Predictive and Prognostic Biomarkers for IO: Value of TMB, MSI, PD-L1 and Others in Liquid and Solid Tumor Specimens

Alberto Chiappori, MD
Senior Member
Department of Thoracic Oncology
Moffitt Cancer Center

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Biomarkers
Definitions

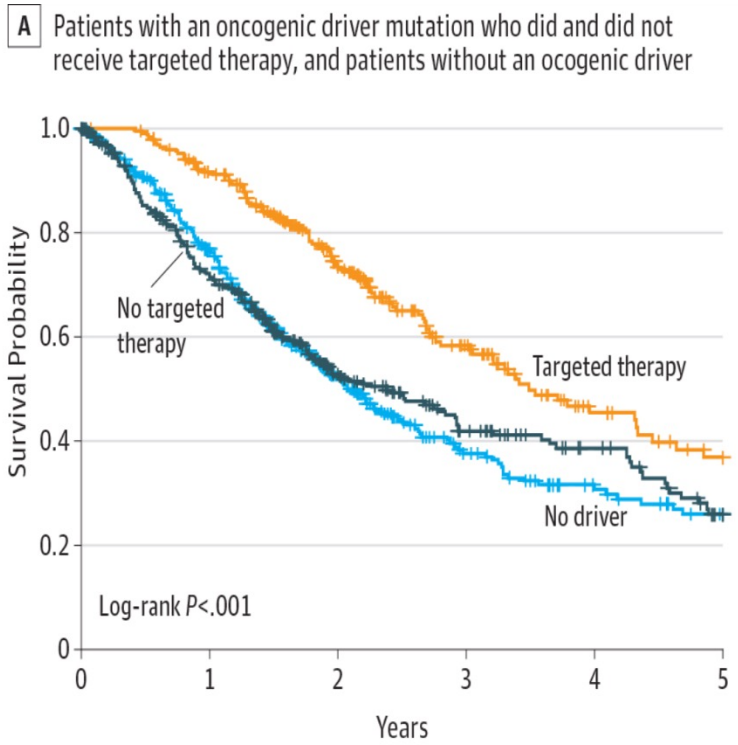
- **Prognostic** biomarkers are associated with the clinical outcome and are used to identify patien… …ows for the selection of "stronger" or more "aggressive" treatments (including higher toxicities).
- **Predictive** biomarkers are associated with the likelihood of response to a particular therapy and al… …fit from that therapy, thus sparing other patients from toxicities of ineffective therapies.
  - **PPV** positive predictive value is referring to the number of correctly predicted responders or survivors divided by the total number of patients with a positive biomarker result,
  - **NPV** negative predictive value is referring to the number of correctly predicted non-responders or non-survivors divided by the total number of patients with a negative biomarker result.

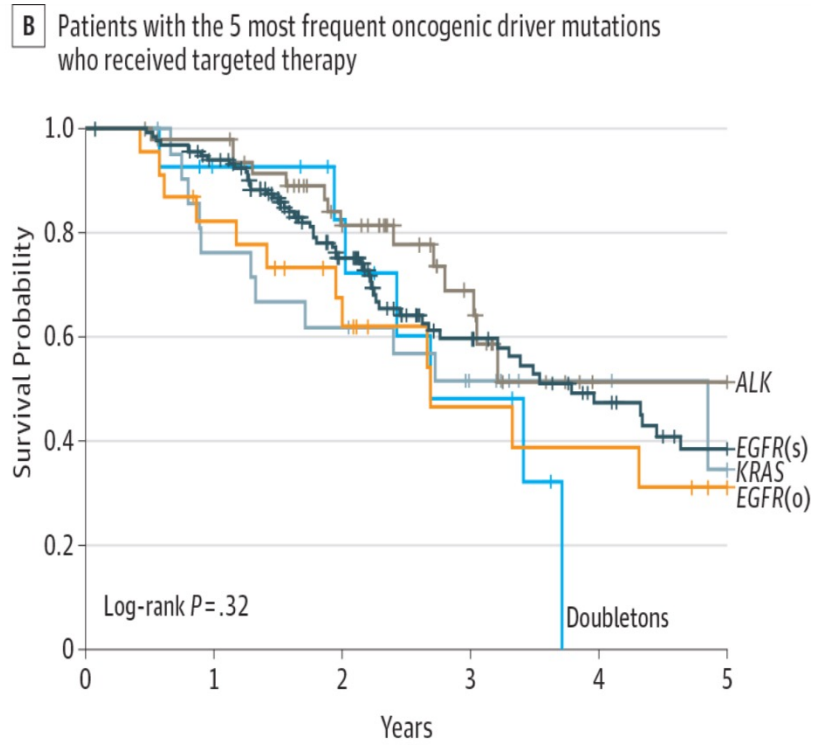
SELECT POPULATIONS
OUTCOME INDEPENDENT OF TREATMENT

SELECT TREATMENTS
OUTCOME DEPENDENT OF TREATMENT

Targeted Therapies

Oncogenic Drivers – Predictive Biomarkers





Driver Mutation identified Targeted Treatment Leads to Improved Survival

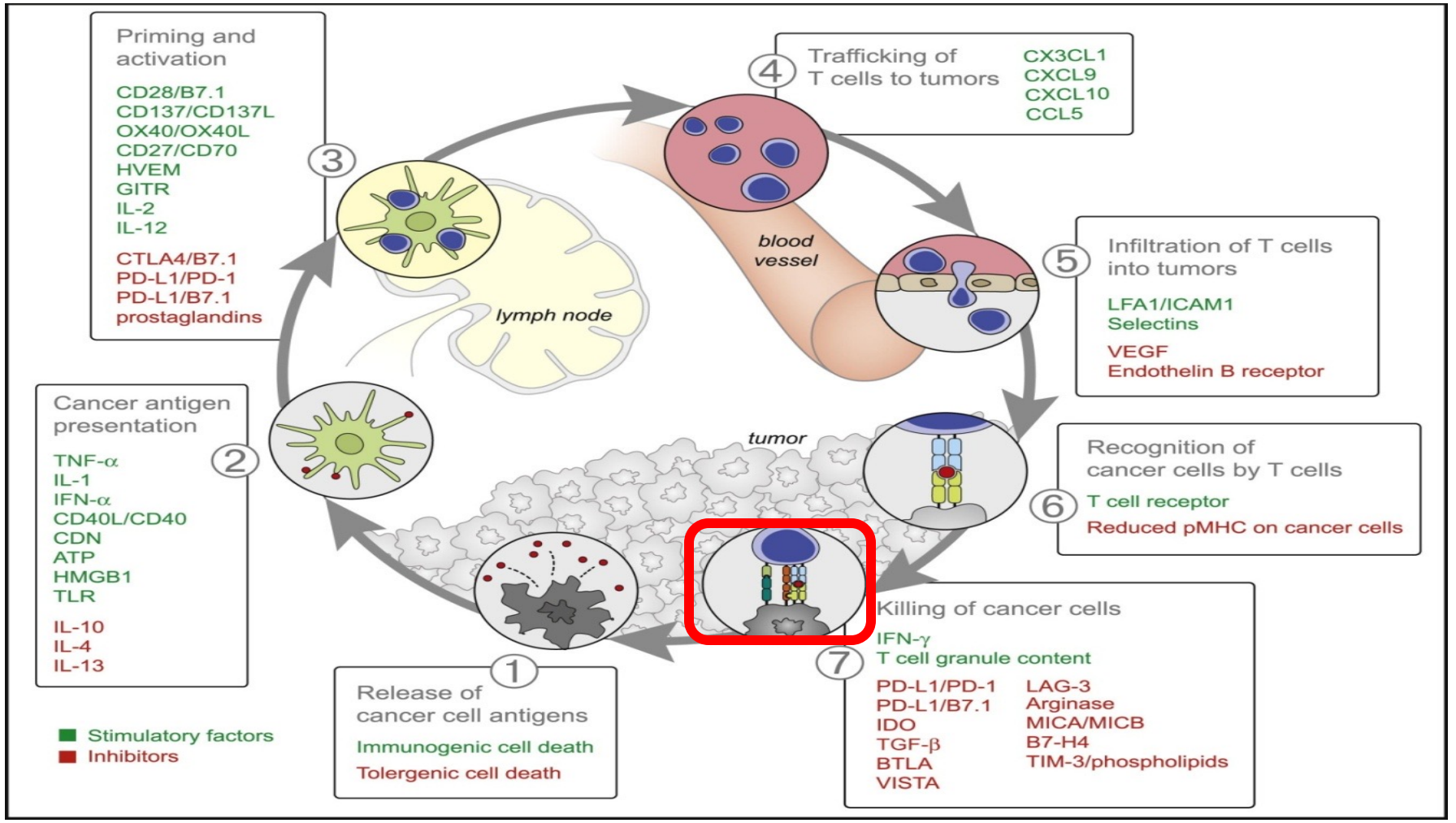
JAMA. 2014;311(19):1998-2006. doi:10.1001/jama.2014.3741

NSCLC

Prognostic and Predictive Biomarkers for Immunotherapy

Immunotherapy

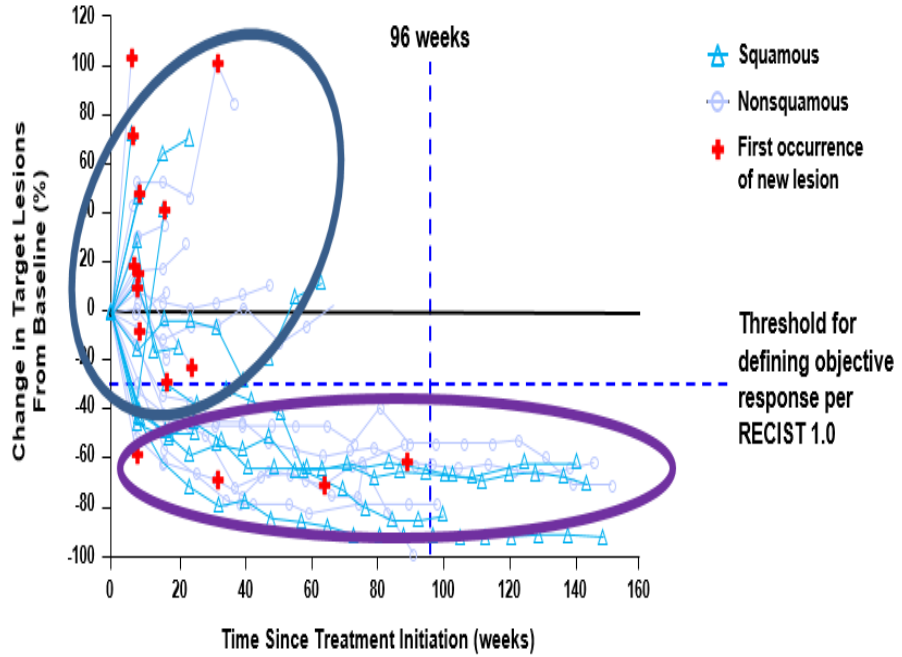
Anti-Tumor Immune Response



Chen DS, et al. Immunity. 2013;39:1-10.

Anti-Tumor Immune Response Identifiable NSCLC Sub-populations

Tumor burden kinetics in patients with NSCLC treated with Nivolumab 3 mg/kg (N=37)



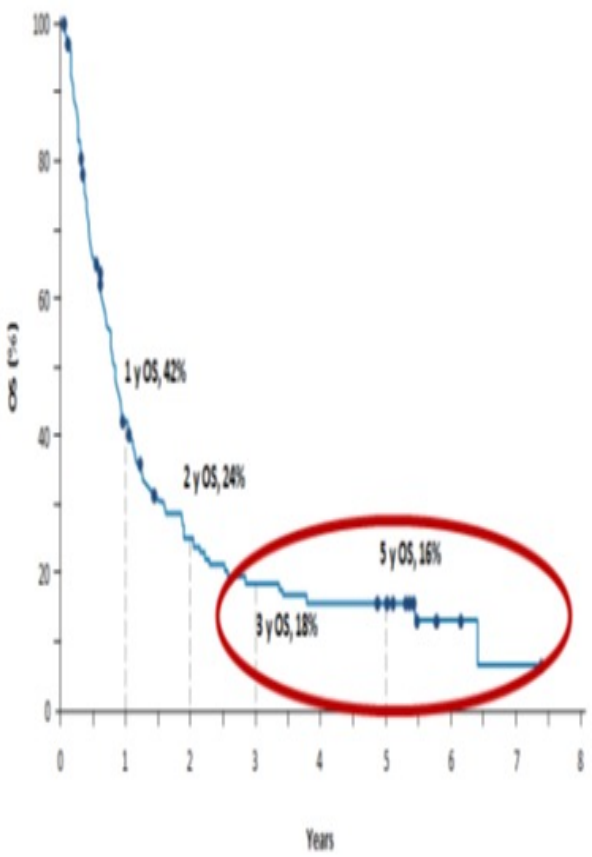
- Tumor burden was measured as sum of longest diameters of target lesions compared with baseline
  - Eleven (50%) responses were documented at the first 8-week tumor assessment

NSCLC = non small cell lung cancer; RECIST = response evaluation criteria in solid tumors.

Gettinger SN, et al. *J Clin Oncol*. 2015;33:2004–2012.

