	CPS	Cervical Cancer	Pembrolizumab	6/12/2018

Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

- Companion vs Complementary Diagnostic Tests
- Immunohistochemistry
- Genomic testing (targeted and TMB)
- Expression (mRNA) signatures
- Multiplex Fluorescence
- cfDNA and other circulating markers (NLR, LIPI)

Assay Comparison Literature:

Journal of Thoracic Oncology Vol. 12 No. 2: 208-222

ORIGINAL ARTICLE

PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project

Fred R. Hirsch, MD, PhD,[a,b,*] Abigail McElhinny, PhD,[c] Dave Stanforth, MBA,[d] James Ranger-Moore, PhD,[e] Malinka Jansson, MA,[d] Karina Kulangara, PhD,[d] William Richardson, BA,[e] Penny Towne, BS, MBA,[e] Debra Hanks, MD,[d] Bharathi Vennapusa, MD,[e] Amita Mistry, MD,[e] Rasika Kalamegham, PhD,[f,g] Steve Averbuch, MD,[h] James Novotny, PhD,[h] Eric Rubin, MD,[i] Kenneth Emancipator, MD,[i] Ian McCaffery, PhD,[j,k] J. Andrew Williams, PhD,[j] Jill Walker, PhD,[l] John Longshore, PhD,[m] Ming Sound Tsao, MD,[n] Keith M. Kerr, MB, FRCPath[o]

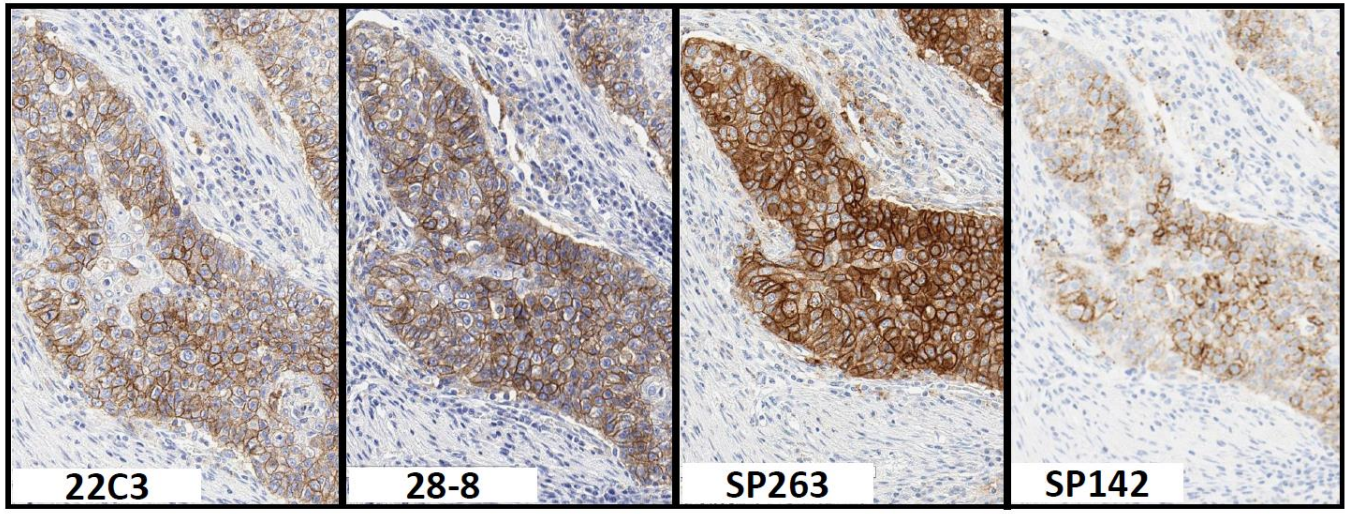
JAMA Oncology | Original Investigation

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

David L. Rimm, MD, PhD; Gang Han, PhD; Janis M. Taube, MD; Eunhee S. Yi, MD; Julia A. Bridge, MD; Douglas B. Flieder, MD; Robert Homer, MD, PhD; William W. West, MD; Hong Wu, MD; Anja C. Roden, MD; Junya Fujimoto, MD; Hui Yu, MD; Robert Anders, MD; Ashley Kowalewski, MS; Christopher Rivard, PhD; Jamaal Rehman, MD; Cory Batenchuk, PhD; Virginia Burns, PhD; Fred R. Hirsch, MD, PhD; Ignacio I. Wistuba, MD, PhD

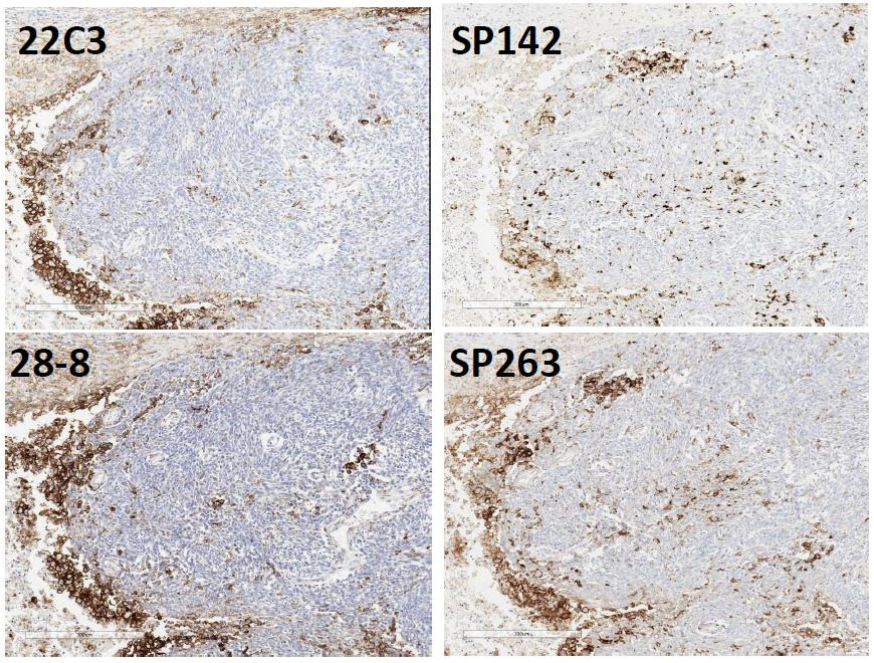
JAMA Oncol. doi:10.1001/jamaoncol.2017.0013
Published online March 9, 2017.