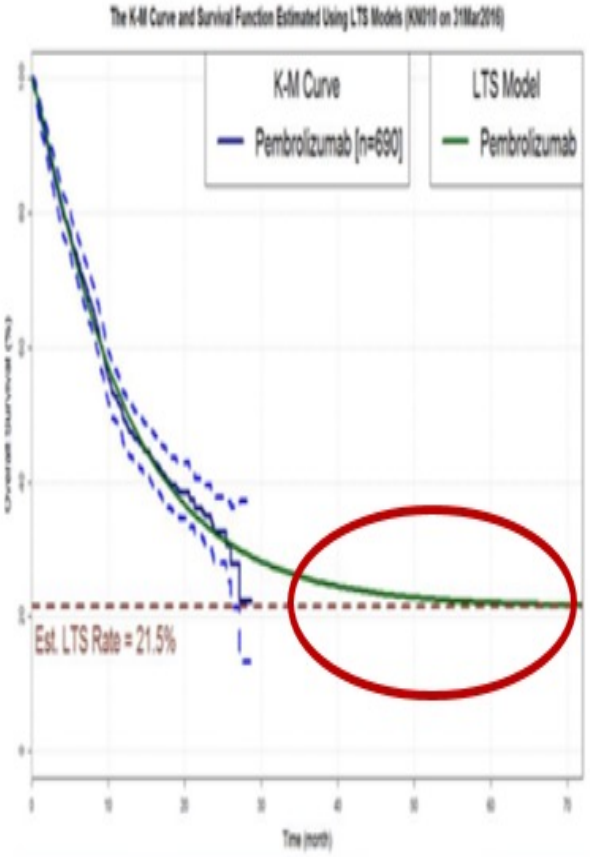
Nivolumab

Prolonged (5-year) Survival in ≥2nd Line NSCLC



Pembrolizumab

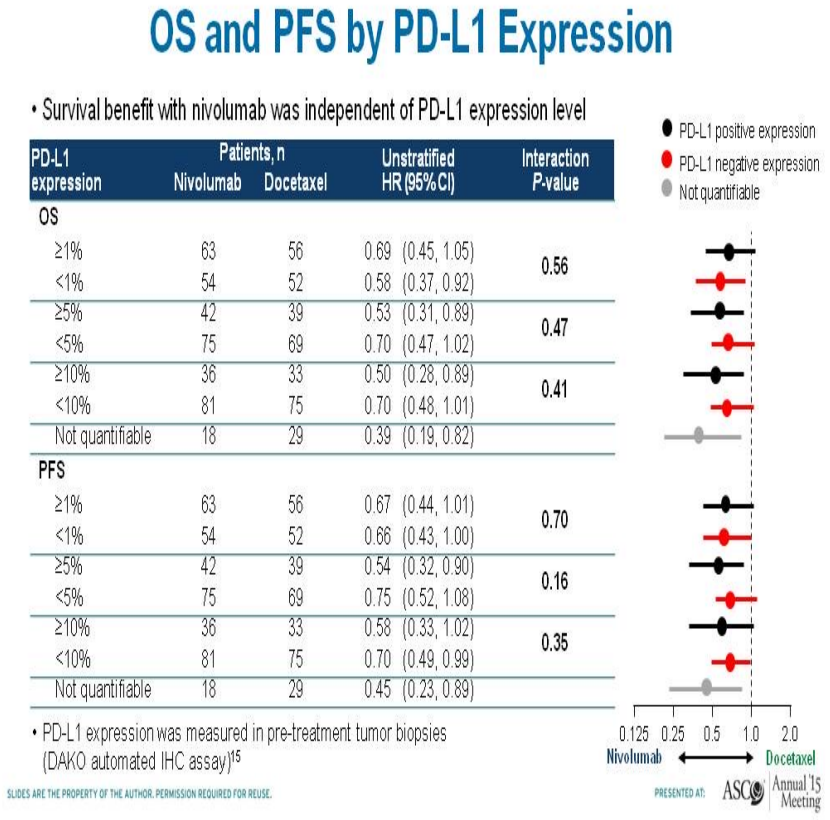
Prolonged (3-year) Survival in ≥2nd Line NSCLC



Clinical Biomarkers

Histology

Squamous - CheckMate 017



OS and PFS by PD-L1 Expression

• Survival benefit with nivolumab was independent of PD-L1 expression level

PD-L1 expression	Patients, n Nivolumab	Docetaxel	Unstratified HR (95% CI)	Interaction P-value
OS				
≥1%	63	56	0.69 (0.45, 1.05)	0.56
<1%	54	52	0.58 (0.37, 0.92)	
≥5%	42	39	0.53 (0.31, 0.89)	0.47
<5%	75	69	0.70 (0.47, 1.02)	
≥10%	36	33	0.50 (0.28, 0.89)	0.41
<10%	81	75	0.70 (0.48, 1.01)	
Not quantifiable	18	29	0.39 (0.19, 0.82)	
PFS				
≥1%	63	56	0.67 (0.44, 1.01)	0.70
<1%	54	52	0.66 (0.43, 1.00)	
≥5%	42	39	0.54 (0.32, 0.90)	0.16
<5%	75	69	0.75 (0.52, 1.08)	
≥10%	36	33	0.58 (0.33, 1.02)	0.35
<10%	81	75	0.70 (0.49, 0.99)	
Not quantifiable	18	29	0.45 (0.23, 0.89)	

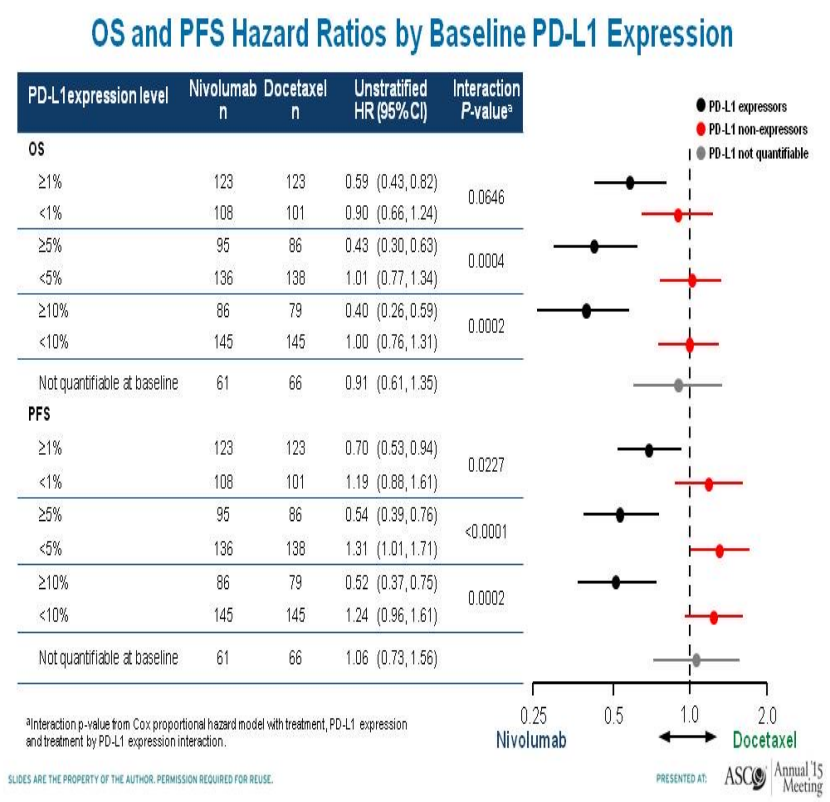
• PD-L1 expression was measured in pre-treatment tumor biopsies (DAKO automated IHC assay)[15]

PD-L1 positive expression
PD-L1 negative expression
Not quantifiable

0.125 0.25 0.5 1.0 2.0
Nivolumab ⟷ Docetaxel

PRESENTED AT: ASCO Annual '15 Meeting

Non-squamous - CheckMate 057



OS and PFS Hazard Ratios by Baseline PD-L1 Expression

PD-L1 expression level	Nivolumab n	Docetaxel n	Unstratified HR (95% CI)	Interaction P-value[a]
OS				
≥1%	123	123	0.59 (0.43, 0.82)	0.0646
<1%	108	101	0.90 (0.66, 1.24)	
≥5%	95	86	0.43 (0.30, 0.63)	0.0004
<5%	136	138	1.01 (0.77, 1.34)	
≥10%	86	79	0.40 (0.26, 0.59)	0.0002
<10%	145	145	1.00 (0.76, 1.31)	
Not quantifiable at baseline	61	66	0.91 (0.61, 1.35)	
PFS				
≥1%	123	123	0.70 (0.53, 0.94)	0.0227
<1%	108	101	1.19 (0.88, 1.61)	
≥5%	95	86	0.54 (0.39, 0.76)	<0.0001
<5%	136	138	1.31 (1.01, 1.71)	
≥10%	86	79	0.52 (0.37, 0.75)	0.0002
<10%	145	145	1.24 (0.96, 1.61)	
Not quantifiable at baseline	61	66	1.06 (0.73, 1.56)	

[a]Interaction p-value from Cox proportional hazard model with treatment, PD-L1 expression and treatment by PD-L1 expression interaction.

PD-L1 expressors
PD-L1 non-expressors
PD-L1 not quantifiable

0.25 0.5 1.0 2.0
Nivolumab ⟷ Docetaxel

PRESENTED AT: ASCO Annual '15 Meeting

[J Thorac Oncol. 2013; 8(6): 803–805.]

Clinical Biomarkers

Histology

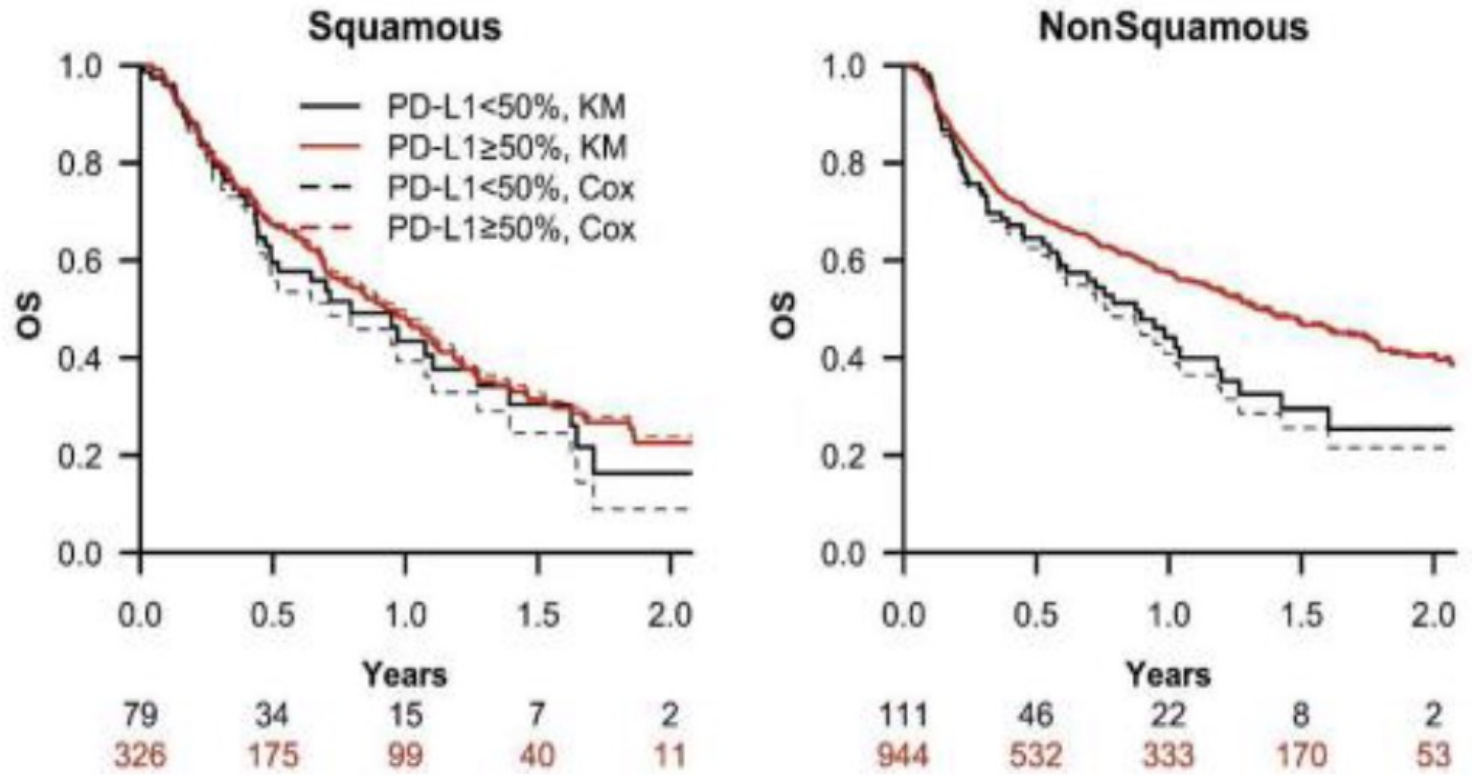
BRIEF REPORT

IASLC

Programmed Death-Ligand 1 Tumor Proportion Score and Overall Survival From First-Line Pembrolizumab in Patients With Nonsquamous Versus Squamous NSCLC

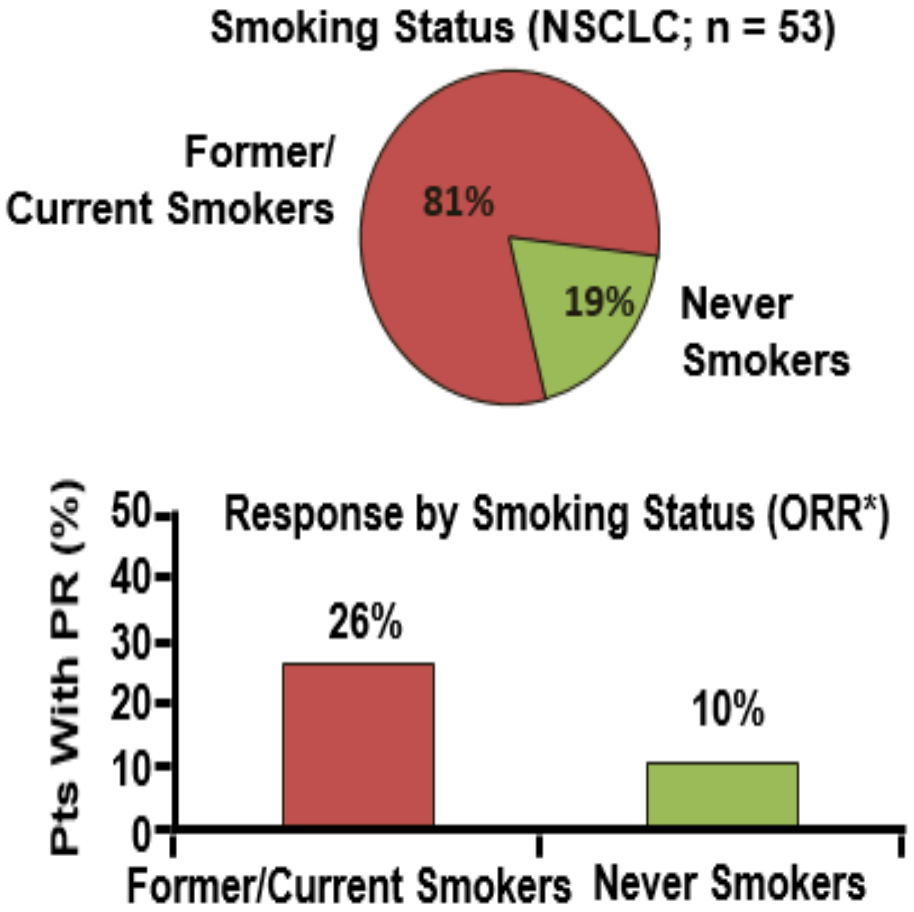
Deborah B. Doroshow, MD, PhD,[a,*] Wei Wei, MD, PhD,[b] Swati Gupta, PhD,[c] Jon Zugazagoitia, MD,[d] Charles Robbins, BS,[e] Blythe Adamson, PhD,[f] David L. Rimm, MD, PhD[e,g,h]

Journal of Thoracic Oncology Vol. 16 No. 12

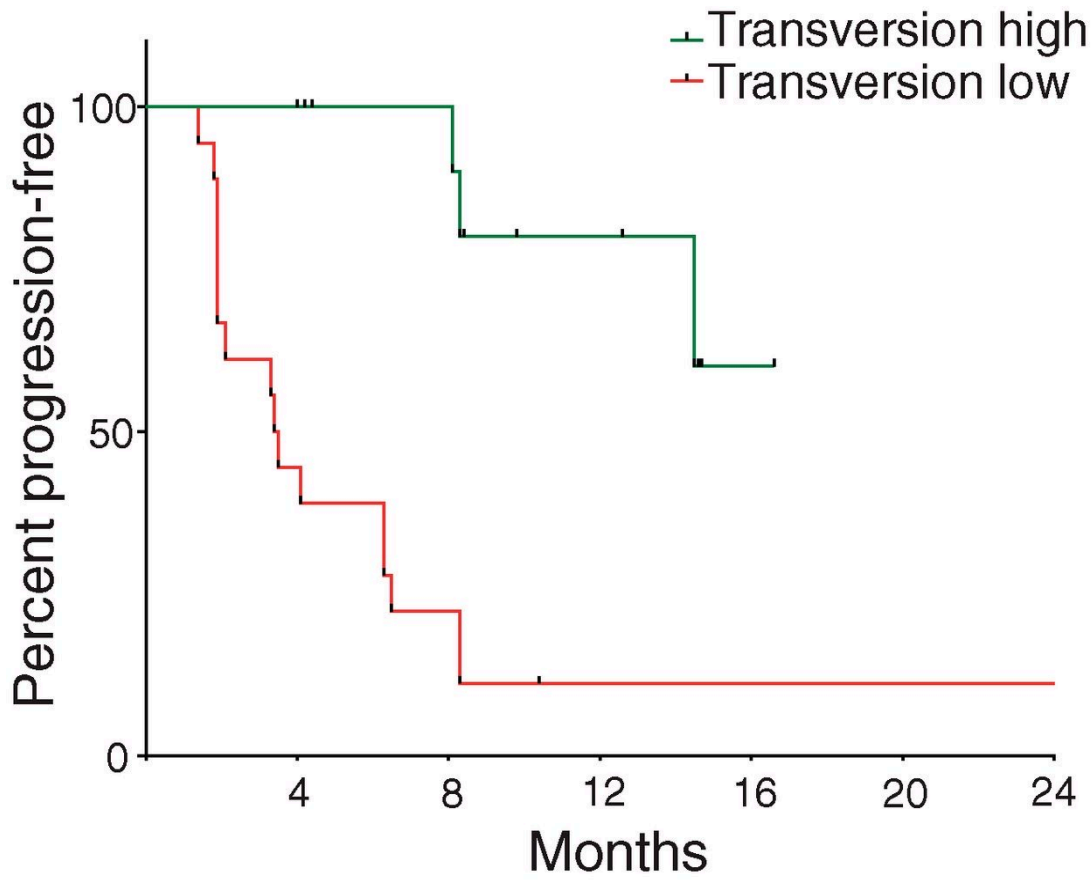


Clinical Biomarkers

Smoking status and Molecular smoking signature



Horn L, et al. WCLC 2013. Abstract MO18.



Molecular smoking signature is significantly associated with improved PFS in NSCLC patients treated with pembrolizumab.

Naiyer A. Rizvi et al. Science 2015;348:124-128

Clinical Biomarkers

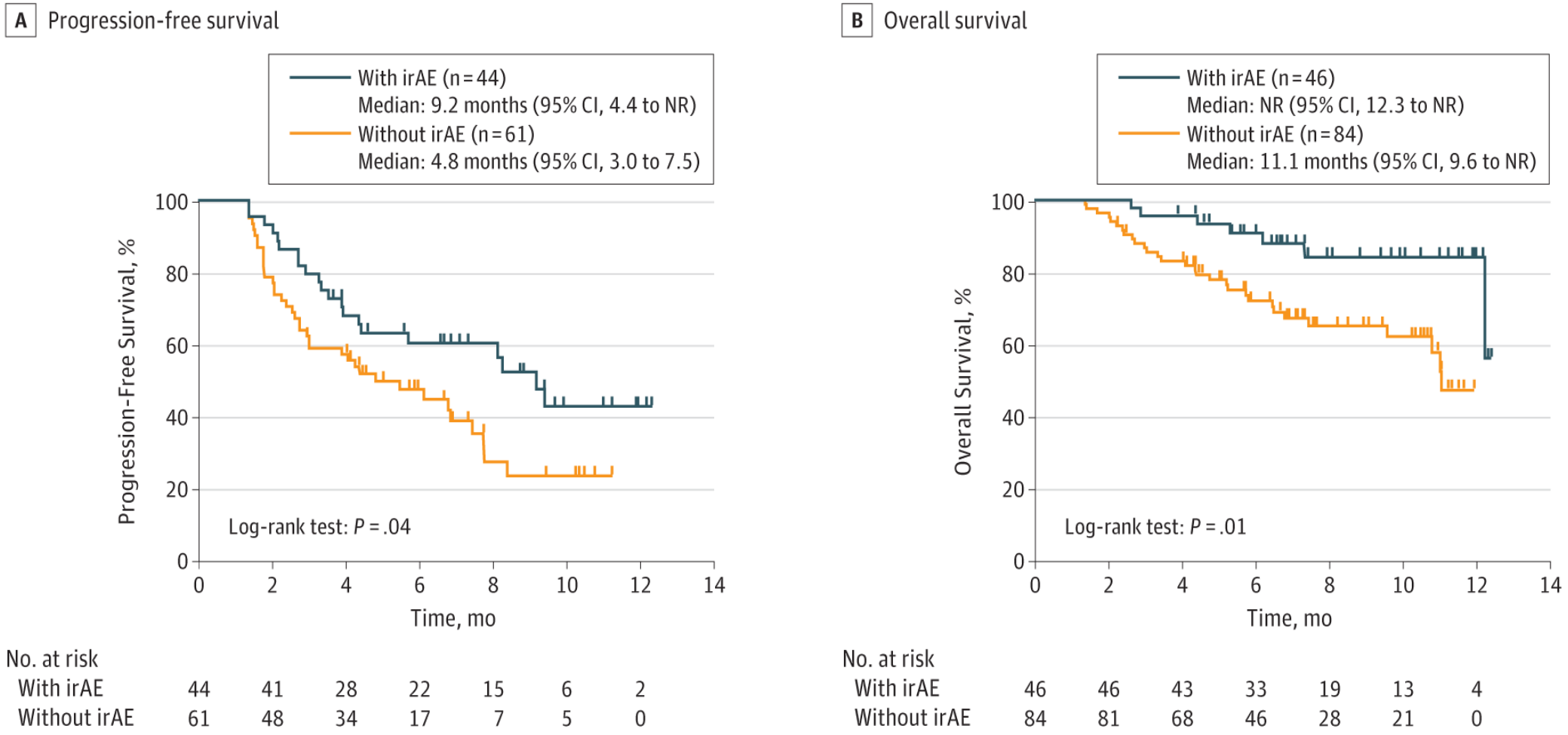
Immune Related Adverse Events

Study	n=	IO Drug	irAE (% of patients)	OS in irAE+ (months)	OS in irAE- (months)	PFS in irAE+ (months)	PFS in irAE- (months)	ORR in irAE+ (%)	ORR in irAE- (%)	Comments
Topalian et al [9]	129	Nivolumab	70.50%	11	7.5	–	–	23%	3%	
Fujimoto et al [46]	613	Nivolumab	10%	–	–	5.8	2.1	37%	18%	
Haratani et al [52]	134	Nivolumab	51%	Not reached	11.1	9.2	4.8	–	–	
Hasan Ali et al [21]	40	Nivolumab	17%	–	–	–	–	42%	7%	Skin Toxicity only
Osorio et al [22]	48	Pembrolizumab	21%	40	14	8	2	–	–	Thyroid toxicity only
Teraoka et al [47]	43	Nivolumab	44%	–	–	6.4	1.5	37%	17%	
Sato et al [49]	38	Nivolumab	28.90%	–	–	not reached (HR 0.10)	1.63	63.60%	7.40%	
Ricciuti et al [43]	195	Nivolumab	43.60%	17.80	4	8.50	2.00	43.50%	10%	
Cortellini et al [44]	559	Nivolumab Pembrolizumab	41.30%	20.50	8.50	10.10	4.10	46.50%	25.70%	
Campredon et al [25]	105	Nivolumab	14.30%	Please see comments		–	–	–	–	
Owen et al [45]	91	Nivolumab Pembrolizumab Atezolizumab	30%	24.30	5.3	–	–	–	–	
Toi et al [48]	70	Nivolumab	40%	–	–	12	3.6	57%	12%	
Ahn et al [50]	155	Nivolumab Pembrolizumab	38.1%	24.05	11.63	7.39	3.27	–	–	
Berner et al [53]	73	Nivolumab Pembrolizumab	34.2%	HR 0.29		HR 0.22		–	–	Skin toxicity only
Fukihara et al [26]	170	Nivolumab Pembrolizumab	16%	8.7	23	3.4	6.1	43%	25%	Pneumonitis only
Jin Lee et al [17]	211	Nivolumab Pembrolizumab	16.4%	HR 0.29		–	–	–	–	Skin Toxicity only
Peiro et al [18]	55	Nivolumab	14.6%	HR 0.40		–	–	–	–	Thyroid toxicity only
Sugano et al [51]	130	Nivolumab Pembrolizumab Atezolizumab	30%	–	–	15.9	3.3	63%	22%	