Example of PD-L1 Tumor Expression

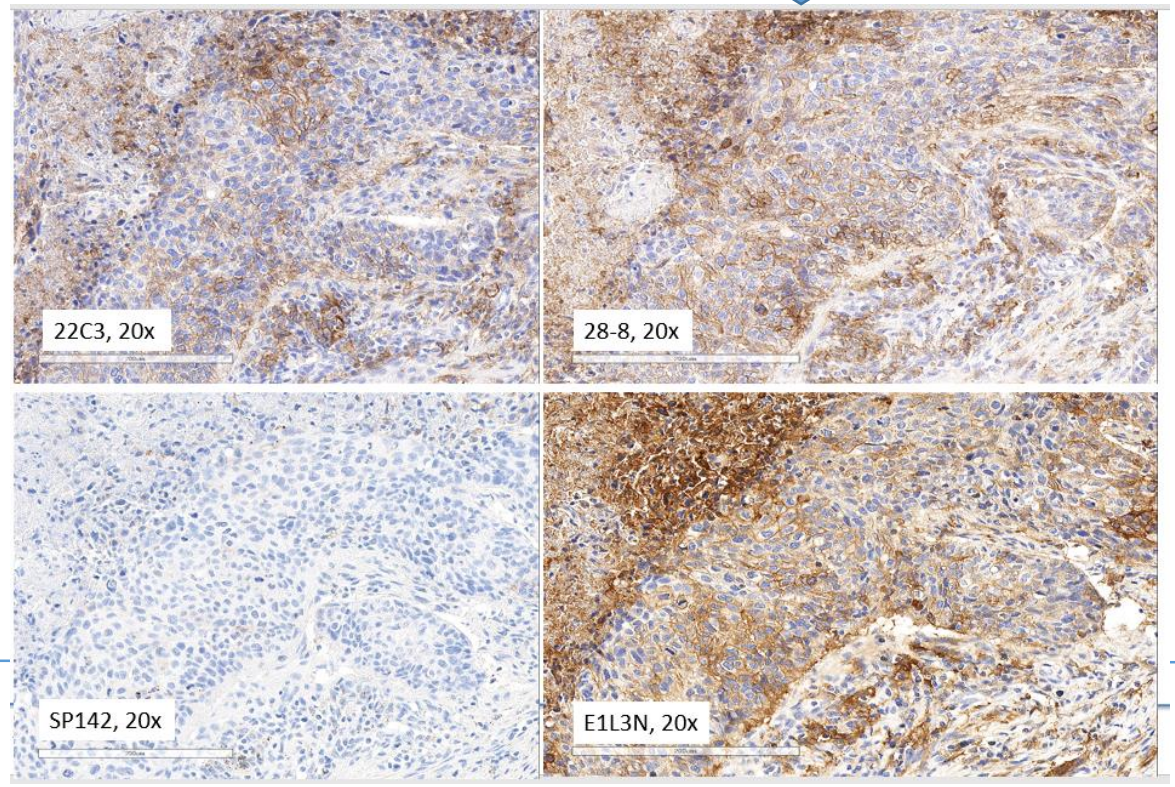


Blueprint

NCCN

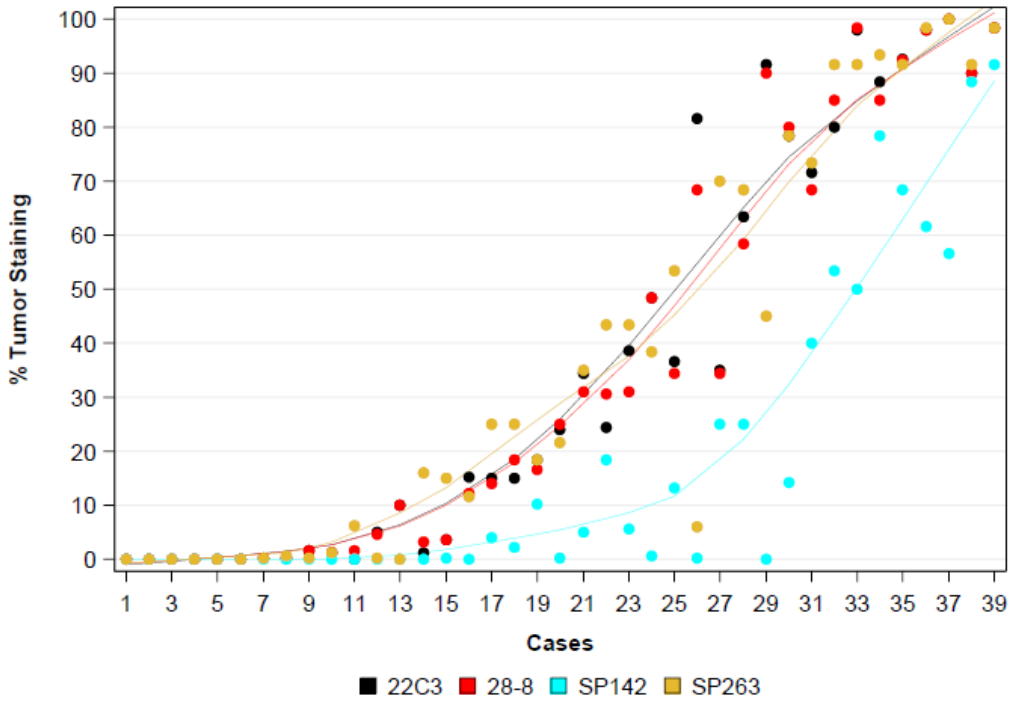


Yale School of Medicine



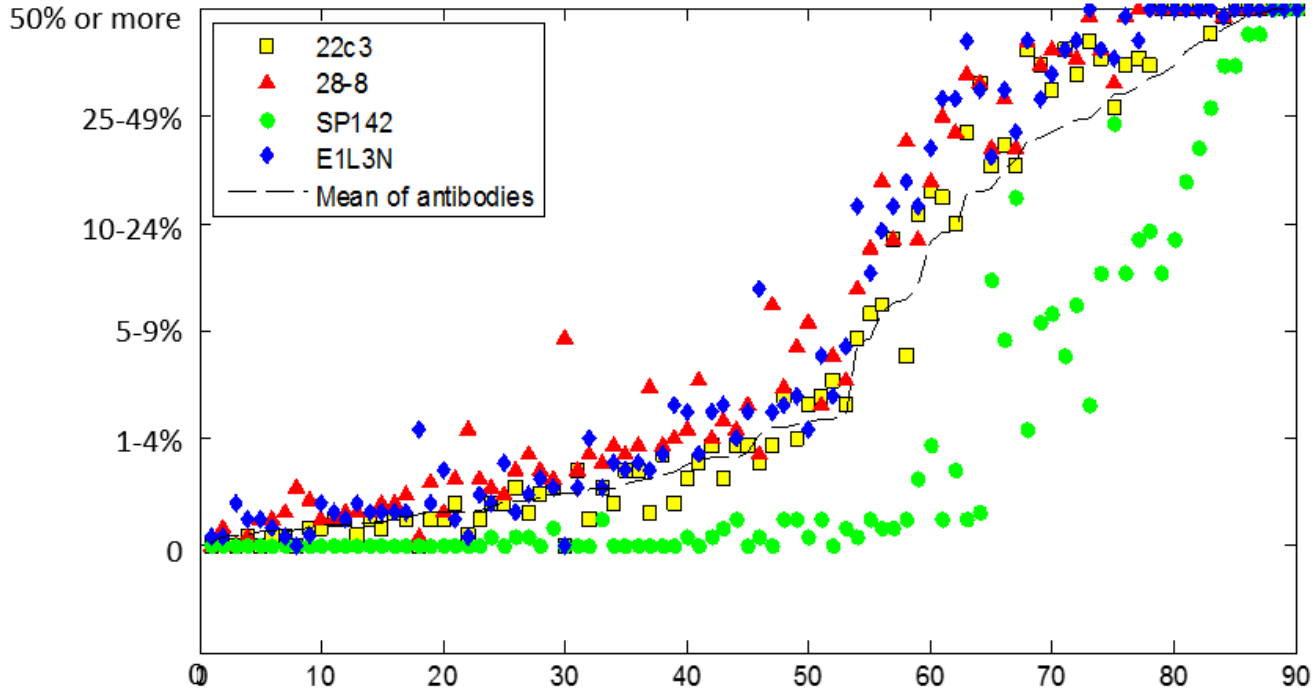
BLUEPRINT-1:

- 39 Cases – no outcome data
- 3 pathologists – from Dako and Ventana
- 4 Assays – FDA/IUO
- Not statistically powered
- By agreement of 6 companies (BMS, Merck, Genentech, AZ, Dako, Ventana)



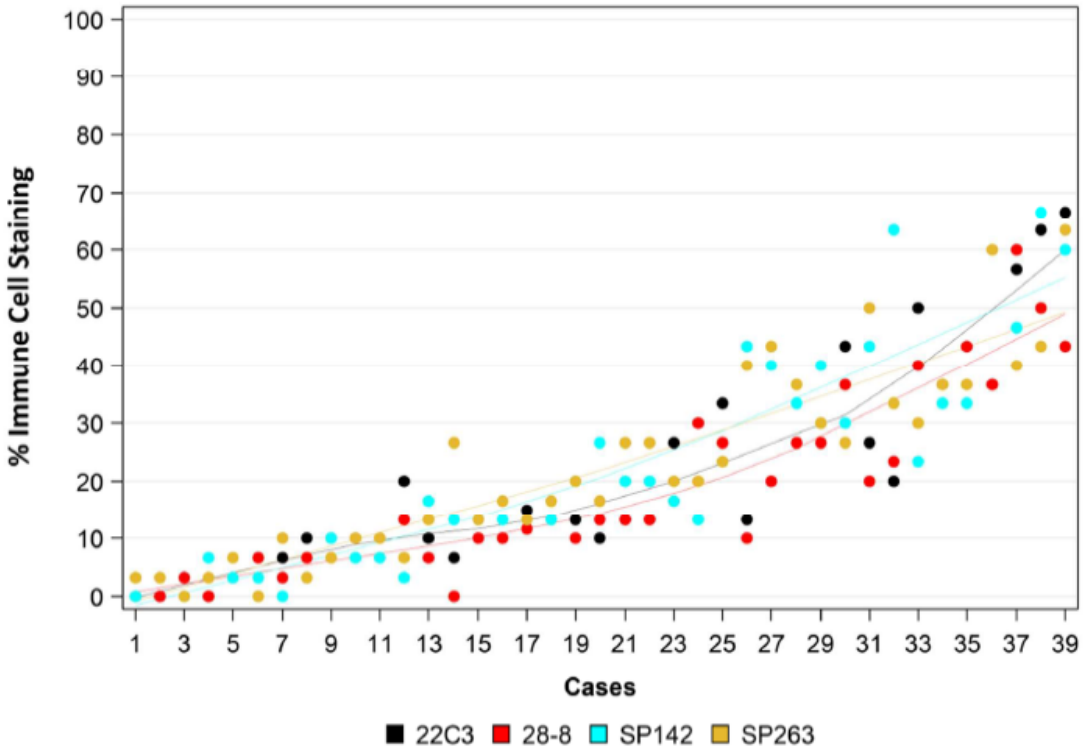
NCCN:

- 90 Cases- no outcome data
- 13 pathologists – from 7 academic sites
- 4 Assays – 3FDA/IUO and 1 LDT (E1L3N on Leica Bond)
- Prescribed, powered, statistical protocol for ICC between pathologists, assays and localization.
- Led by NCCN, sponsored by BMS

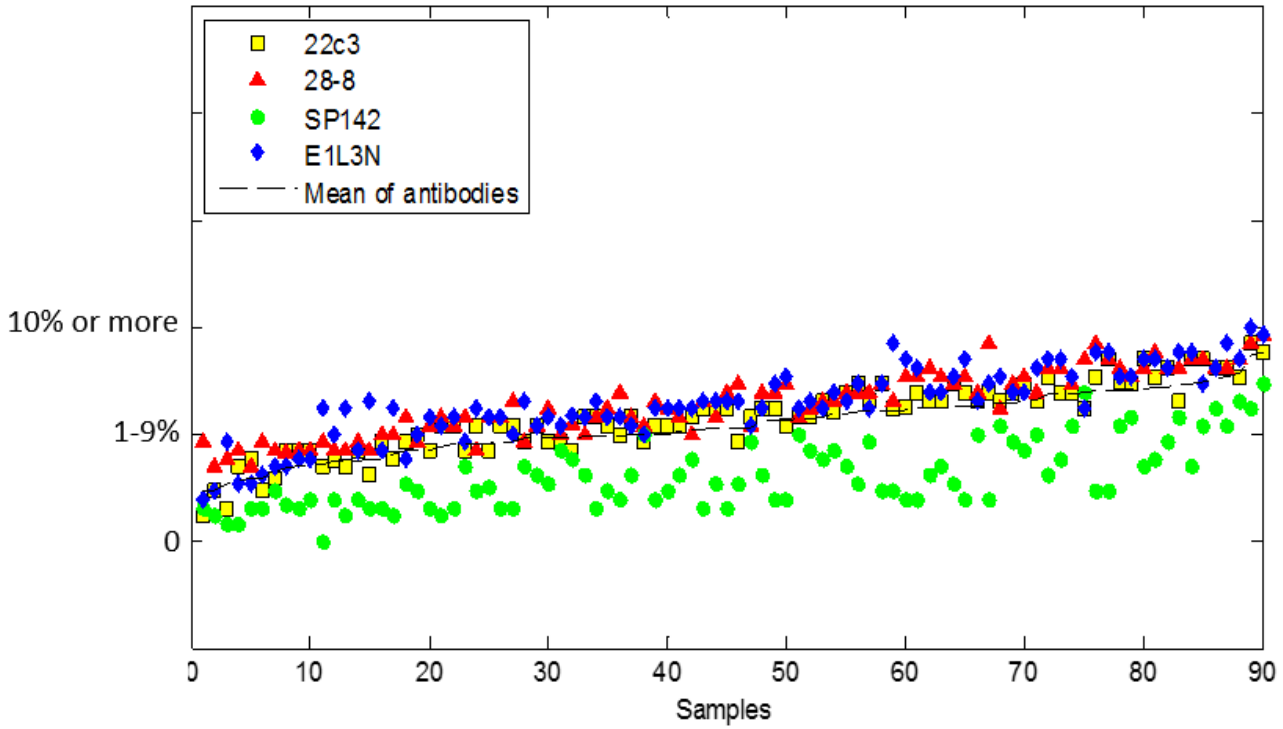


Comparison of Immune Cell Scores

BLUEPRINT-1:



NCCN:



Released 5/22/2018

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Article in Press

PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project

Ming Sound Tsao, MD, Keith M. Kerr, MD, Mark Kockx, MD, PhD, Mary-Beth Beasley, MD, Alain C. Borczuk, MD, Johan Botling, MD, Lukas Bubendorf, MD, Lucian Chirieac, MD, Gang Chen, MD, Teh-Ying Chou, MD, PhD, Jin-Haeng Chung, MD, PhD, Sanja Dacic, MD, PhD, Sylvie Lantuejoul, MD, Mari Mino-Kenudson, MD, Andre L. Moreira, MD, Andrew G. Nicholson, DM, Masayuki Noguchi, MD, PhD, Giuseppe Pelosi, MD, Claudia Poleri, MD, Prudence A. Russell, MD, Jennifer Sauter, MD, Erik Thunnissen, MD, PhD, Ignacio Wistuba, MD, PhD, Hui Yu, MD, PhD, Murry W. Wynes, PhD, Melania Pintilie, MSc, Yasushi Yatabe, MD, PhD, Fred R. Hirsch, MD, PhD

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- P. Russell (Melbourne)

25 pathologists reading 81 cases (including some cytology specimens) after a 1.5 day training course

PD-L1 immunohistochemistry (IHC) assays

Drug	PD-L1 IHC Assay	PD-L1 scoring	Cut-offs reported in clinical trials	FDA Diagnostic Status
Nivolumab	28-8	Tumor cells	1%, 5%, 10%	Complementary
Pembrolizumab	22C3	Tumor cells (TPS)	1%, 50%	Companion
Atezolizumab	SP142	Tumor cells (TC)	1%, 5%, 50%	Complementary
		Immune cells (IC)	1%, 5%, 10%	
Durvalumab	SP263	Tumor cells	25%	Unknown
Avelumab	73-10	Tumor cells	1%, 50%, 80%	Unknown

TPS: tumor proportional score; TC: staining on tumor cell; IC: staining on immune cells

M. Tsao et al, IASLC presentation at WCLC 2017

Strong reliability among all pathologists on tumor cell scoring

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

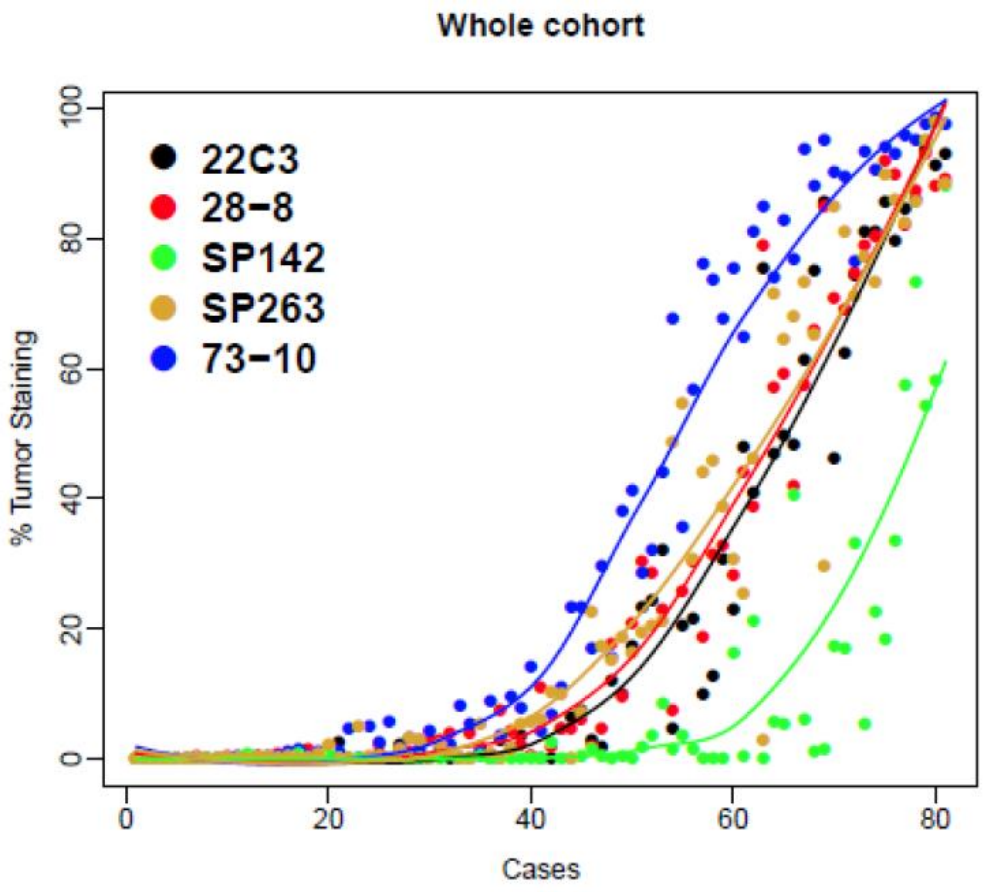
0.75-0.9: good

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

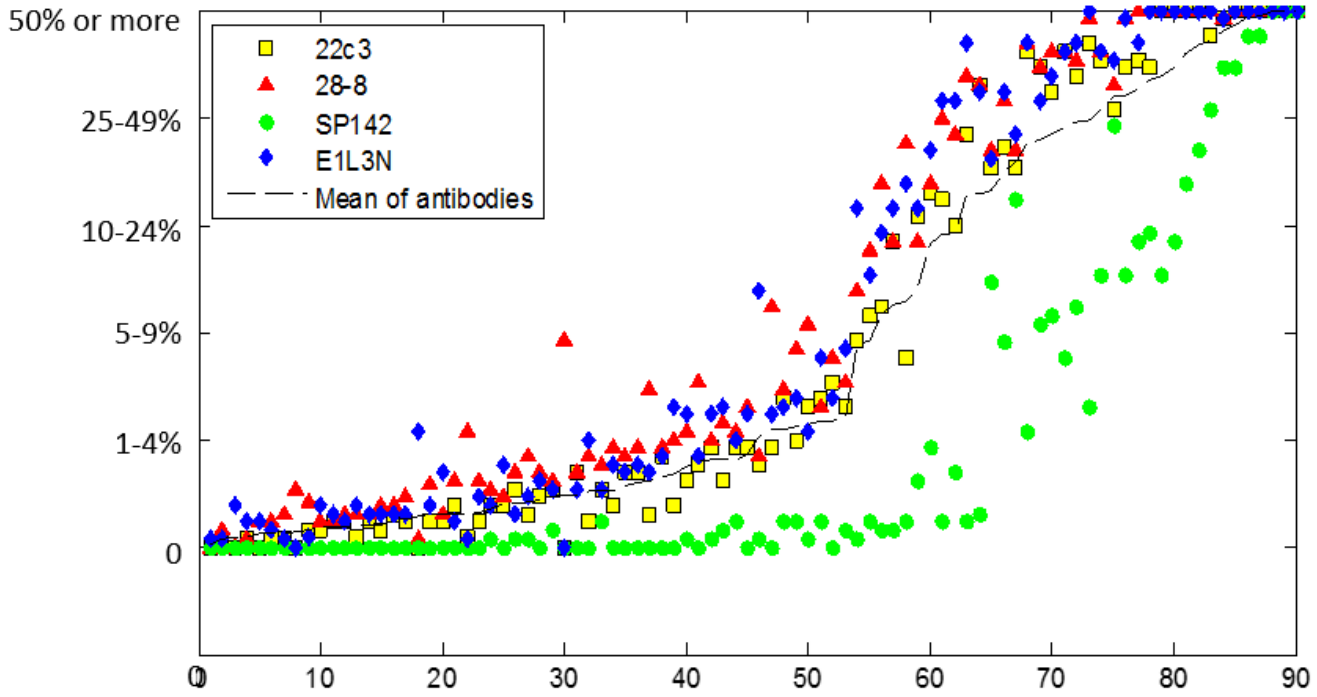
M. Tsao et al, IASLC presentation at WCLC 2017

Blueprint 2 results similar to NCCN study

Blueprint 2 Study



NCCN Study



M. Tsao et al, IASLC presentation at WCLC 2017

Poor reliability among all pathologists on immune cell scoring

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**

M. Tsao et al, IASLC presentation at WCLC 2017

Summary:

Two statistically powered, multi-institutional studies (NCCN and Blueprint 2) and a number of smaller studies have shown

1. The 22c3, 28-8 and SP263 assays are practically equivalent, while the SP142 assay shows uniformly lower scores for both tumor cells and immune cells
2. Cytology specimens, although not included in the label for the FDA approved assay, are practically equivalent to surgical biopsy specimens although there is just slightly lower concordance in the TPS scoring.
3. Pathologist can read TPS (tumor proportion scores) with high concordance, but even with training, are not concordant in reading of immune cell scores.
4. ICC is higher when assessing higher percentages of cells

Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

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- Immunohistochemistry
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- Expression (mRNA) signatures
- Multiplex Fluorescence
- cfDNA and other circulating markers (NLR, LIPI)

RESEARCH ARTICLE

STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in *KRAS*-Mutant Lung Adenocarcinoma

Ferdinandos Skoulidis[1], Michael E. Goldberg[2], Danielle M. Greenawalt[3], Matthew D. Hellmann[4], Mark M. Awad[5], Justin F. Gainor[6], Alexa B. Schrock[2], Ryan J. Hartmaier[2], Sally E. Trabucco[2], Laurie Gay[2], Siraj M. Ali[2], Julia A. Elvin
Han Chang[3], Ariella Sasson[3], Sujaya S
Robin Edwards[3], Jose A. Bufill[7], Neele
Hira Rizvi[4], Elizabeth Jimenez Aguilar
Andrew J. Plodkowski[14], Niamh M. Lor
Haifa Hamdi[1], Taghreed Hirz[1], Pan Tor
Edwin R. Parra[17], Neda Kalhor[18], Lyne
Mari Mino-Kenudson[21], Roxana Azimi
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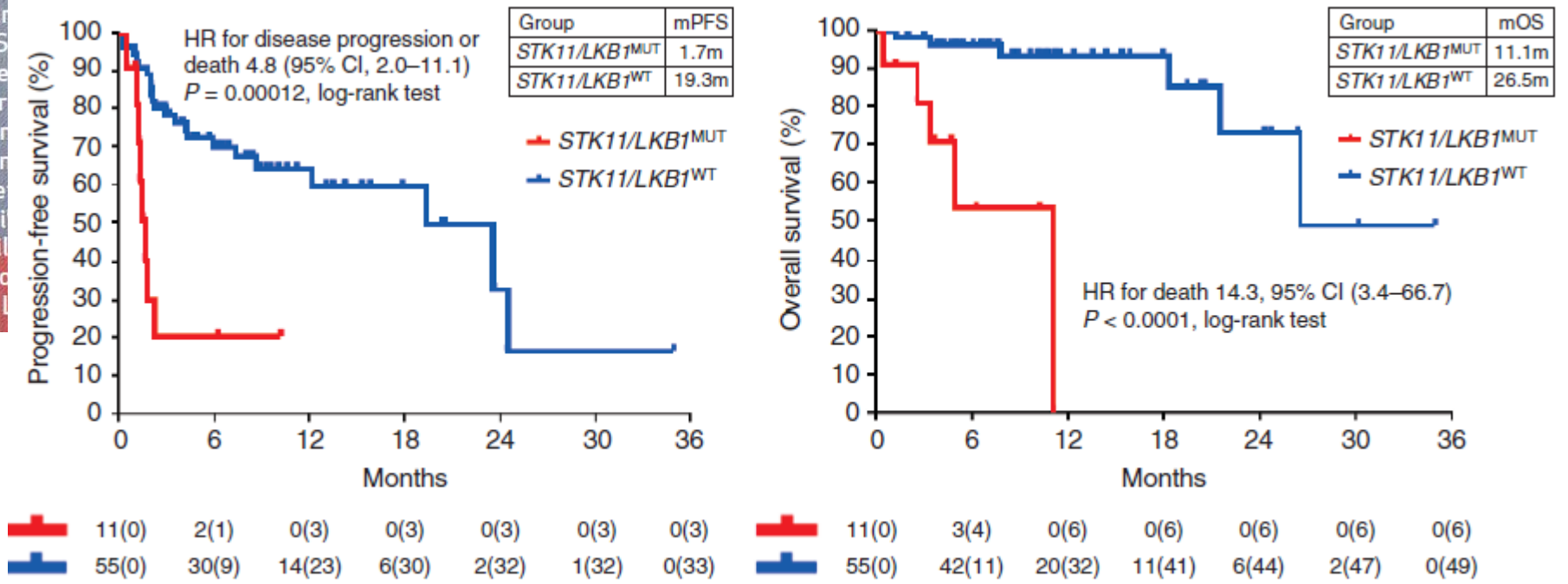
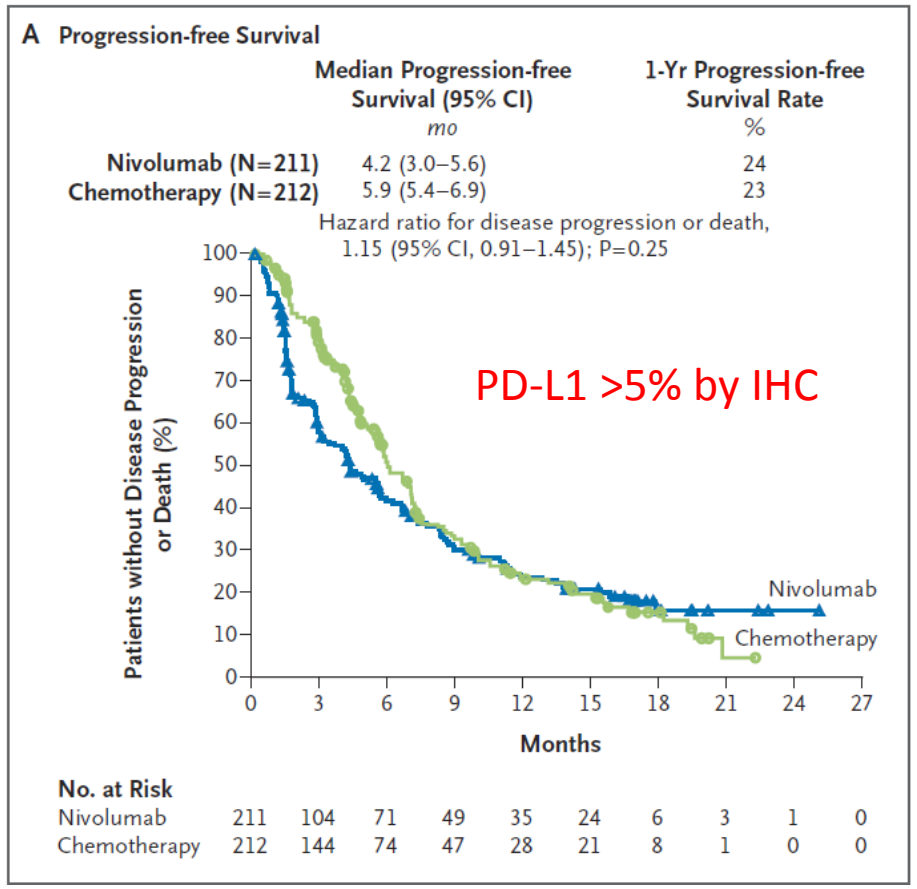
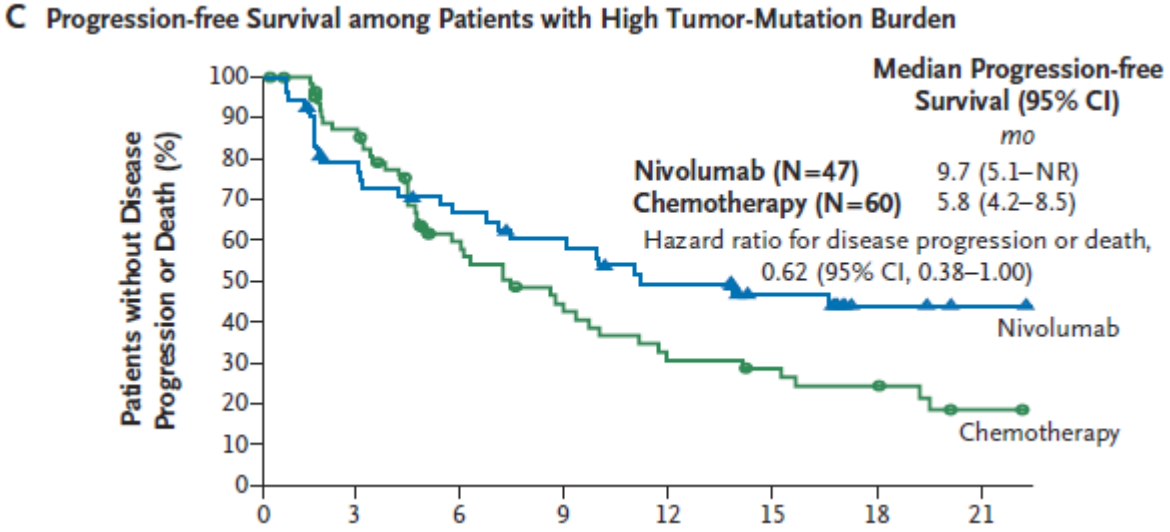
Charles M. Rudin[4], William J. Geese[3],