Cancer Treatment Reviews 92 (2021) 102134

Clinical Biomarkers

Immune Related Adverse Events



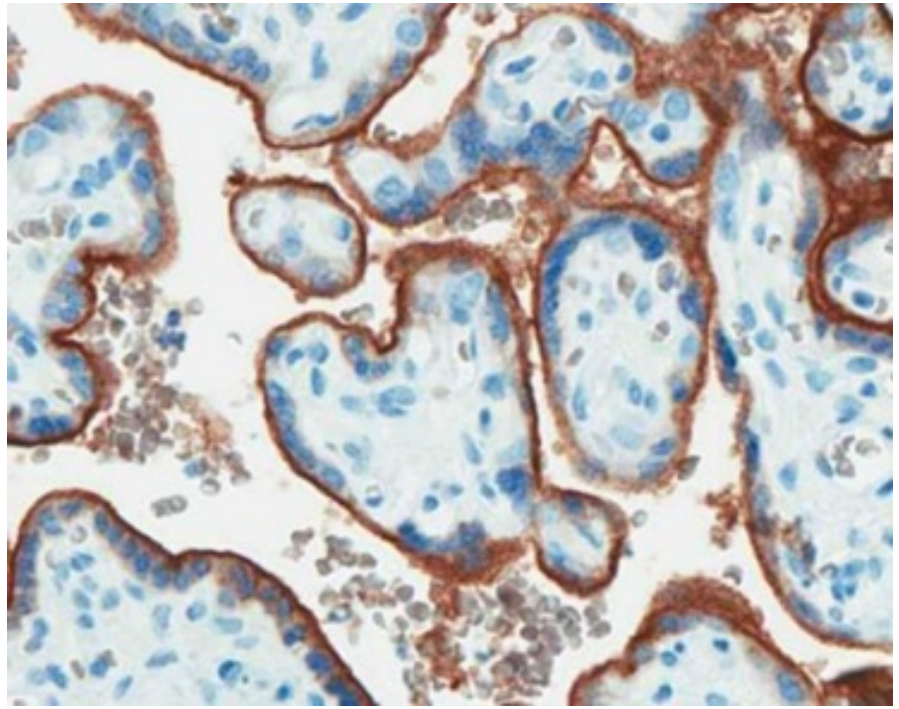
JAMA Oncol. 2018 Mar 1;4(3):374-378

PD-1/PD-L1 Pathway

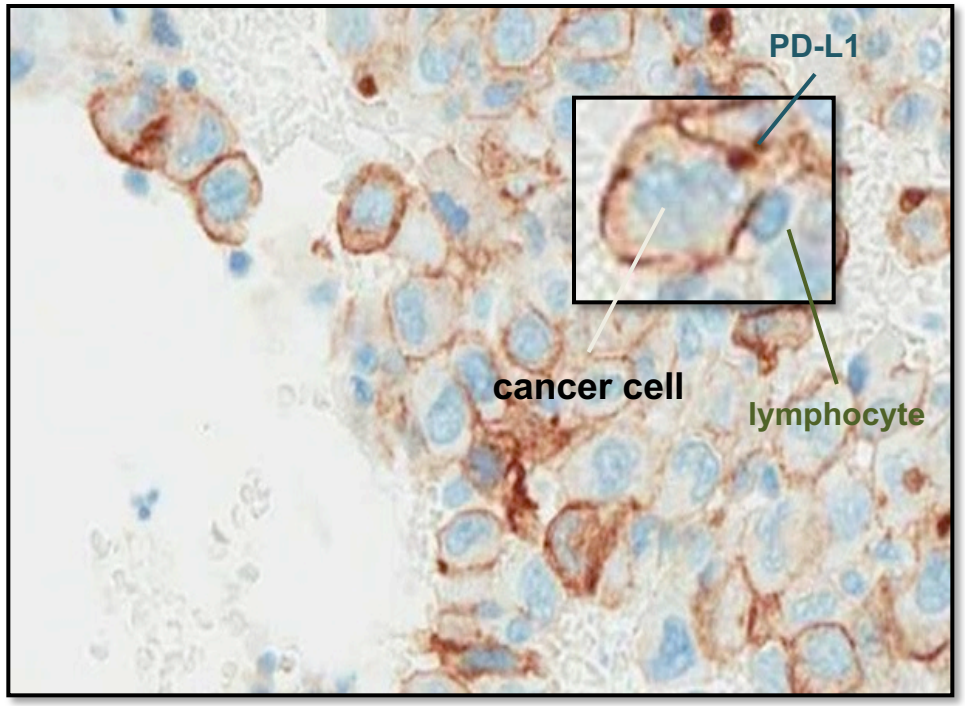
Effector Phase

Placenta and Tumors Express PD-L1 to Evade Immune Recognition

Placenta



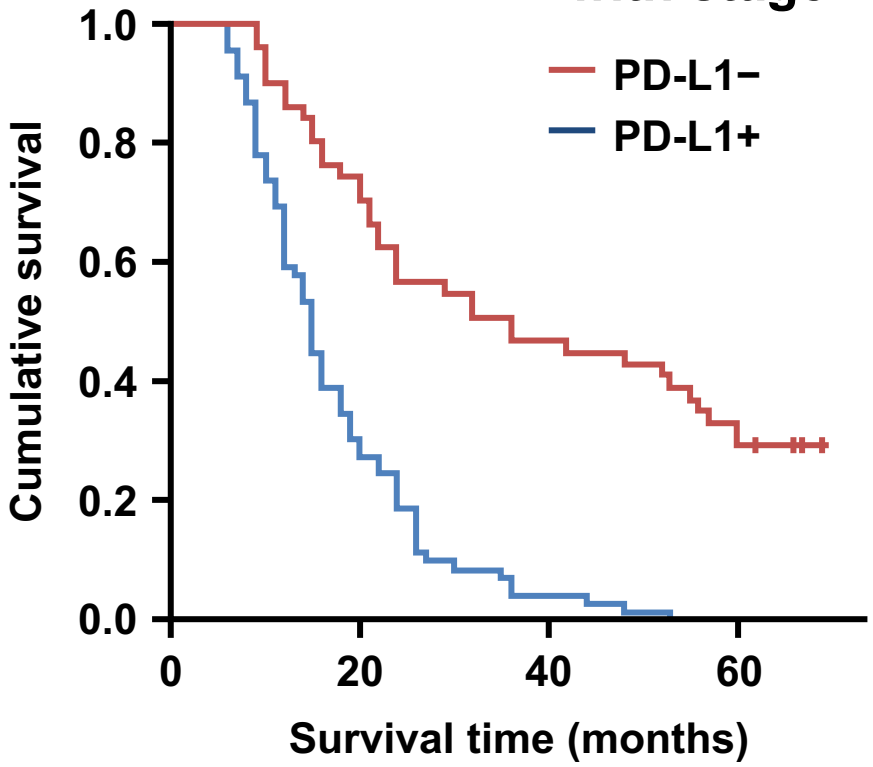
Tumor

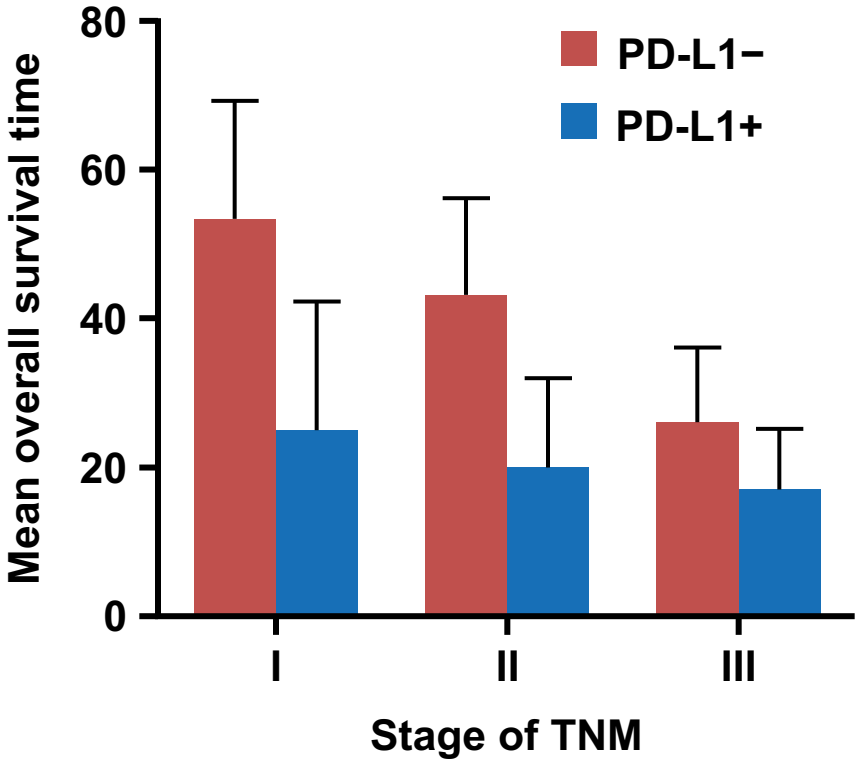


Biomarker

PD-L1 expression in NSCLC - Prognostic

No apparent differential PD-L1 expression with stage



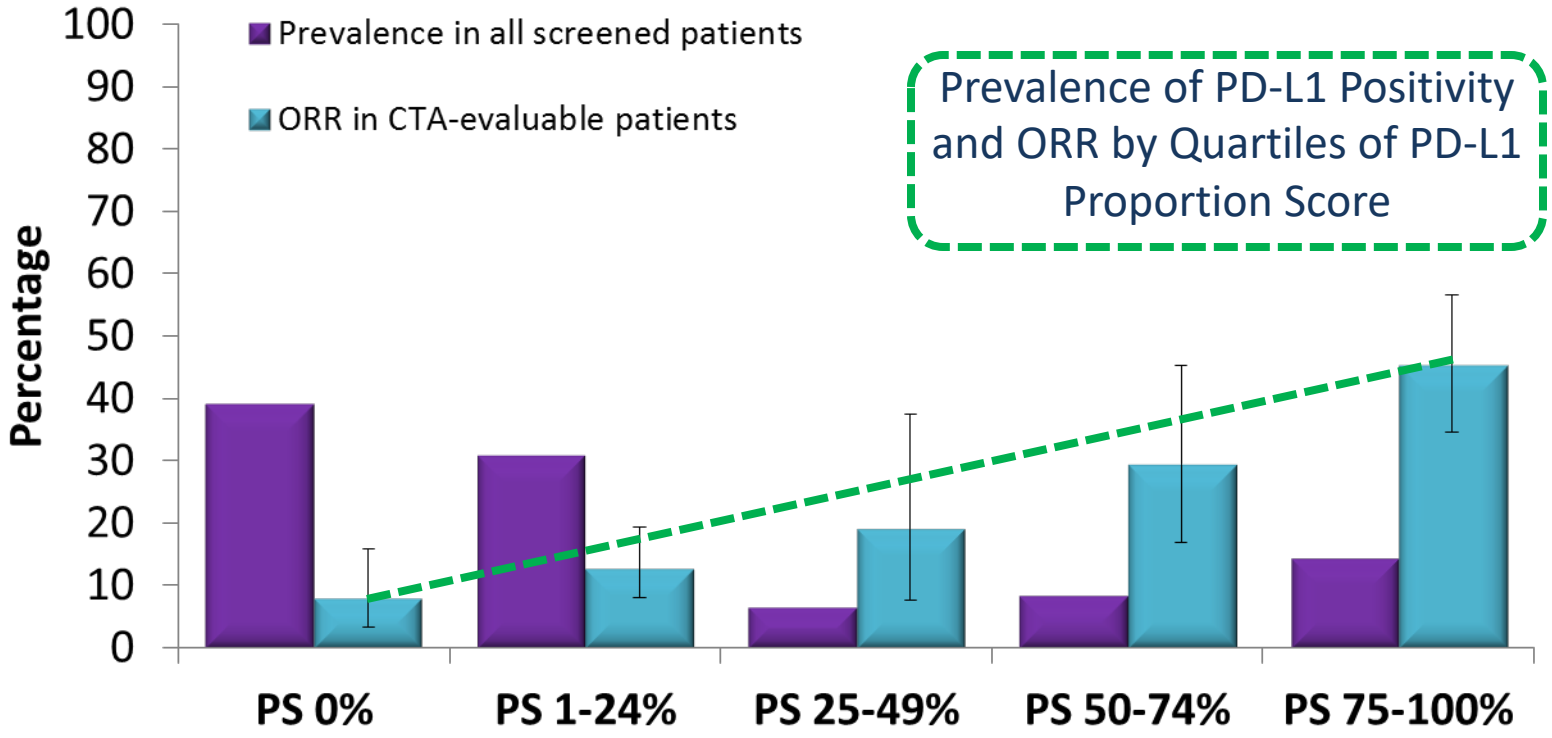


PD-L1 expression is a negative prognostic for patient survival

Chen, et al. Tumori 2012

PD-L1 expression - Predictive

Pembrolizumab



	PS 0%	PS 1-24%	PS 25-49%	PS 50-74%	PS 75-100%
Prevalence, all screened pts,[a] n (%)	323 (39.2)	255 (31.0)	55 (6.7)	71 (8.6)	120 (14.6)
ORR in CTA-evaluable pts, n (%) [95% CI]	7 (8.1) [3.3-15.9]	19 (12.9) [8.0-19.4]	6 (19.4) [7.5-37.5]	13 (29.6) [16.8-45.2]	39 (45.4) [34.6-56.5]

[a]Prevalence and ORR (RECIST v1.1 by central review) assessed in patients whose samples were evaluable by the CTA, regardless of the interval between cutting and staining.
Analysis cut-off date: August 29, 2014.

Garon EB et al, AACR 2015/NEJM 2015

Biomarker

PD-L1 expression - Predictive

Author	Phase	Disease Type	Drug	Treatment Line	PD-L1Cut-Off (%)	PD-L1 Subgroup	Sample size	ORR (%)
Weber et al, JCO 2013 [39]	I	Melanoma	Nivolumab	≥2°	5	Positive	12	67.0
						Negative	32	19.0
Hamid et al, ASCO 2013 [33]	I	Melanoma	MPDL3280A	Mixed	5	Positive	15	27.0
						Negative	15	20.0
Hodi et al, ASCO 2014 [35]	I	Melanoma	Nivolumab	≥2°	5	Positive	18	44.0
						Negative	23	13.0
Sznol et al, ASCO 2014 [38]	I	Melanoma	Nivolumab	≥2°	5	Positive	22	35.0
						Negative	57	59.0
Robert et al, NEJM 2015 [24]	III	Melanoma	Nivolumab	1°	5	Positive	74	52.7
						Negative	136	33.1
Weber et al, LO 2015 [10]	III	Melanoma	Nivolumab	≥2°	5	Positive	55	43.6
						Negative	64	20.3
Kefford et al, ASCO 2014 [36]	I	Melanoma	Pembrolizumab	Mixed	1	Positive	83	49.0
Rizvi et al, CMSTO 2014 [43]	I	NSCLC	Nivolumab	1°	5	Positive	26	31.0
						Negative	21	10.0
Antonia et al, CMSTO 2014 [44]	I	NSCLC	Nivolumab	1°	5	Positive	16	19.0
						Negative	22	14.0
Herbst et al, Nature 2014 [34]	I	NSCLC	MPDL3280A	≥2°	5	Positive	9	27.0
						Negative	37	24.0
Gettinger et al, JCO 2015 [45]	I	NSCLC	Nivolumab	≥2°	5	Positive	33	15.0
						Negative	35	14.0
Rizvi et al, LO 2015 [11]	II	NSCLC	Nivolumab	≥2°	5	Positive	25	24.0
						Negative	51	14.0
Garon et al, NEJM 2015 [46]	I	NSCLC	Pembrolizumab	≥2°	1	Positive	159	23.0
						Negative	35	9.0
Rizvi et al, ASCO 2014 [37]	I	NSCLC	Pembrolizumab	1°	1	Positive	42	26.0
Cho et al, ASCO 2013 [42]	I	GU	MPDL3280A	Mixed	5	Positive	10	20.0
						Negative	21	10.0
Powles et al, Nature 2014 [15]	I	GU	MPDL3280A	≥2°	5	Positive	7	29.0
						Negative	58	26.0
Motzer et al, JCO 2014 [14]	II	GU	Nivolumab	≥2°	5	Positive	29	31.0
						Negative	78	18.0
Choueiri et al, ESMO 2014 [32]	I	GU	Nivolumab	Mixed	5	Positive	18	22.0
						Negative	38	8.0
Hammers et al, ESMO 2014 [40]	I	GU	Nivolumab	Mixed	1	Positive	16	50.0
						Negative	20	55.0
Plimack et al, ESMO 2014 [41]	I	GU	Pembrolizumab	Mixed	1	Positive	33	24.1

PD-L1: programmed death-ligand-1; ORR: overall response rate; NSCLC: non-small cell lung cancer.

doi:10.1371/journal.pone.0130142.t001

Table 1. Trials' Characteristics (selected arms for the analysis).

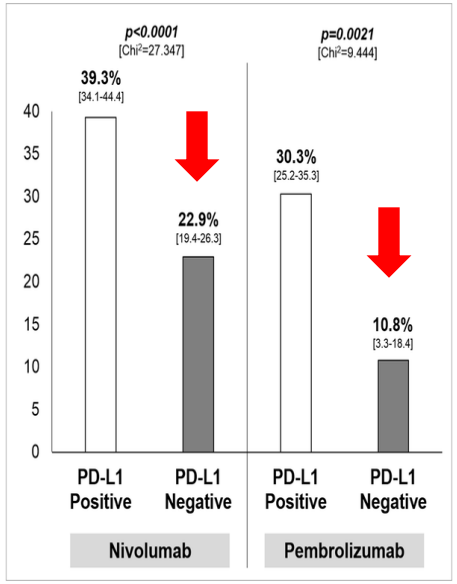
PLOS ONE | TENTH ANNIVERSARY

Carbognin L, et al. PLOS ONE 10(6): e0130142. https://doi.org/10.1371/journal.pone.0130142

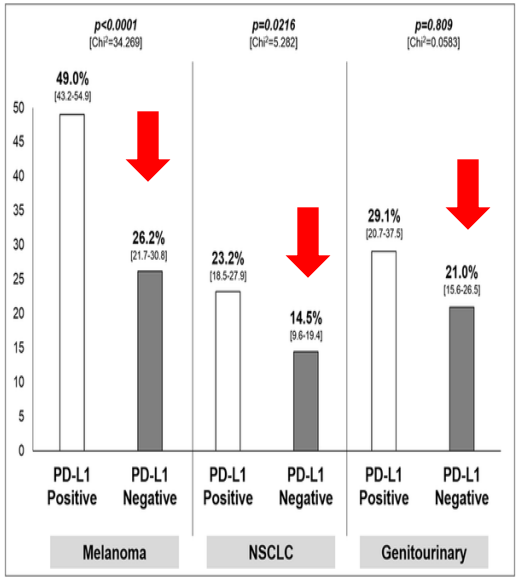
PD-L1 Biomarker

Negative Expression

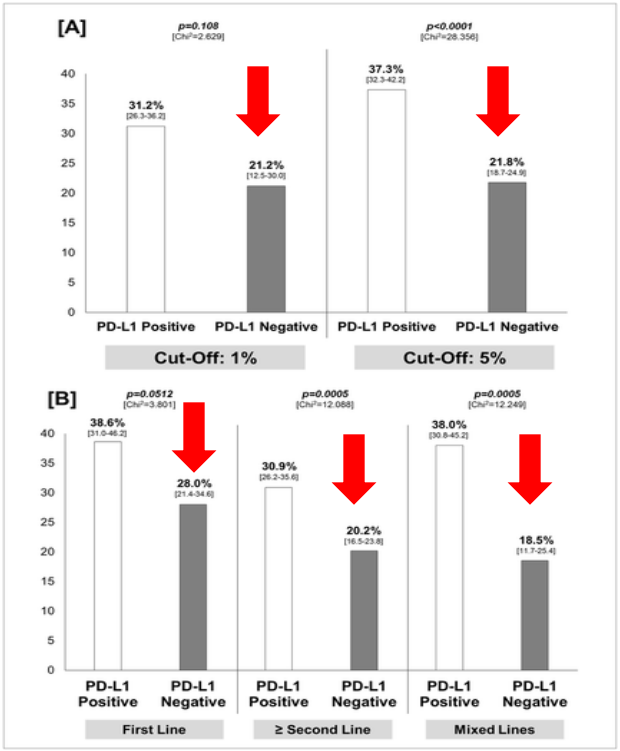
Overall response rate, according to adopted drug.