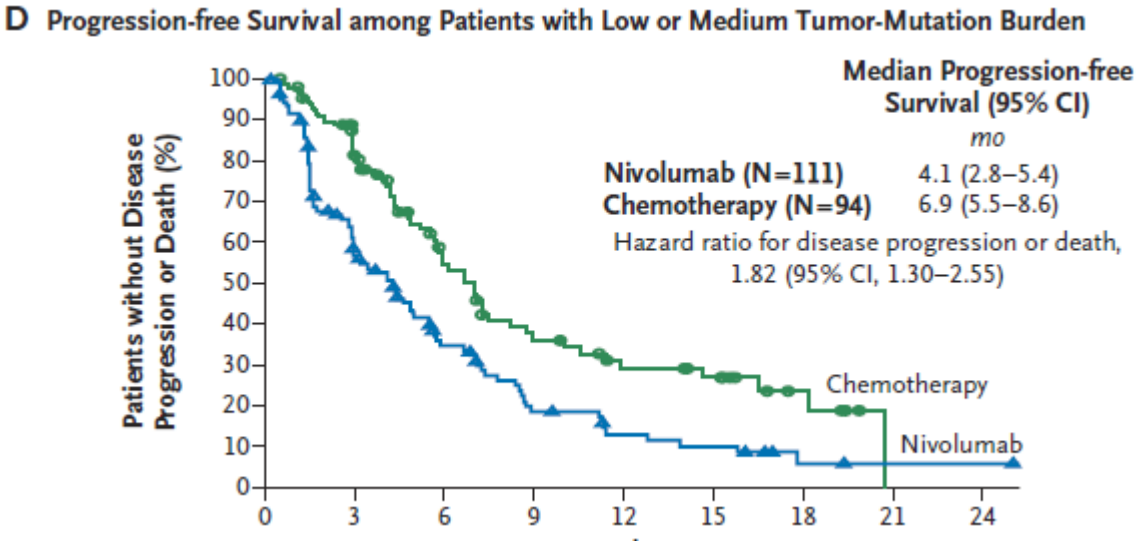


Checkmate 26 – the most promising TMB data



Carbone et al, NEJM 2017





TMB does not predict Overall Survival

Figure S8. Kaplan–Meier Plot Overall Survival in Evaluable Patients with High Tumor Mutation Burden.

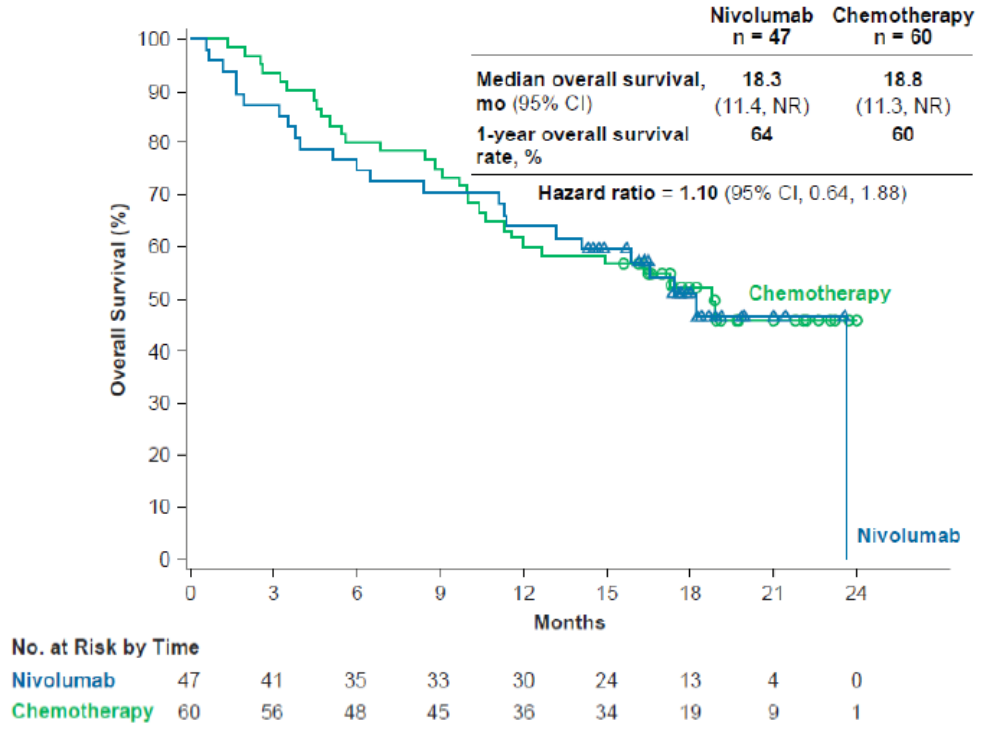
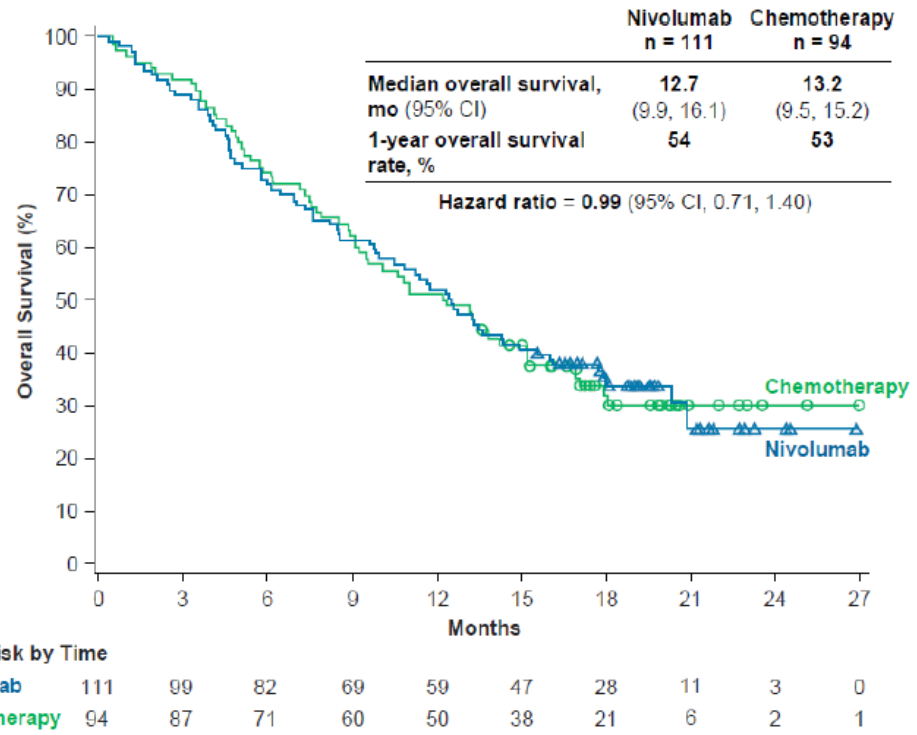


Figure S9. Kaplan–Meier Plot of Overall Survival in Evaluable Patients with Low or Medium Tumor Mutation Burden.

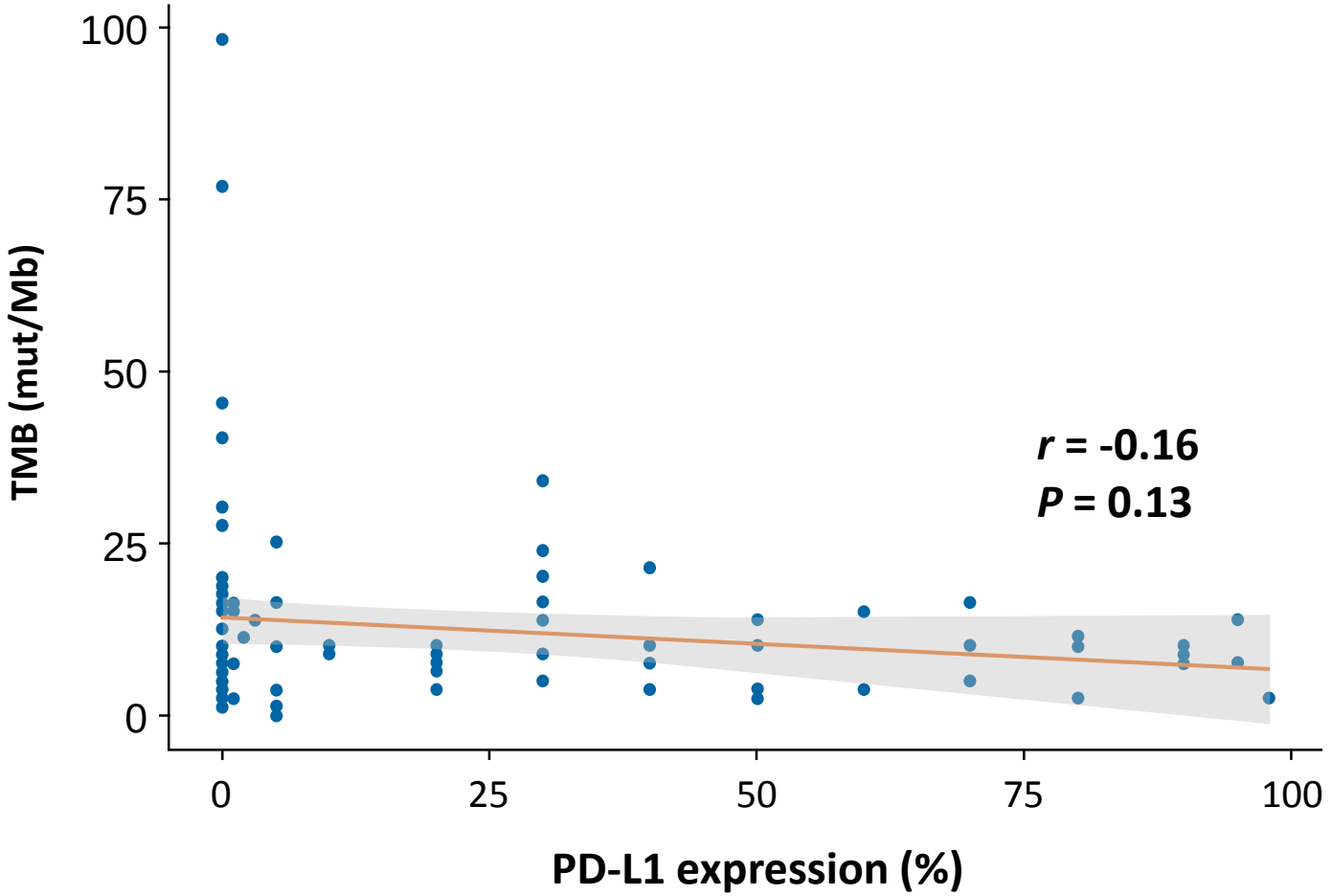


Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

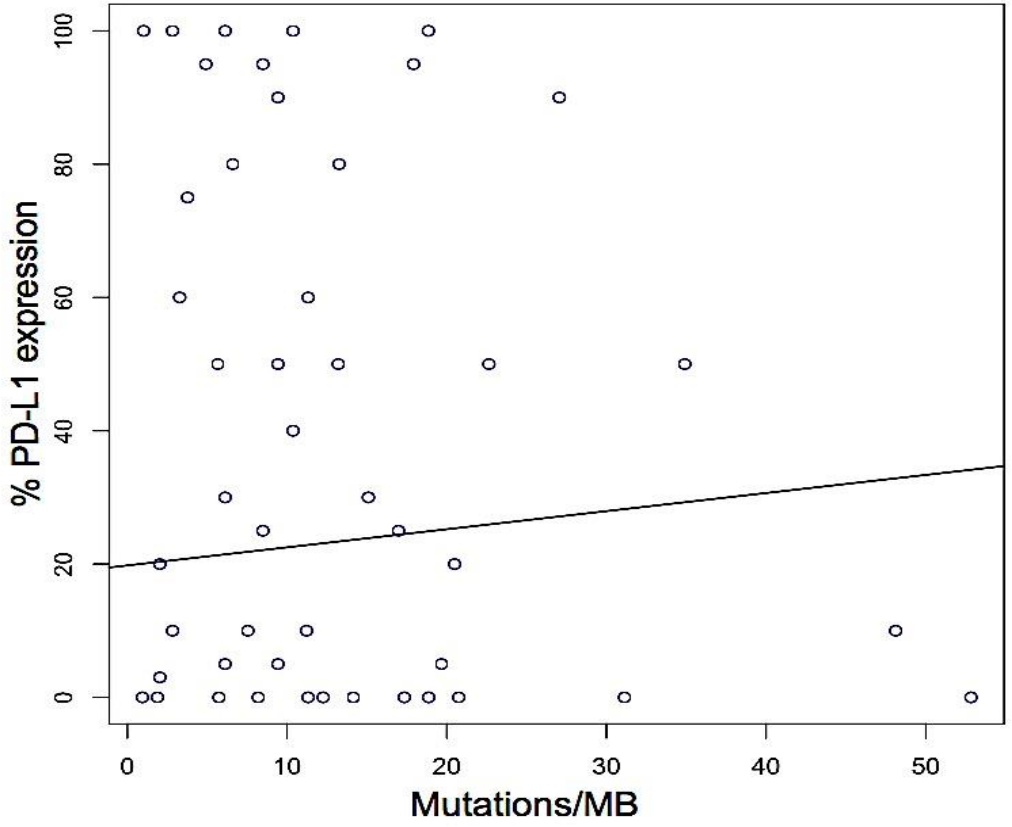
Supplement to: Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage IV or recurrent non–small-cell lung cancer. N Engl J Med 2017;376:2415-26. DOI: 10.1056/NEJMoa1613493

In BMS 568 TMB and PD-L1 Identify Distinct and Independent Populations of NSCLC



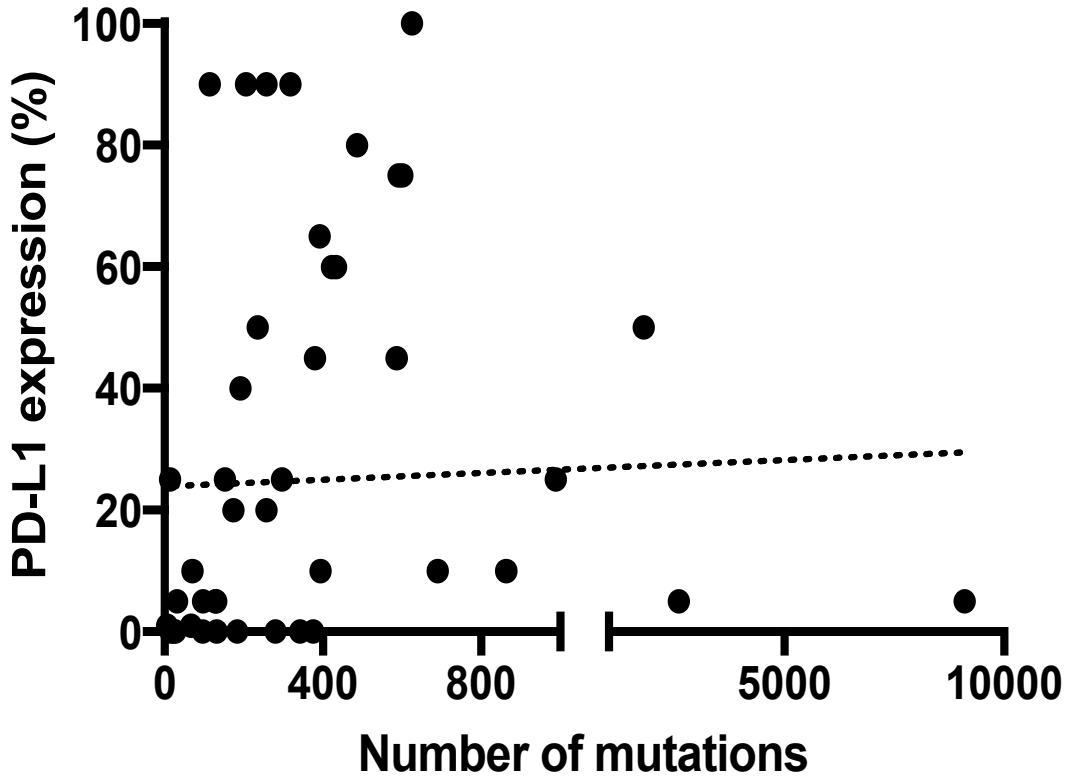
- No association between PD-L1 expression and TMB levels was observed

Inverse Relationship between PD-L1 and TMB in the literature



86 NSCLC cases analyzed with MSK-IMPACT panel (341-468 genes)

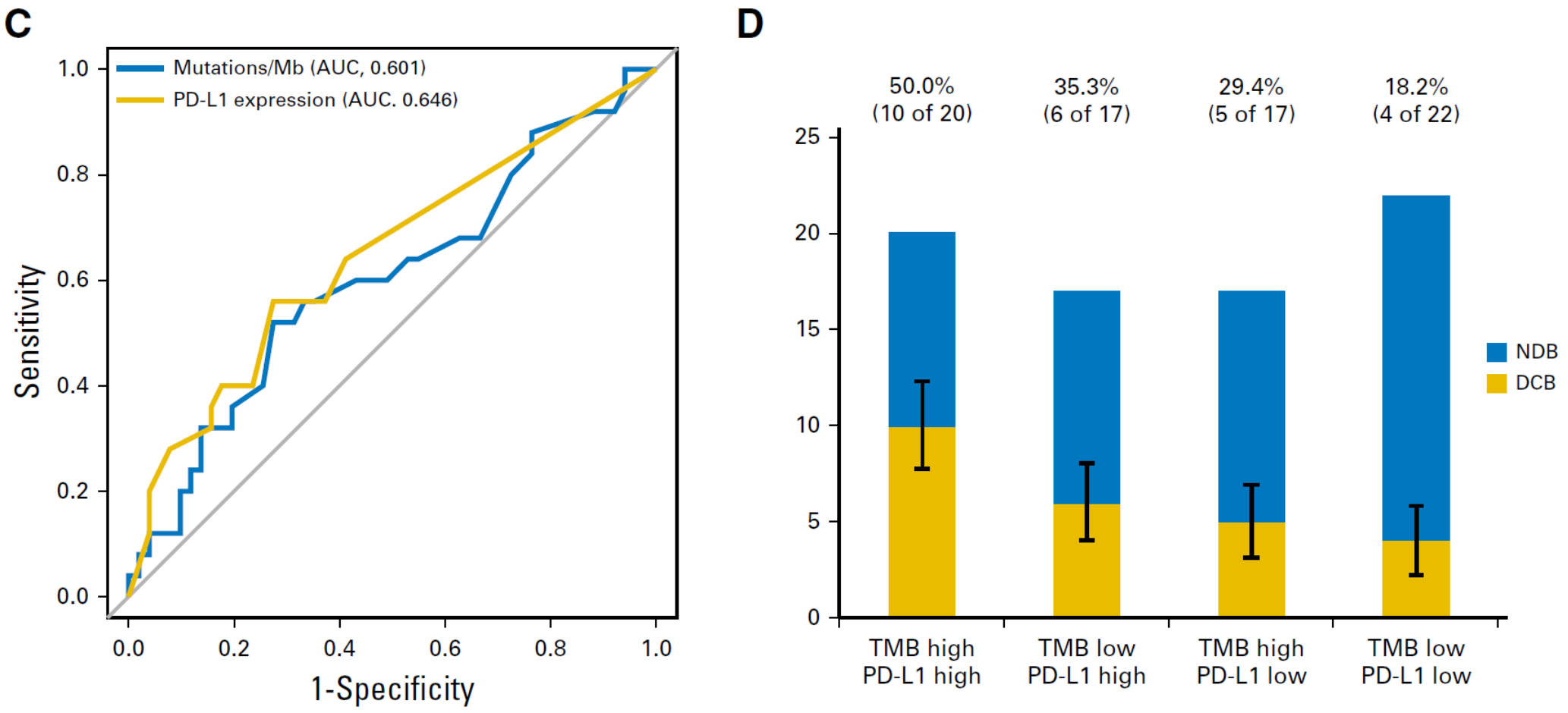
Rizvi H. et al., 2017, JCO



49 NSCLC cases analyzed with whole exome sequencing

Gettinger. et al., 2018, Nat Comm (in press)