Overall response rate, according to tumor type.



Sensitivity Analysis



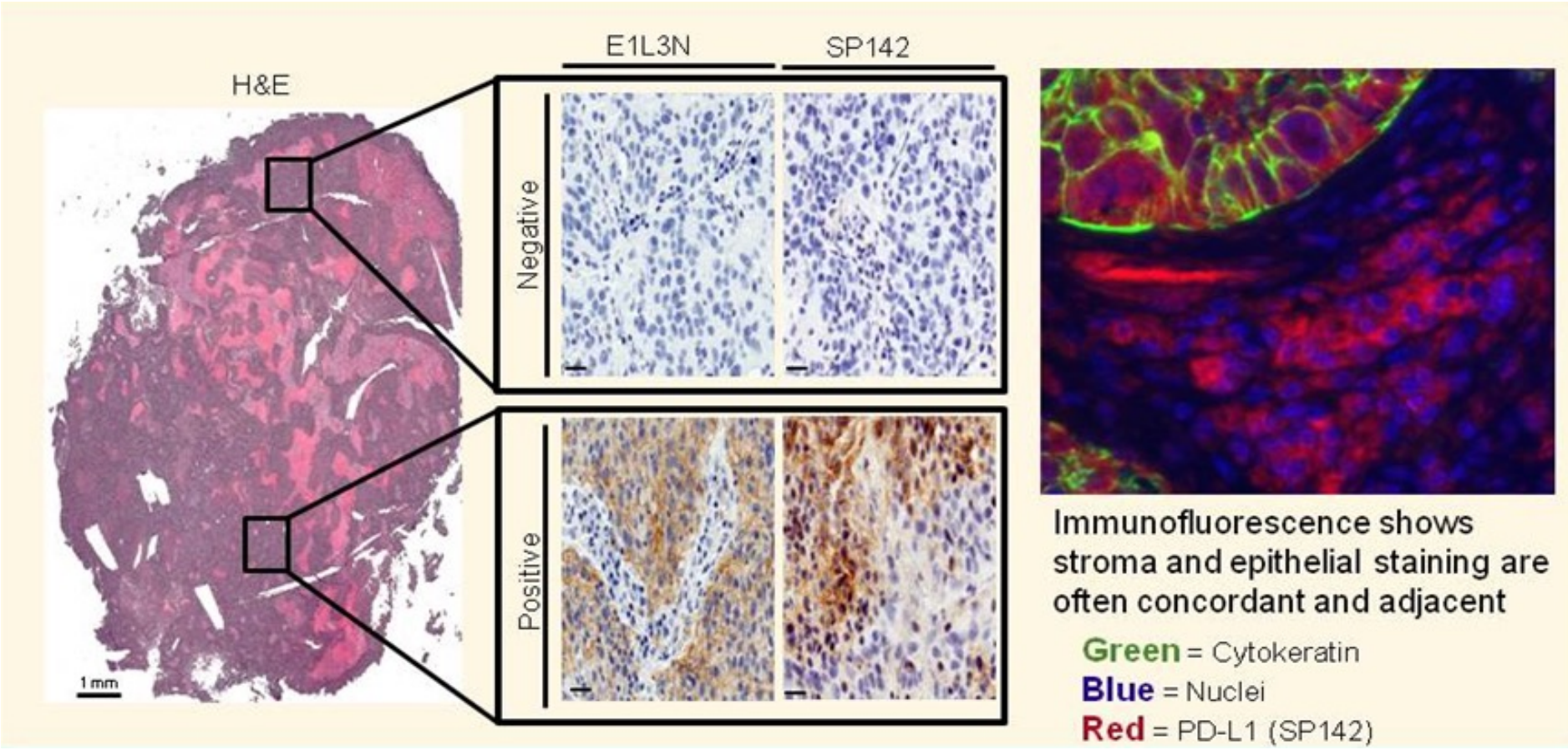
Carbognin L, et al. PLOS ONE 10(6): e0130142.
https://doi.org/10.1371/journal.pone.0130142

PLOS ONE
TENTH ANNIVERSARY

Biomarker

PD-L1 expression in NSCLC

Expression of PD-L1 is heterogeneous and varies with antibody



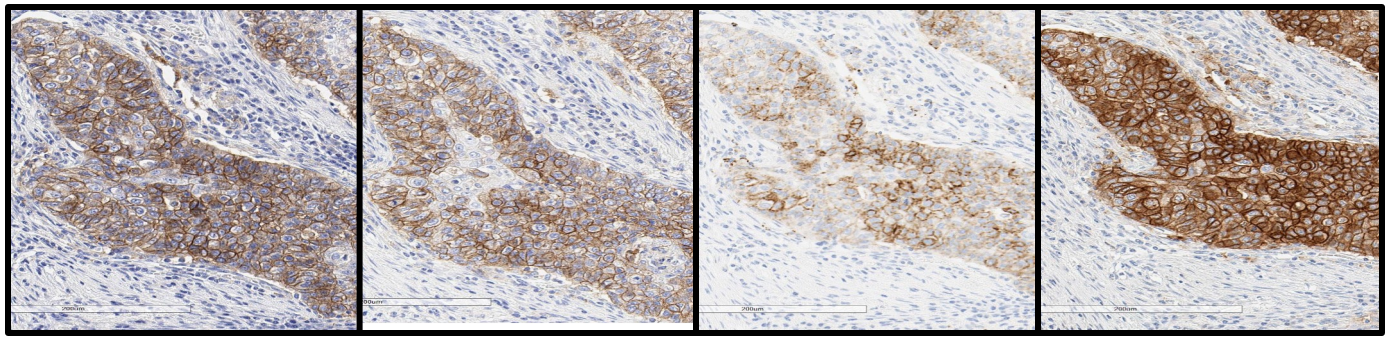
Presented By Roy Herbst at 2015 ASCO Annual Meeting

PD – L1 Biomarker

IASLC 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				

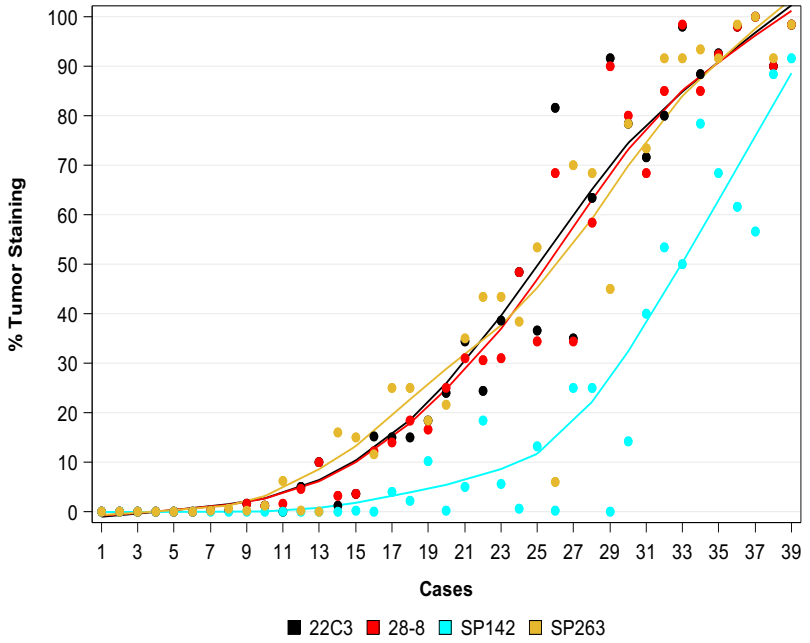
J Thorac Oncol. 2017 Feb;12(2):208-222



PD-L1 Biomarker

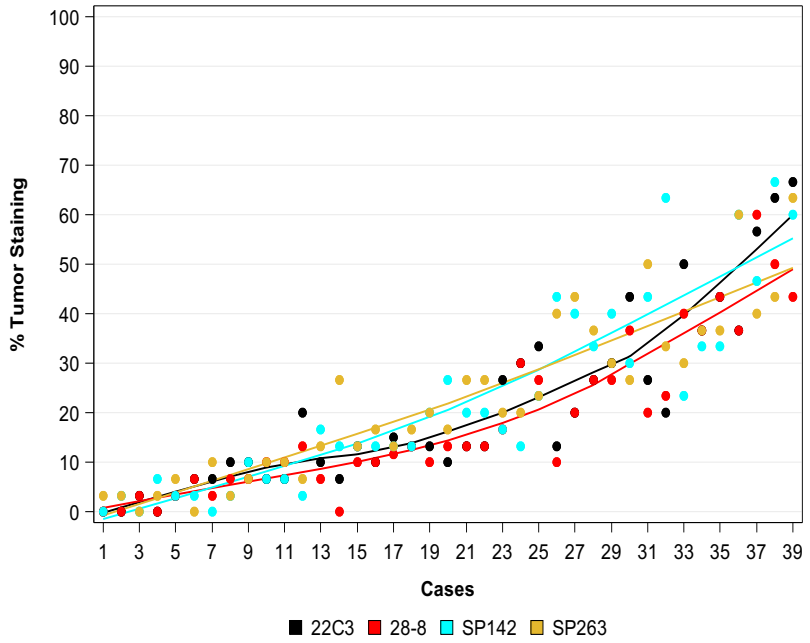
Agreement Rates: IHC PD-L1 Assays

Analytical comparison of % tumor cell staining (TPS) by case for each assay



Three assays (22C3, 28-8, SP263) demonstrate similar analytical performance with respect to percentages of tumor cells positive and dynamic range

Analytical comparison of % immune cell staining (ICPS) by case for each assay

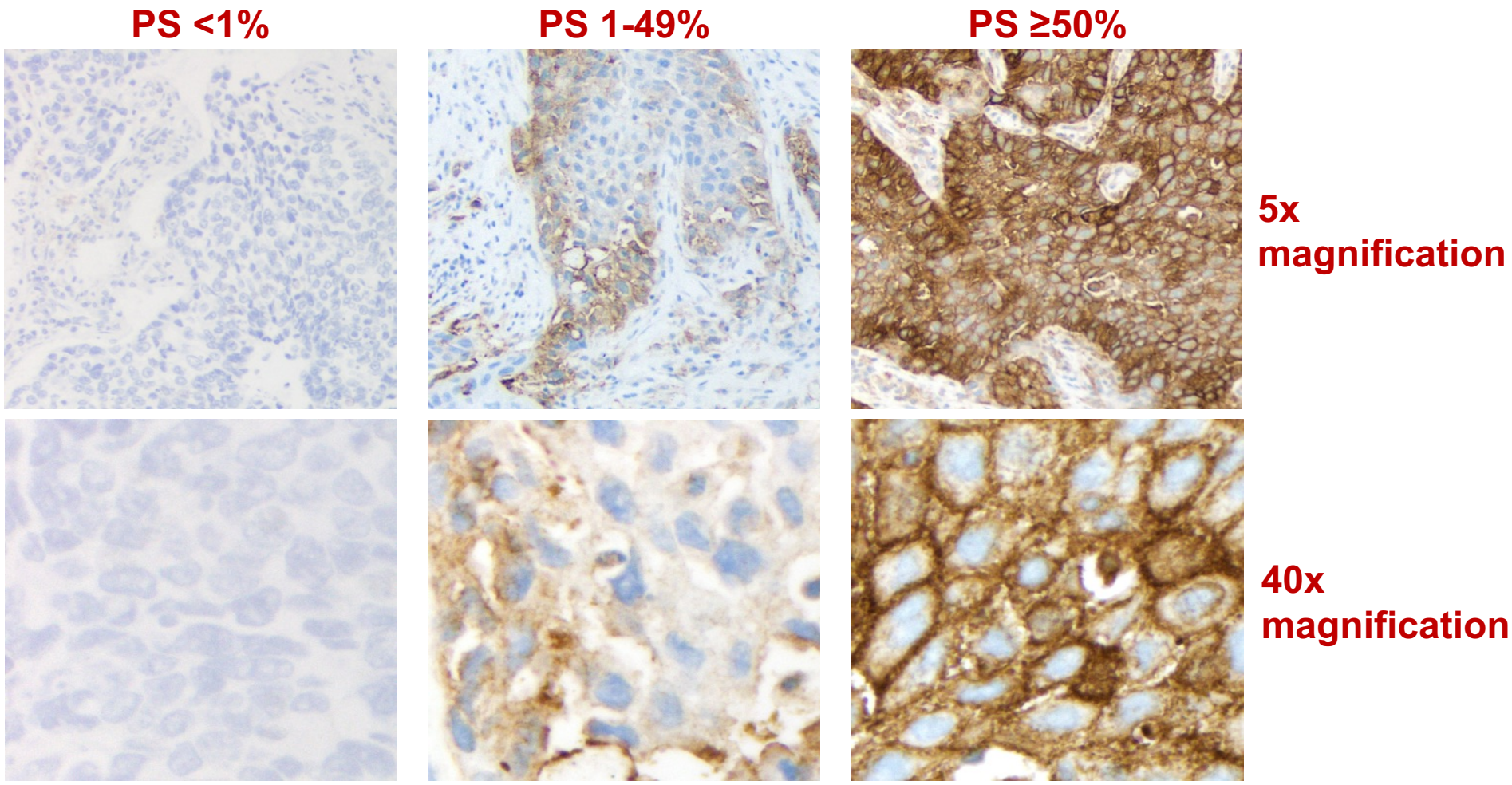


All assays label immune cells but there is less precision in analytical performance than with tumor cells

J Thorac Oncol. 2017 Feb;12(2):208-222

Biomarker

PD-L1 expression in NSCLC



Brown chromogen: PD-L1 staining.
Blue color: hematoxylin counterstain.

Garon EB et al, AACR 2015/NEJM 2015

Biomarker
PD-L1 selection - Trials

KEYNOTE-024

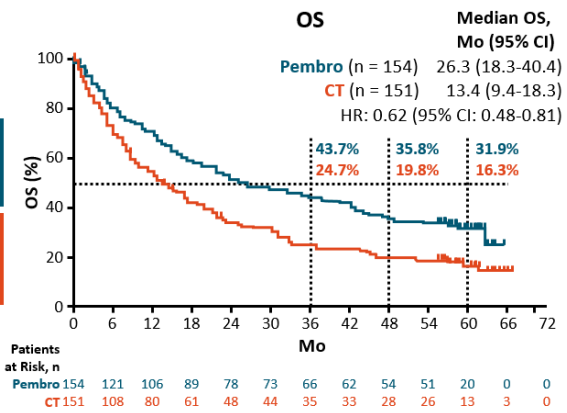
- Open-label, randomized phase III study

Stratified by ECOG PS (0 vs 1), histology (squamous vs nonsquamous), and enrollment site

Chemotherapy-naive, stage IV NSCLC; PD-L1 selected (TPS ≥50%*); ECOG PS 0/1; no *EGFR/ALK* mutations; no untreated CNS mets or autoimmune disease requiring tx (N = 305)

- **Pembrolizumab** 200 mg IV Q3W for up to 35 cycles (n = 154)
- **Platinum-Doublet CT** (histology based) for 4-6 cycles (n = 151)

*Staining of ≥50% tumor cells by 22C3 companion diagnostic IHC assay.



Reck. JCO. 2021;21:2339.

EMPOWER-Lung 1

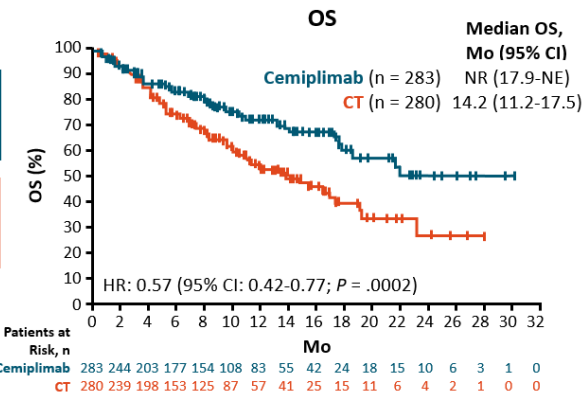
- Randomized phase III study

Stratified by histology (squamous vs nonsquamous) and region (Europe, Asia, vs ROW)

Treatment naive advanced NSCLC, PD-L1 ≥50%*; no *EGFR/ALK/ROS1* mutations, ECOG PS 0/1 (N = 563†)

- **Cemiplimab** 350 mg Q3W Until PD or 108 wk
- **Platinum-Doublet CT** (histology based) for 4-6 cycles

*Staining of ≥50% tumor cells using 22C3 companion diagnostic IHC assay.
†710 patients enrolled in overall ITT population.



Sezer. Lancet. 2021;397:592.

IMpower110

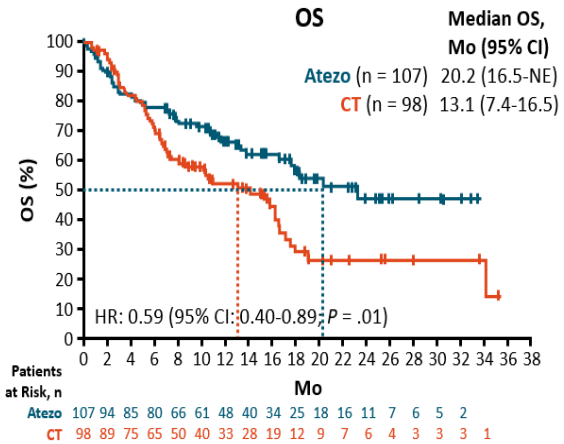
- Randomized phase III study

Stratified by sex, ECOG PS, histology, and tumor PD-L1 status

Untreated LA or metastatic NSCLC of any histology; PD-L1 ≥50% on TC or 10% on IC*; no sensitizing *EGFR* or *ALK* alterations; ECOG PS 0/1; no untreated or unstable CNS mets or pneumonitis requiring tx (N = 205†)

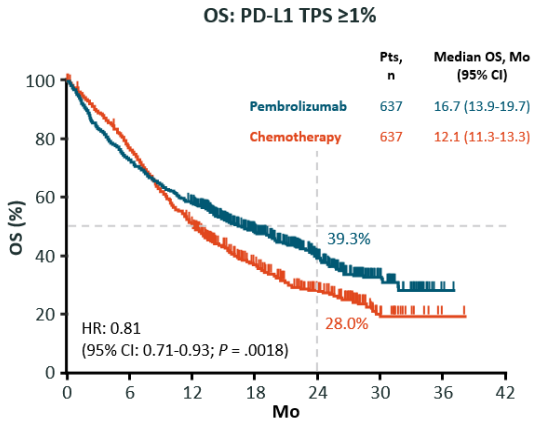
- **Atezolizumab** 1200 mg Q3W until PD (n = 107)
- **Platinum-Doublet CT** (histology based) for 4-6 cycles (n = 98)

*Staining of ≥50% tumor cells (TC3) or ≥10% tumor-infiltrating immune cells (IC3) using SP142 complementary diagnostic IHC assay.
†572 patients with PD-L1 ≥1% on TC or IC enrolled overall.



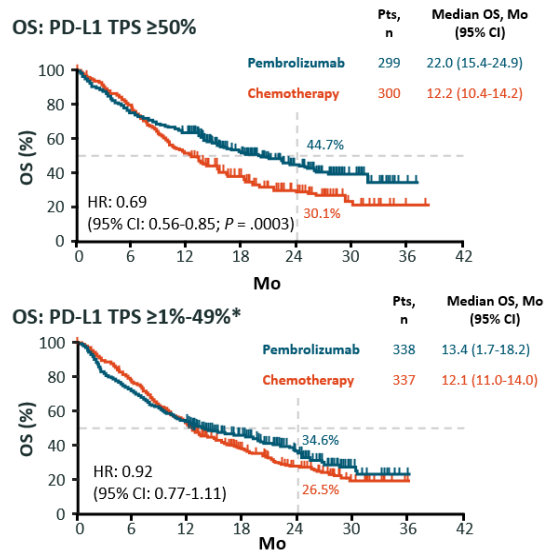
Herbst. NEJM. 2020;383:1328.

KEYNOTE-042 - PD-L1+



Mok. Lancet. 2019;393:1819.

*Exploratory analysis; no alpha allocated to this comparison.



Pembrolizumab – First Line
Responses by (very) High PD-L1 Expression Level

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 30: 1653–1659, 2019
doi:10.1093/annonc/mdz288
Published online 21 August 2019

ORIGINAL ARTICLE

Outcomes to first-line pembrolizumab in patients with non-small-cell lung cancer and very high PD-L1 expression

E. J. Aguilar[1†], B. Ricciuti[1†], J. F. Gainor[2], K. L. Kehl[1], S. Kravets[3], S. Dahlberg[3], M. Nishino[4], L. M. Sholl[5], A. Adeni[1], S. Subegdjo[1], S. Khosrowjerdi[2], R. M. Peterson[2], S. Digumarthy[2], C. Liu[6], J. Sauter[7], H. Rizvi[8], K. C. Arbour[8], B. W. Carter[9], J. V. Heymach[10], M. Altan[10], M. D. Hellmann[8,11] & M. M. Awad[1*]

Annals of Oncology

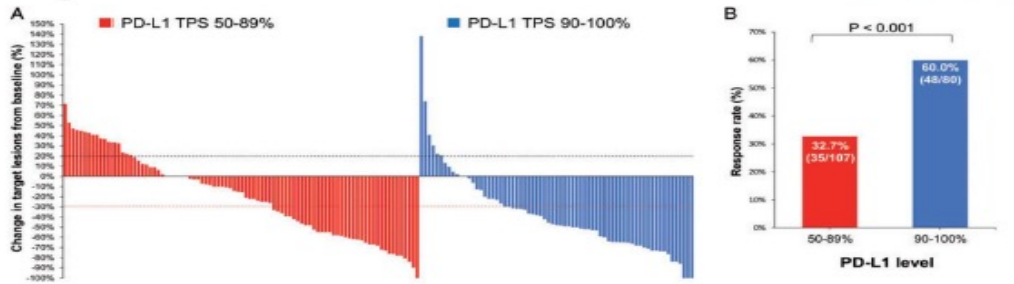
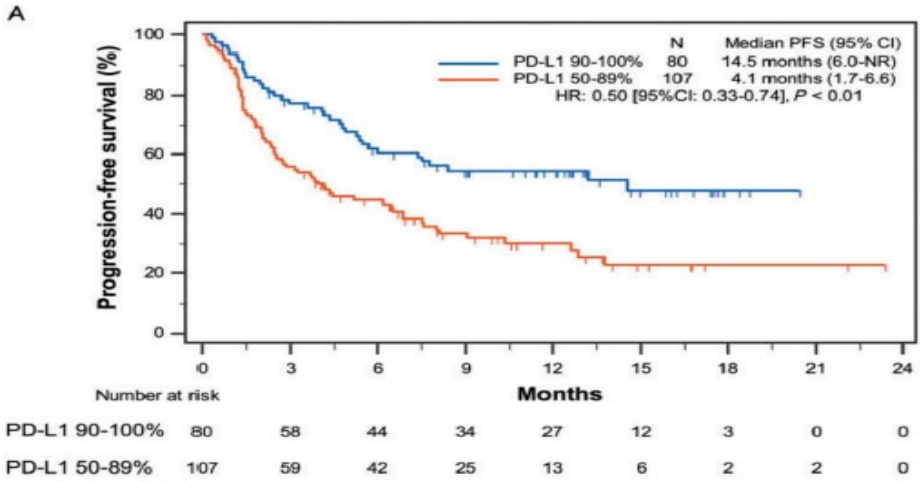
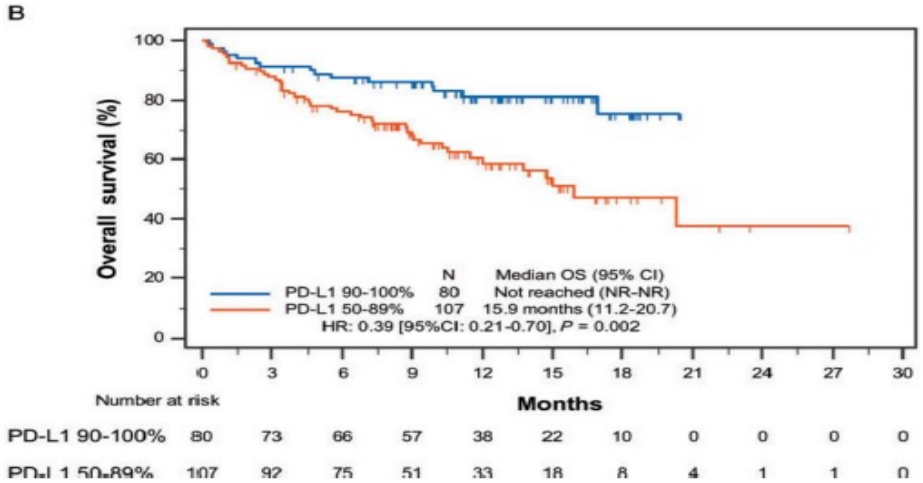


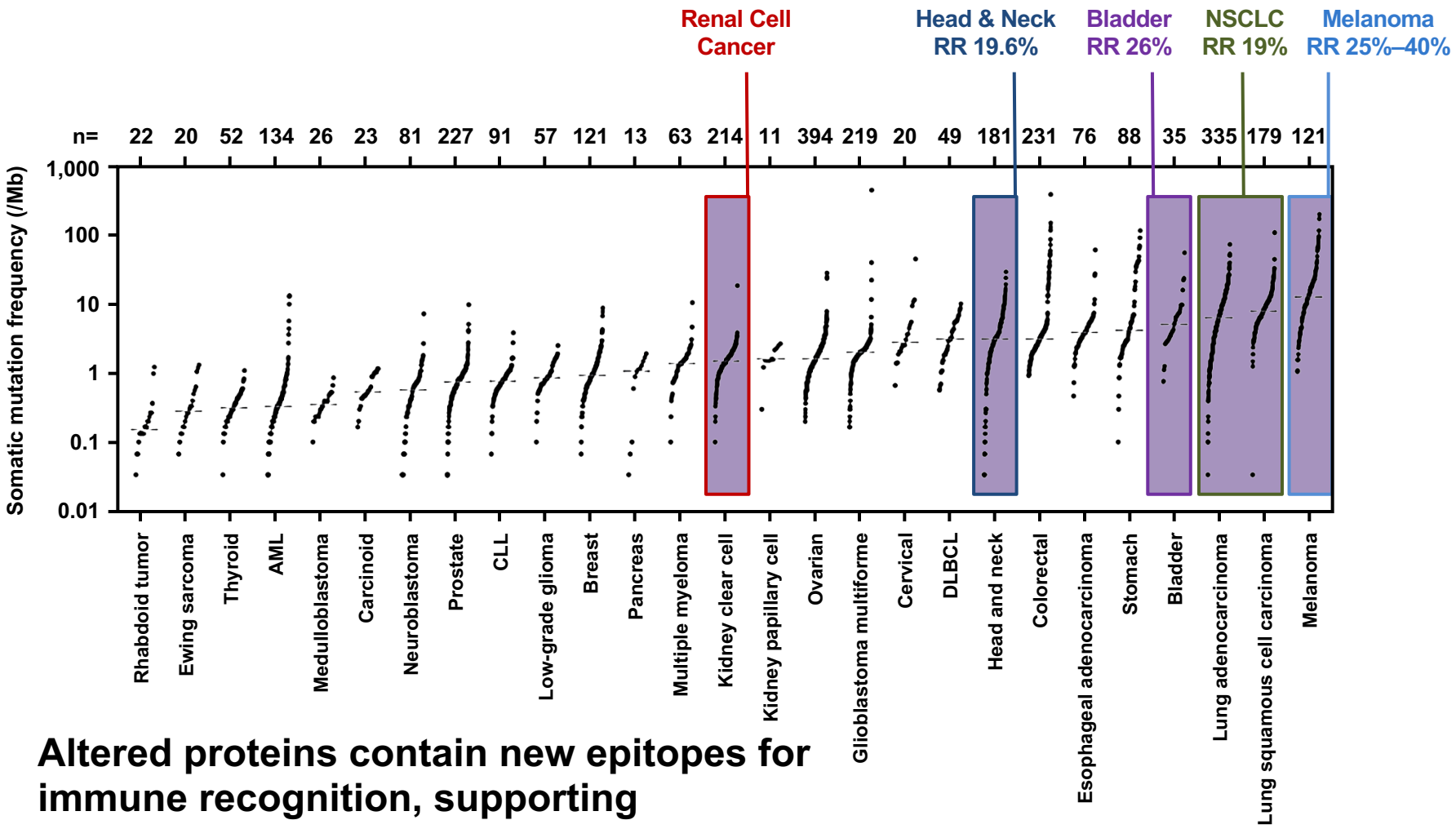
Figure 2. (A) The best objective response to pembrolizumab is shown as a percent change of target lesions from baseline in evaluable patients in patients with a non-small-cell lung cancer programmed death-ligand 1 (PD-L1) expression level of 50%–89% versus 90%–100%. (B) Histograms showing the response rate to first-line pembrolizumab in the PD-L1 expression 50%–89% versus 90%–100% groups.