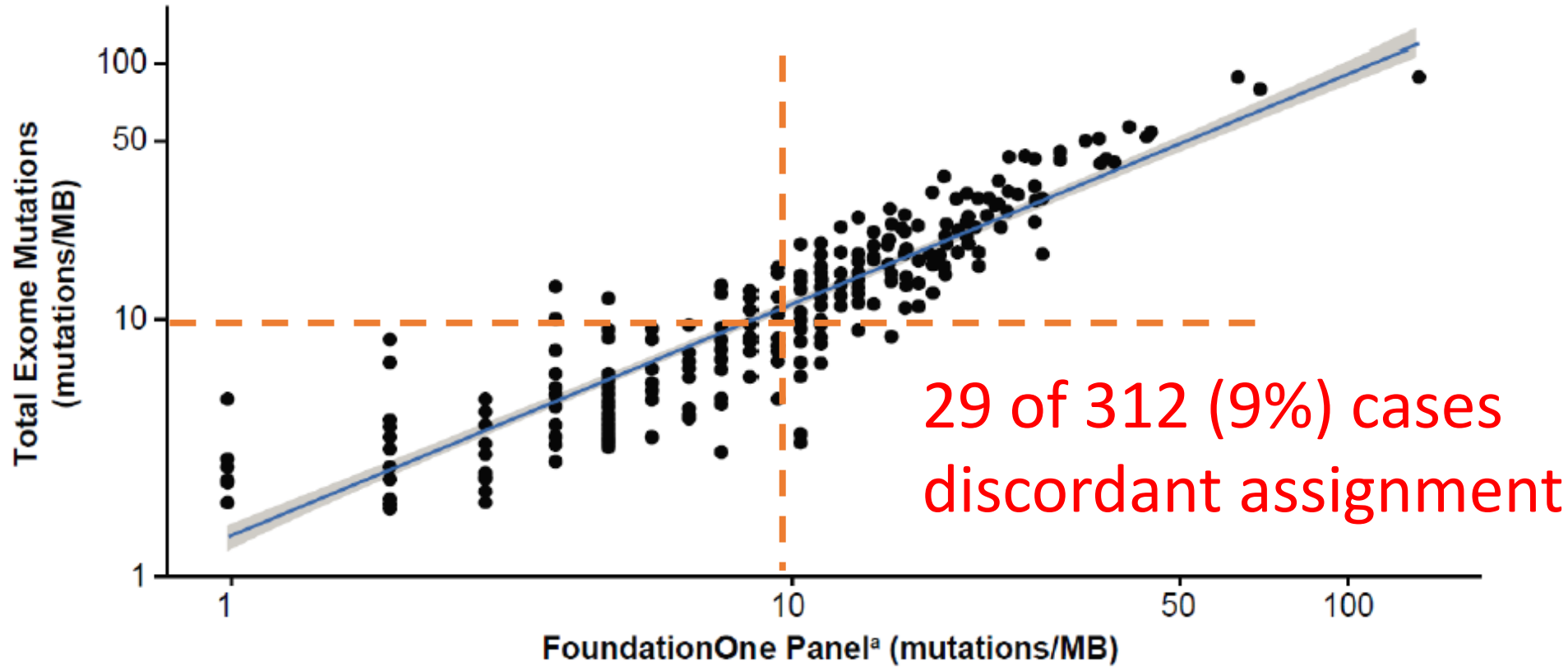
PD-L1 Expression and TMB are complementary



H. Rizvi et al JCO 2018

From Checkmate 26

Figure S5. Total Exome Mutations Versus Genes in FoundationOne Panel[a]



[a]Based on in silico analysis filtering on 315 genes in FoundationOne comprehensive genomic profile (Foundation Medicine, Inc, Cambridge, MA, USA)[4]

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage IV or recurrent non–small-cell lung cancer. N Engl J Med 2017;376:2415-26. DOI: 10.1056/NEJMoa1613493

Summary:

1. TMB is a biomarker for immunotherapy and it is complementary to PD-L1
2. TMB is PREDICTIVE for outcome
3. TMB testing is NOT standardized (Biggest Challenge for TMB)
4. TMB may be associated with PFS but not OS
5. Sensitivity and Specificity (AUC) no better than existing tests
6. TMB costs about 5-10X IHC (actual cost, not charge) and uses 10x as much tissue

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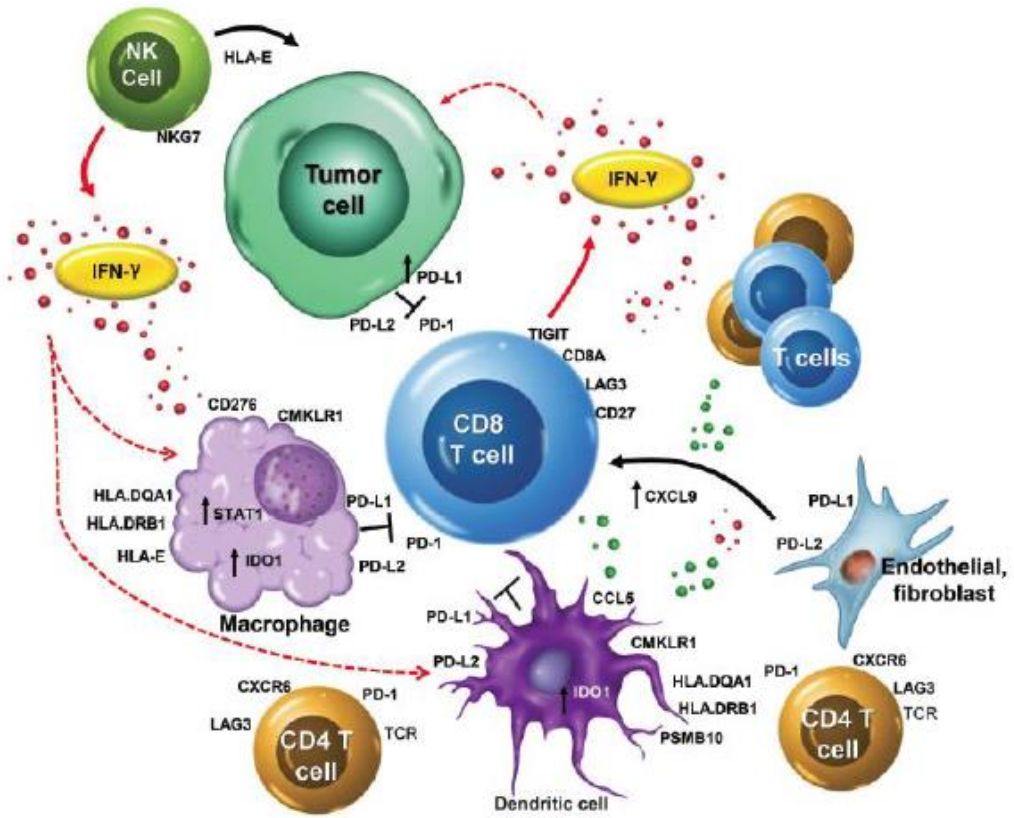
- cfDNA and other circulating markers (NLR, LIPI)

Four Areas of Immune Biology are Represented in the Tissue Inflammation Signature



IFNγ Biology	T Cell Exhaustion
CCL5	TIGIT
CXCL9	CD8A
CD27	LAG3
CXCR6	PD-L1
IDO1	PD-L2
STAT1	CD276

T Cell/NK Signature	Antigen Presenting Cell Signature
HLA-E	PSMB10
NKG7	HLA-DQA1
CMKLR1	HLA-DRB1

ASCO 2016; Poster #1536

TIS has been clinically verified in HNSCC, gastric, TNBC, urothelial, anal, biliary, colorectal, esophageal, and ovarian cancer

 The Tumor Inflammation Signature Assay (TIS) for use on the nCounter Dx Analysis System is for investigational use only. Limited by United Stated law to investigational use

nanoString TECHNOLOGIES®

An RNA-based Signature?

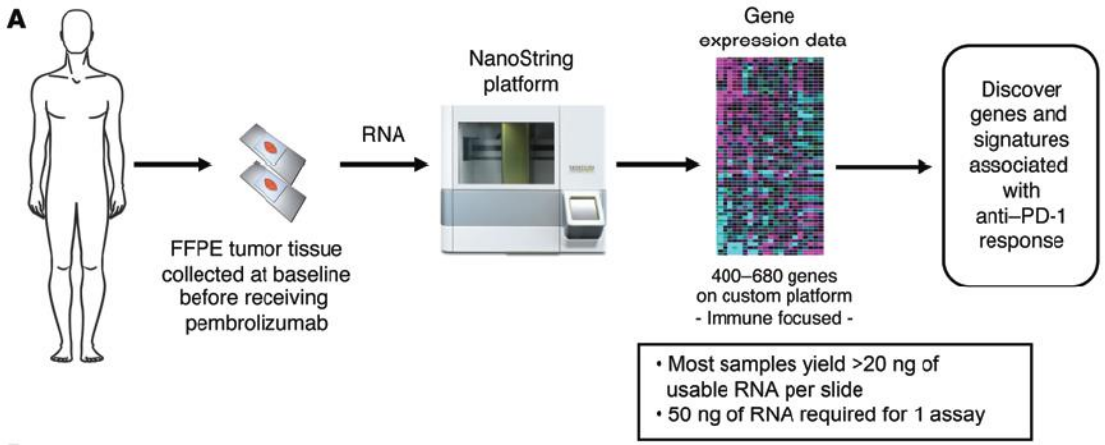
RESEARCH ARTICLE

The Journal of Clinical Investigation

IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade

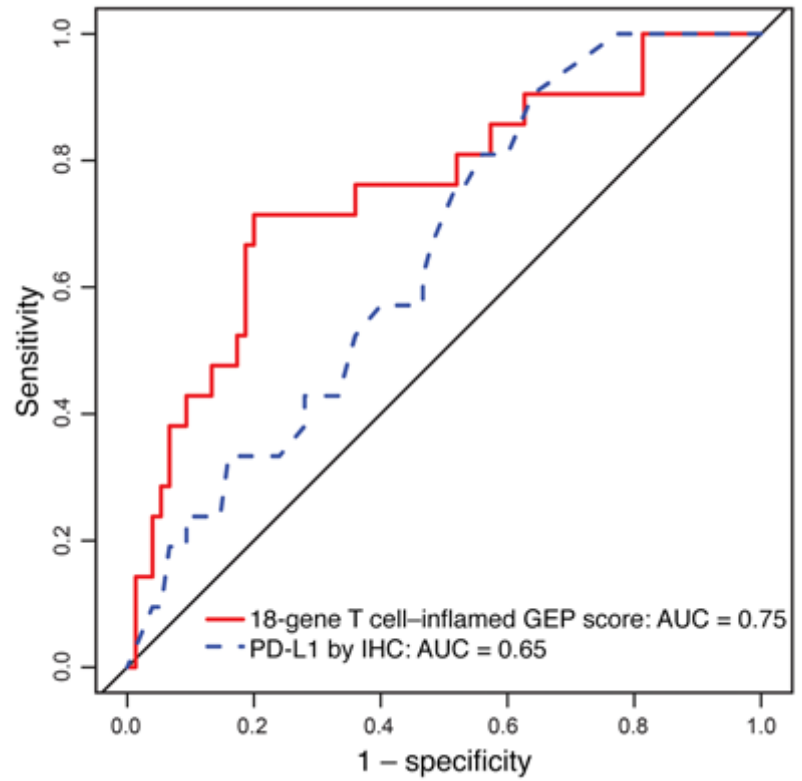
Mark Ayers,[1] Jared Lunceford,[1] Michael Nebozhyn,[1] Erin Murphy,[1] Andrey Loboda,[1] David R. Kaufman,[1] Andrew Albright,[1] Jonathan D. Cheng,[1] S. Peter Kang,[1] Veena Shankaran,[2] Sarina A. Piha-Paul,[3] Jennifer Yearley,[1] Tanguy Y. Seiwert,[4] Antoni Ribas,[5] and Terrill K. McClanahan[1]

[1]Merck & Co. Inc., Kenilworth, New Jersey, USA. [2]University of Washington, Seattle, Washington, USA. [3]University of Texas MD Anderson Cancer Center, Houston, Texas, USA. [4]University of Chicago, Chicago, Illinois, USA. [5]UCLA, Los Angeles, California, USA.