Tumor Mutation Burden

Mutation Heterogeneity



Altered proteins contain new epitopes for immune recognition, supporting immunotherapy approaches

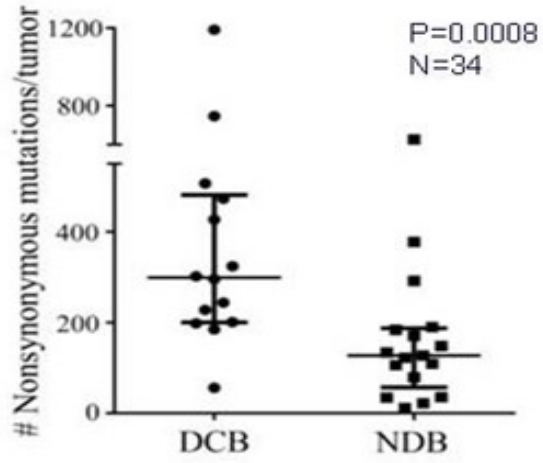
Adapted from Alexandrov et al. *Nature.* 2013.

Biomarkers

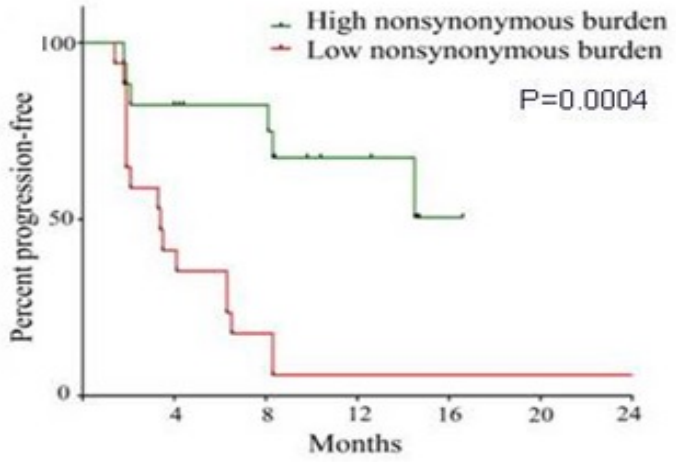
TMB - Predictive

Preliminary findings: Mutational load in NSCLC correlates with response to anti-PD-1 (pembrolizumab) therapy

Endpoint: "durable clinical benefit"



Endpoint: PFS



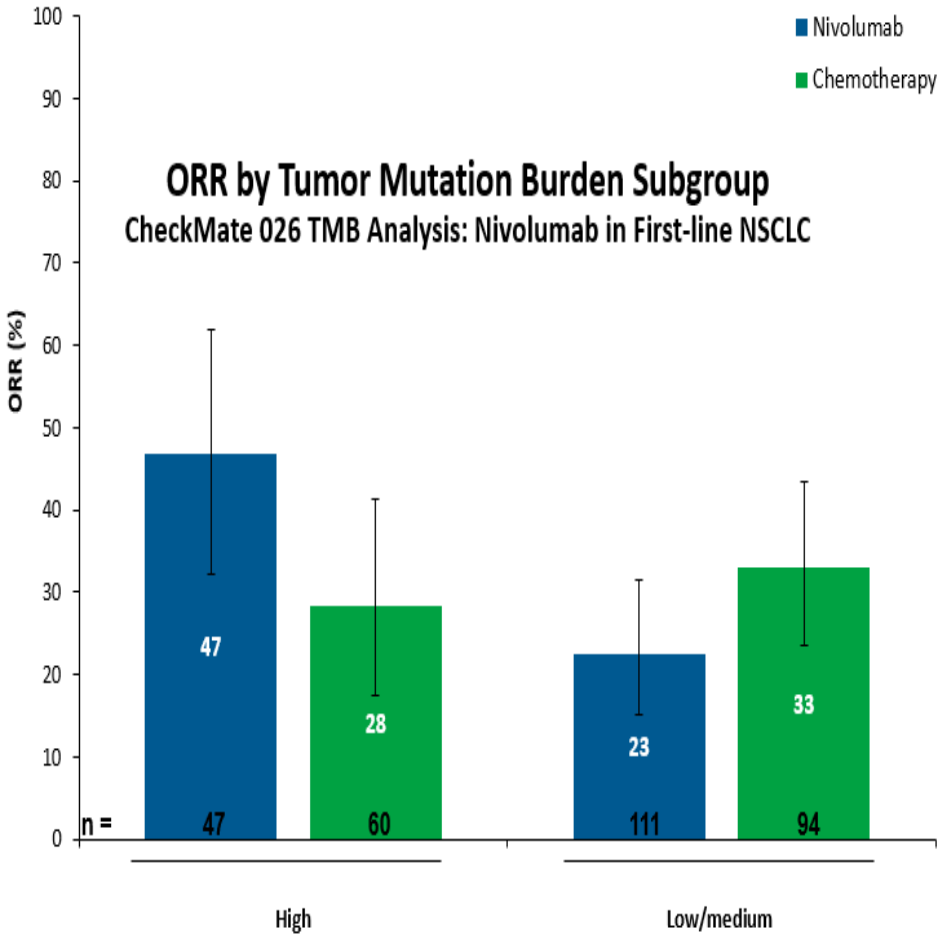
- Somatic exomic mutations create new proteins potentially recognized by the immune system