IFNγ Biology	T Cell Exhaustion
CCL5	TIGIT
CXCL9	CD8A
CD27	LAG3
CXCR6	PD-L1
IDO1	PD-L2
STAT1	CD276

T Cell/NK Signature	Antigen Presenting Cell Signature
HLA-E	PSMB10
NKG7	HLA-DQA1
CMKLR1	HLA-DRB1



Overview of Biomarker Development for Immune PD-1/L1 Checkpoint Blockade

- Companion vs Complementary Diagnostic Tests
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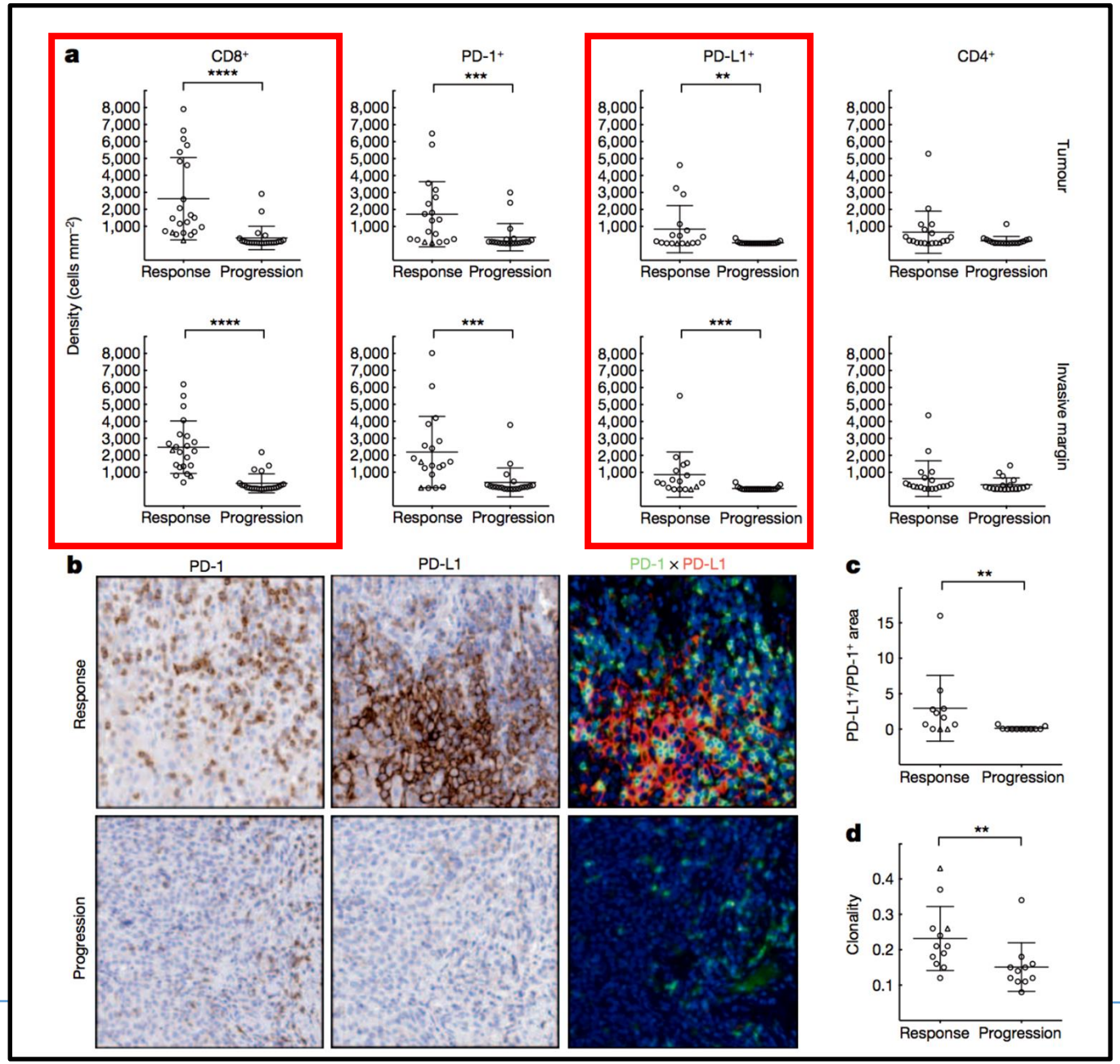
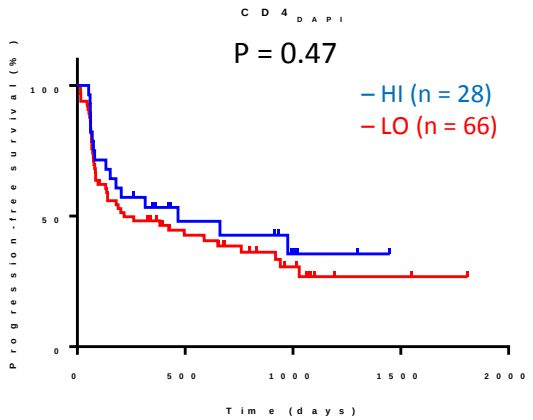
Association between TILs and Response to PD-1 blockade

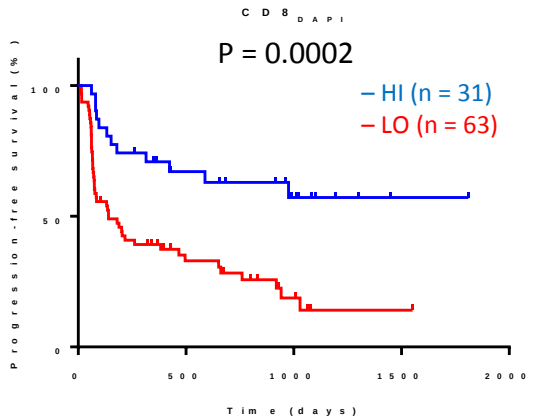


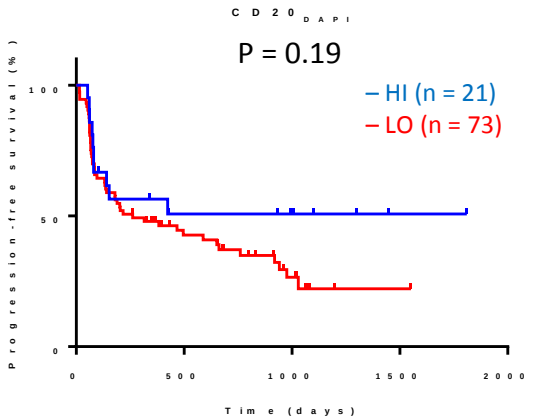
Tumeh et al., 2014 Nature

Testing CD4, CD8 and CD20 for Prediction for Response to Immunotherapy in Melanoma

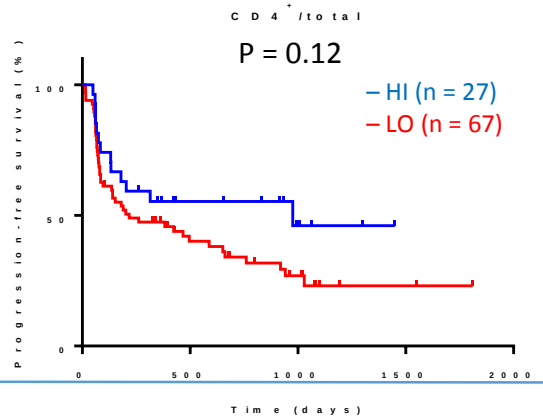
QIF:

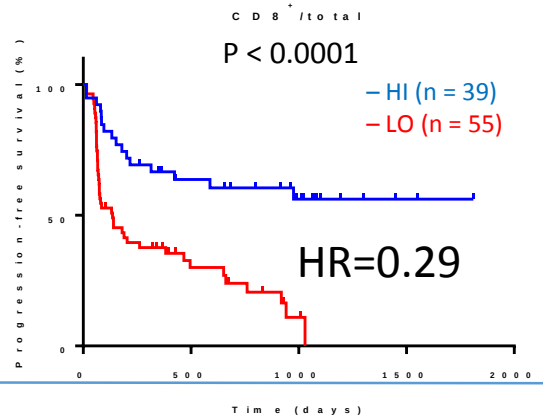


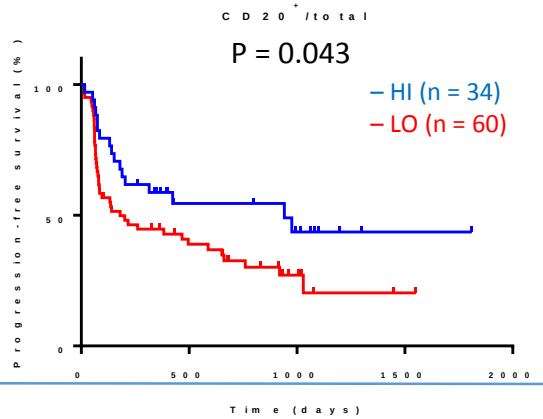




Cell counts:



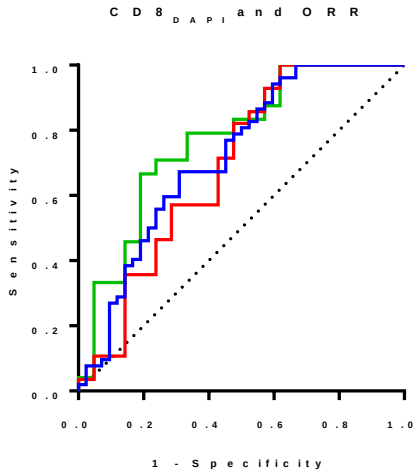




Pok Fai Wong et al, in review

Predictive performance of CD8 evaluated by receiver operating characteristic (ROC) curves in Melanoma

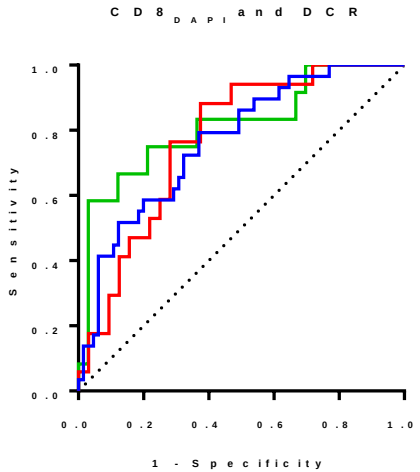
Quantitative Fluorescence



Total, AUC = 0.72
(95% CI, 0.61–0.83)

Mono, AUC = 0.69
(95% CI, 0.53–0.85)

Dual, AUC = **0.77**
(95% CI, 0.63–0.91)

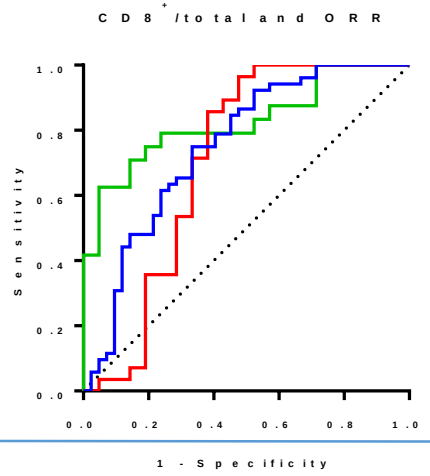


Total, AUC = 0.77
(95% CI, 0.67–0.87)

Mono, AUC = 0.77
(95% CI, 0.64–0.90)

Dual, AUC = **0.81**
(95% CI, 0.66–0.97)

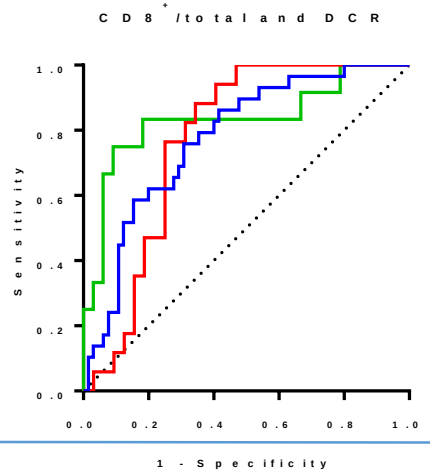
Cell counts



Total, AUC = 0.75
(95% CI, 0.65–0.85)

Mono, AUC = 0.71
(95% CI, 0.54–0.88)

Dual, AUC = **0.83**
(95% CI, 0.70–0.95)



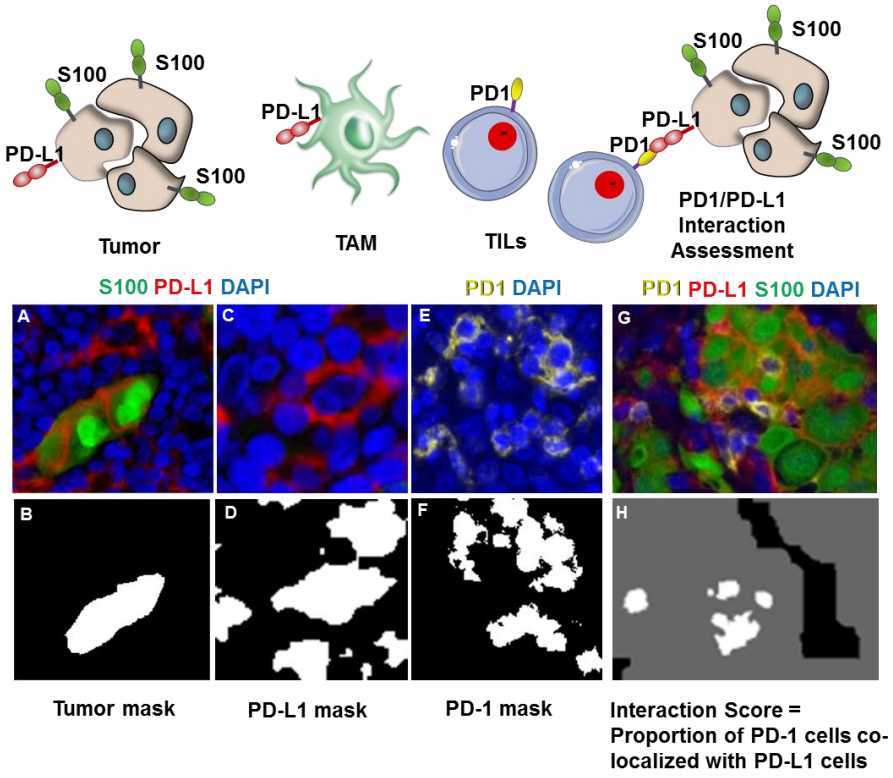
Total, AUC = 0.78
(95% CI, 0.68–0.87)

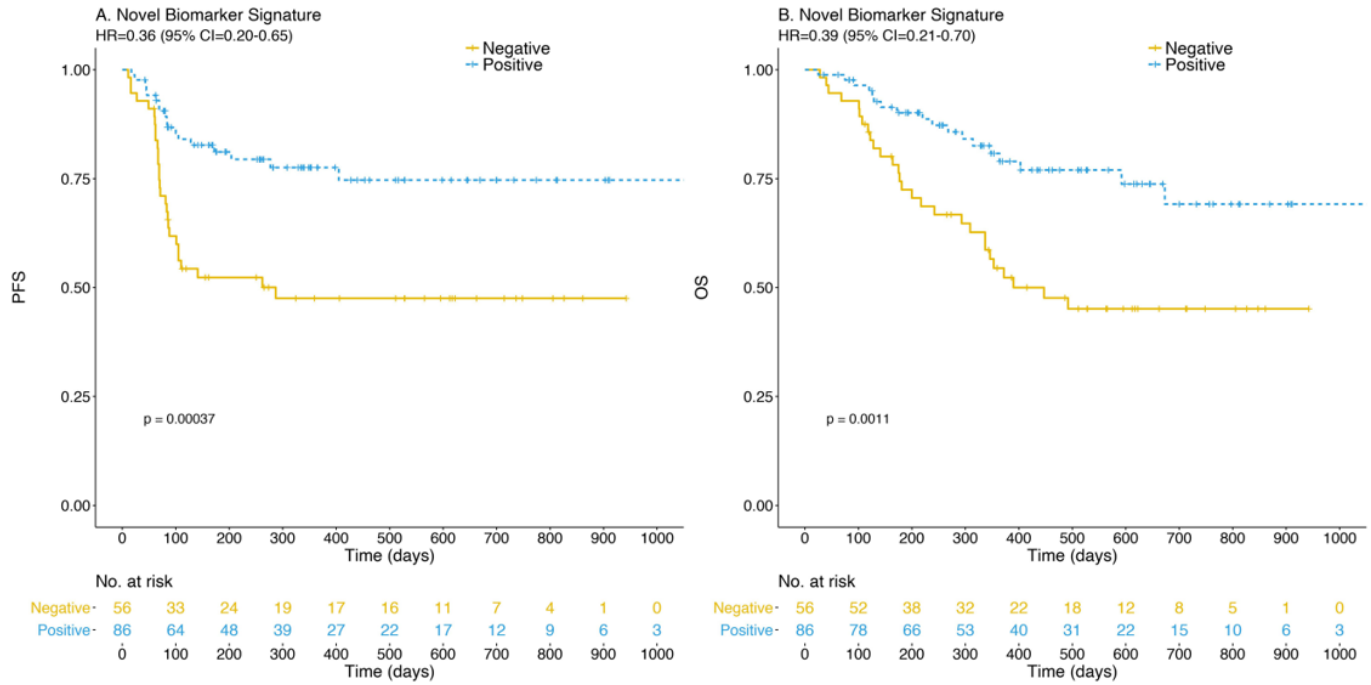
Mono, AUC = 0.77
(95% CI, 0.64–0.90)

Dual, AUC = **0.83**
(95% CI, 0.68–0.99)

Pok Fai Wong et al, in review

PD-L1/PD-1 interaction to predict response





Johnson, Bordeaux…Dakappagari, AACR 2016 and CCR in press

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- Expression (mRNA) signatures
- Multiplex Fluorescence
- cfDNA and other circulating markers (LIPI)

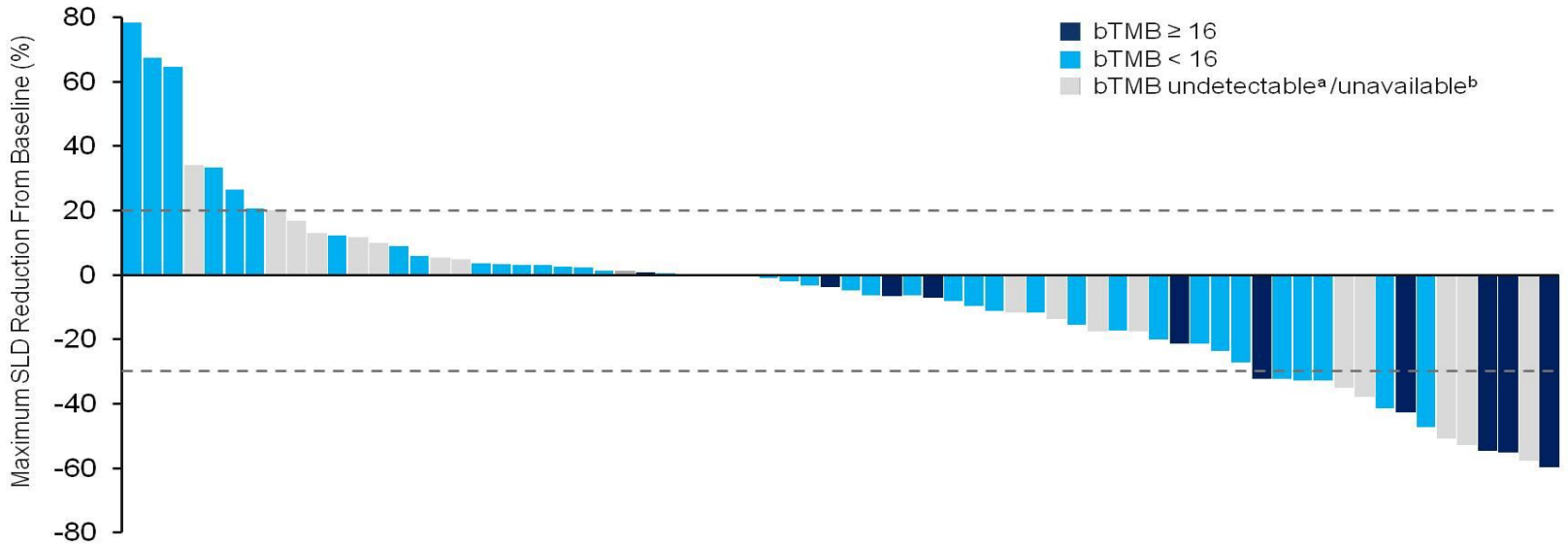
Prospective Clinical Evaluation of Blood-Based Tumor Mutational Burden (bTMB) as a Predictive Biomarker for Atezolizumab in 1L NSCLC: Interim B-F1RST Results

Vamsidhar Velcheti,[1] Edv
Phillip Stella,[5] Vincent Sh
Cindy Y

[1]Taussig Cancer Institute, Cleveland Clir
[3]Florida Hospital Cancer In
[5]St. Joseph Mercy Hospital

PRESENTED AT: 2018 ASCO ANNUAL MEETING #ASCO18

Maximum SLD Reduction From Baseline by bTMB Subgroup in the Interim Analysis Population



Only patients with post-baseline target lesion measurements are shown in plot (n = 70).
[a]15 patients had MSAF < 1%; [b]4 patients without valid sample.
Data cutoff: December 7, 2017.

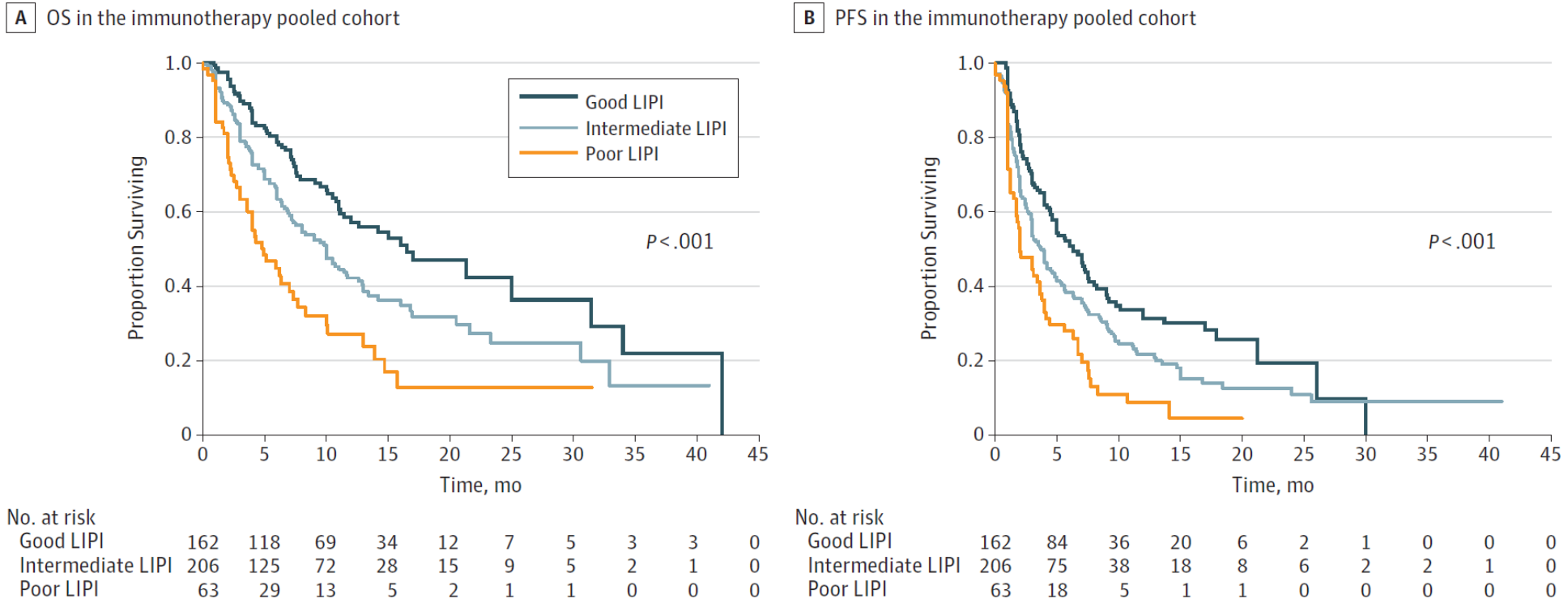
JAMA Oncol. doi:10.1001/jamaoncol.2017.4771
Published online January 11, 2018.

JAMA Oncology | **Original Investigation**

Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non–Small Cell Lung Cancer

Laura Mezquita, MD; Edouard Auclin, MD; Roberto Ferrara, MD; Melinda Charrier, PharmD, PhD; Jordi Remon, MD; David Planchard, MD, PhD; Santiago Ponce, MD; Luis Paz Ares, MD, PhD; Laura Leroy, MD; Clarisse Audigier-Valette, MD; Enriquet Pilar Garrido, MD, PhD; Solenn Brossea Caroline Caramela, MD; Jihene Lahmar, Jean Charles Soria, MD, PhD; Benjamin

Figure. Overall Survival (OS) and Progression-Free Survival (PFS) According to Lung Immune Prognostic Index (LIPI) Groups, in the Immunotherapy Pooled Cohort and in the Chemotherapy Cohort



Yale School of Medicine

Questions – during panel discussion