Rizvi, Chan et al., Science 2015

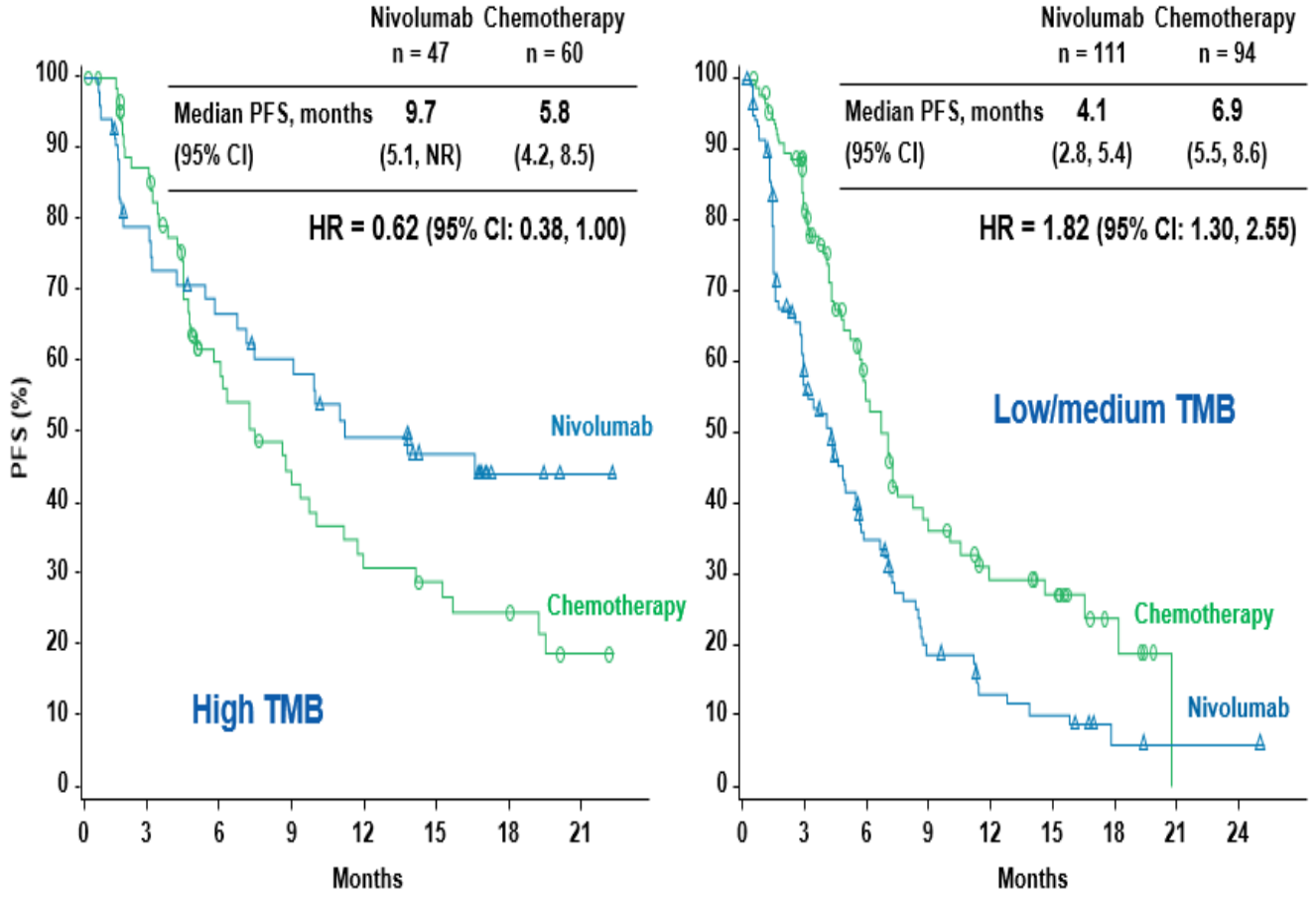
Biomarkers

TMB Analysis: CheckMate 026

ORR

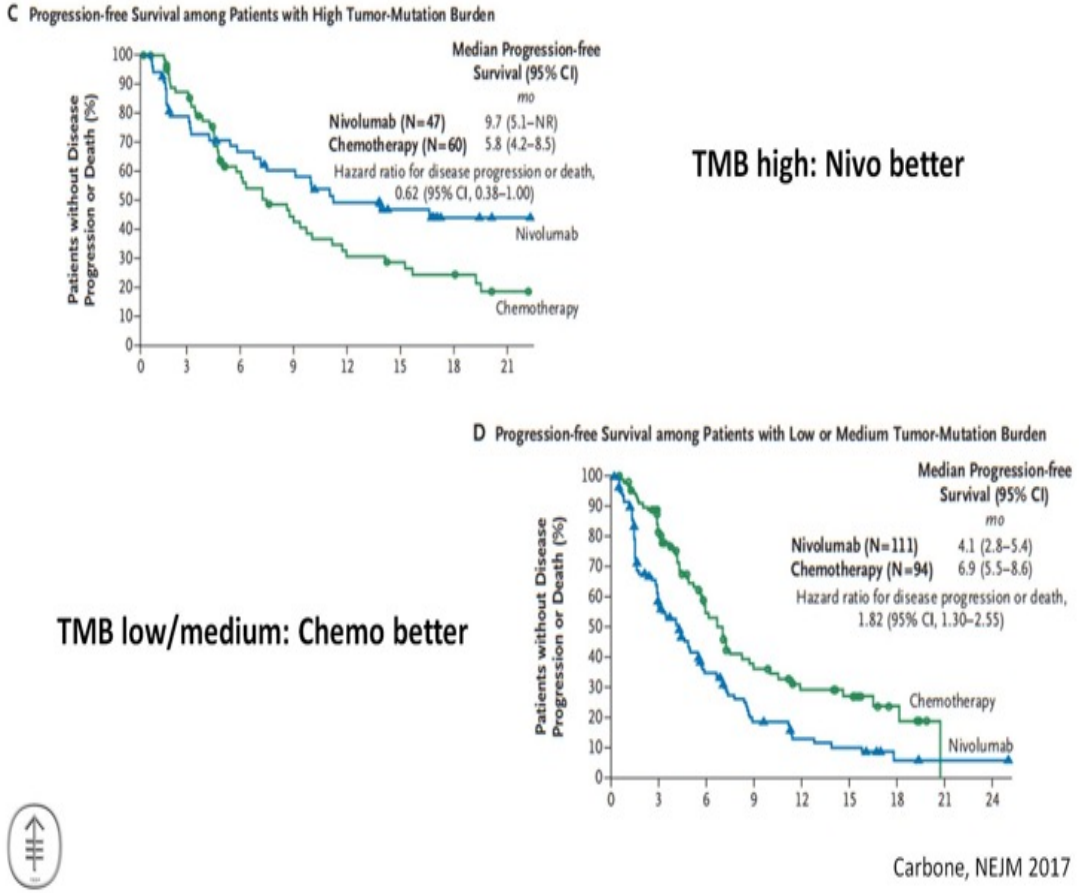


PFS



Biomarker
TMB – CM 026

Predictive

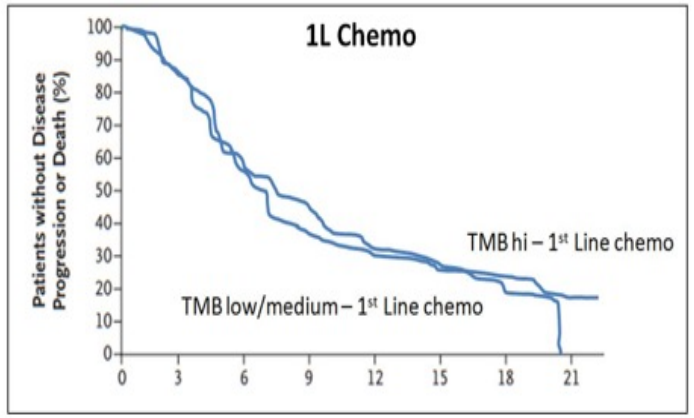


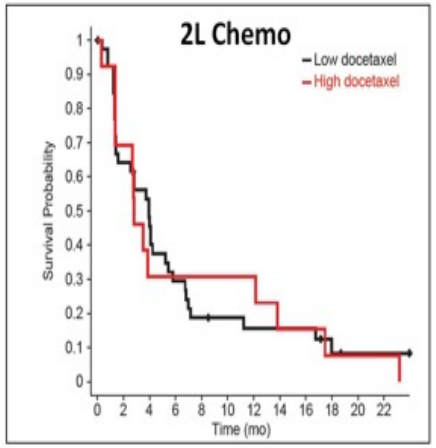
TMB high: Nivo better

TMB low/medium: Chemo better

Carbone, NEJM 2017

Not Prognostic

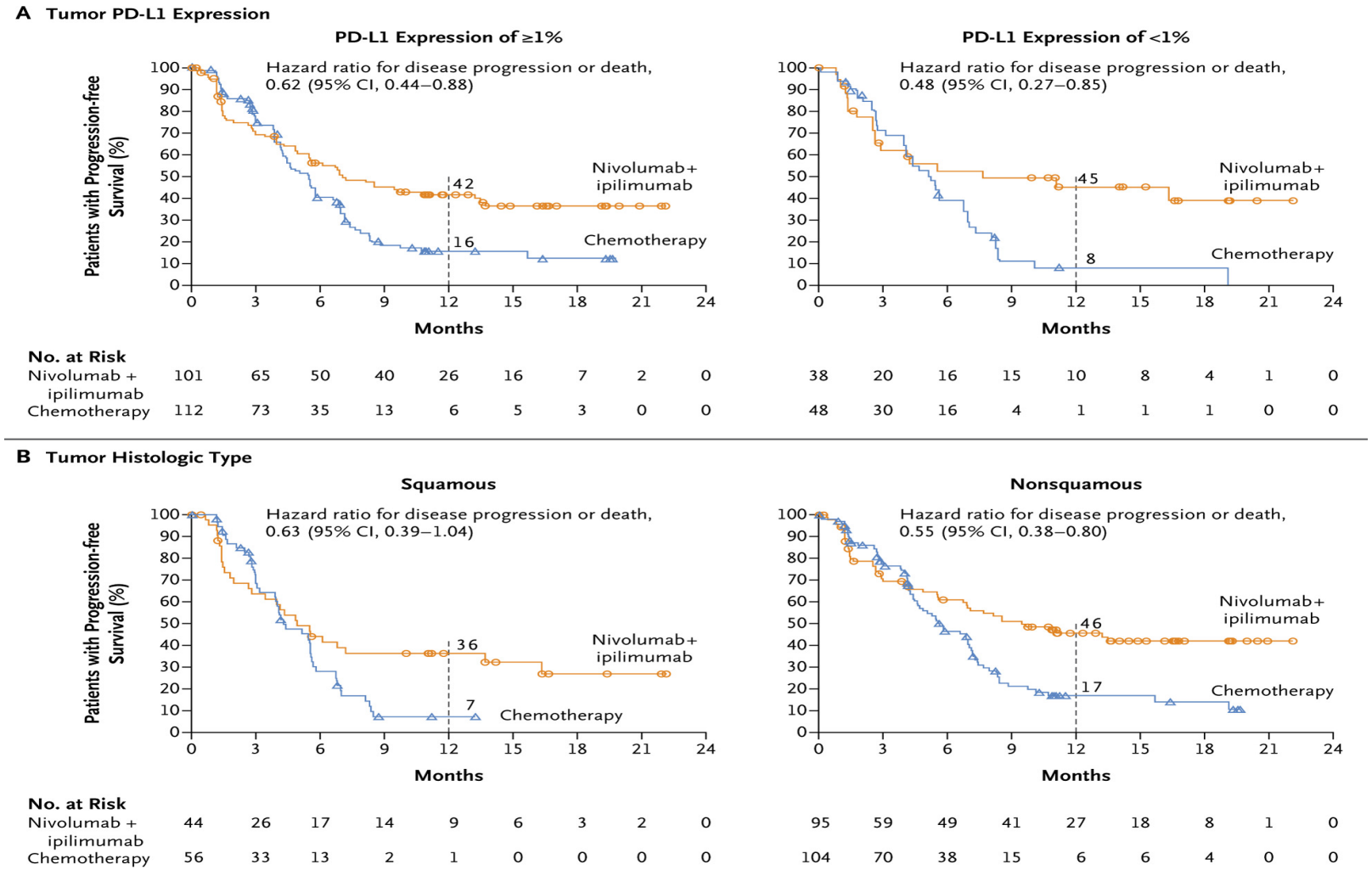




Kowantez ESMO 2016
Carbone, NEJM 2017

TMB

Predictive of benefit to IO – CM 227



PFS among patients with a High TMB according to Tumor PD-L1 Expression and Histologic Type. A high TMB was defined as at least 10 mutations per megabase. The circles (nivolumab plus ipilimumab) and triangles (chemotherapy) indicate censored data.

N Engl J Med 2018 May 31;378(22):2093-2104

TMB

Testing Methodology

- **Only a minority of mutations produce neoantigens**
- TMB cut-off values require validation?

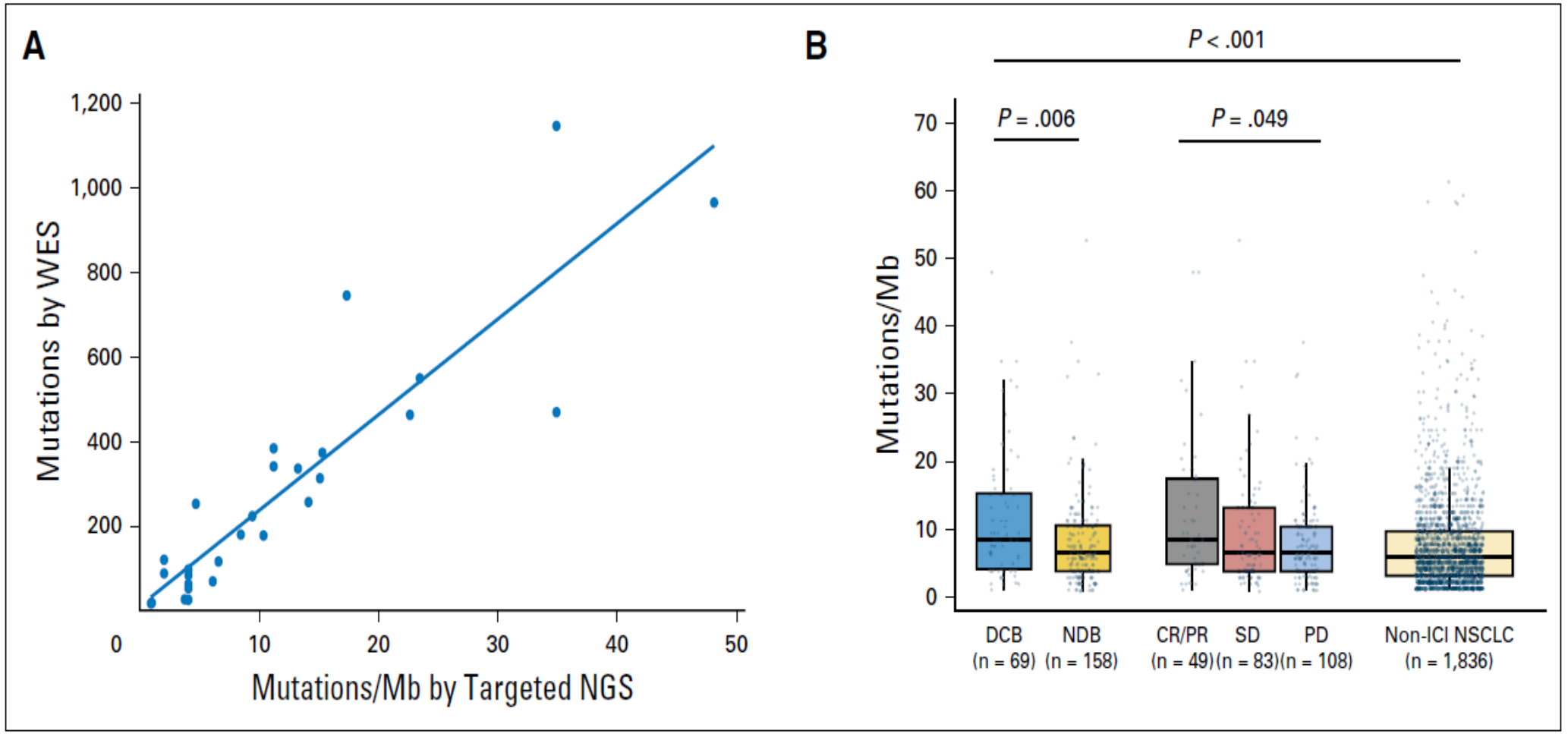
Reference	Sequencing Type	Threshold
Rizvi, Science 2015	WES	5 mut/Mb Nonsynonymous missense
Kowanetz, ESMO 2017	Foundation ONE	10 mut/Mb
Ca[illegible]	[illegible]	[illegible] [illegible]nymous missense
Rizvi, JCO 2018	IMPACT-MSKCC	7 mut/Mb Nonsynonymous
Hellmann, NEJM 2018	Targeted Foundation ONE	10 mut/Mb
Velcheti, ASCO 2018	Genentech	16 mut/Mb

Standardization needed!

Factors to standardize:
- sequencing depth, mutations included, filtering process

TMB

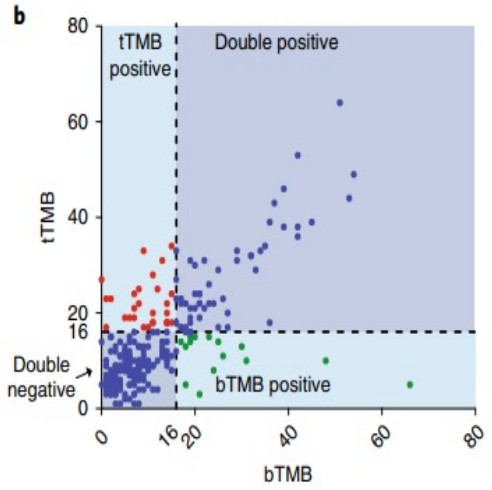
WES vs CGS (Target NGS)

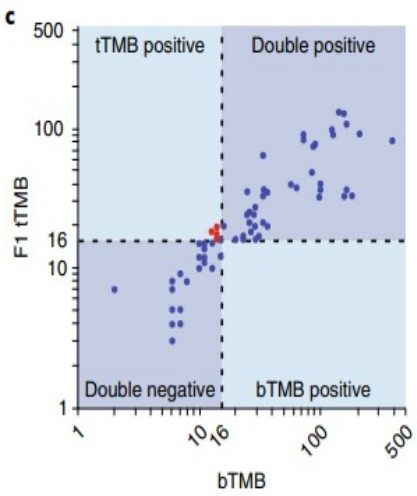


Hira Rizvi, et al. *Journal of Clinical Oncology* 2018 36:633-641.

TMB

Blood DNA (bTMB) vs Tissue DNA (tTMB)



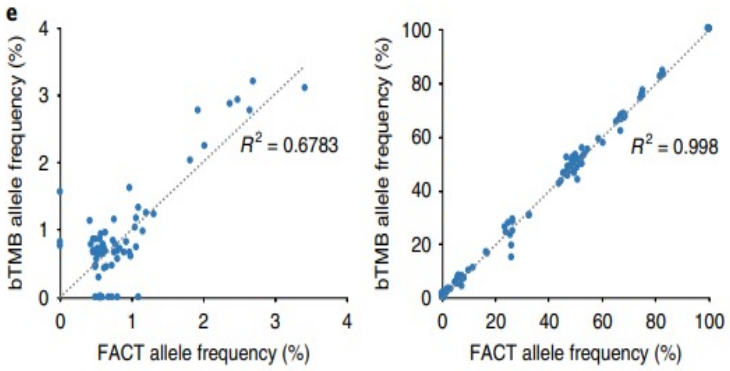


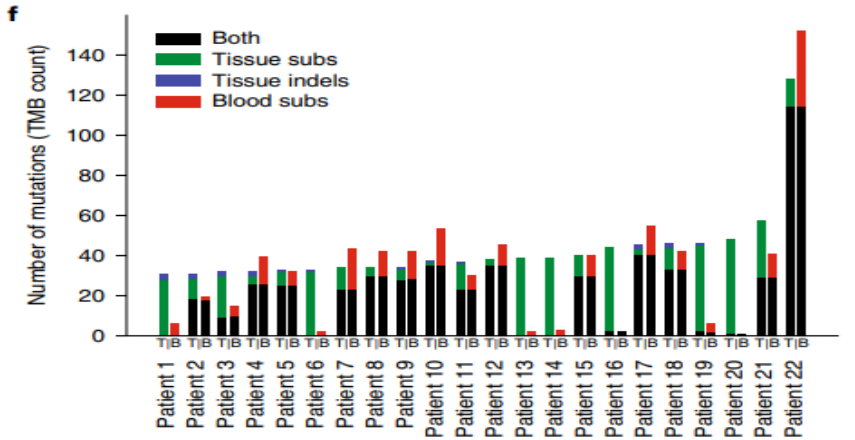
bTMB	PPA (%)	NPA (%)
≥10	100	100
≥11	94.6	92.3
≥12	89.1	92.9
≥13	94	84.2
≥14	90	89.5
≥15	85.7	90
≥16	89.1	100
≥17	90.2	89.3
≥18	92.1	83.9
≥19	94.4	81.8
≥20	97.1	82.4

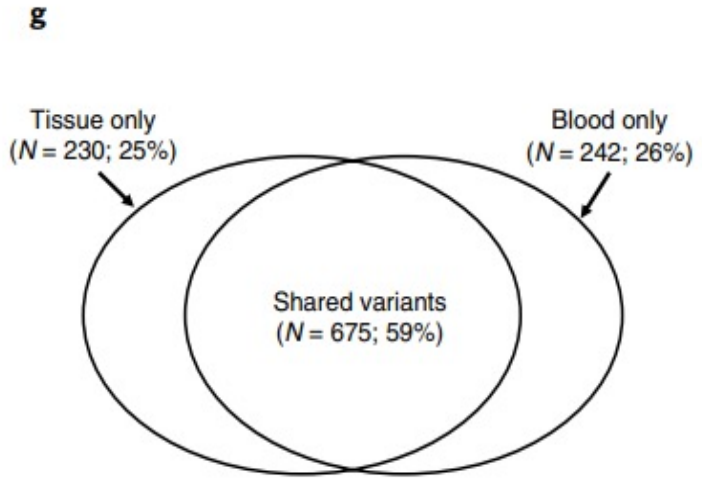
Metric	Performance
PPA	64% (95% CI: 54–74%)
NPA	88% (95% CI: 83–92%)

d

Metric	Value	ctDNA samples	Comments
PPA	93.4% (171/183 variants)	69 samples with variants, 81 total samples	False-negative VAFs range from 0.5% to 1.1% in FACT; probably missed due to low read support
PPV	93.5% (43/46 variants)	33 samples with variants, 81 total samples	False-positive VAFs: 0.76, 0.82 and 1.56% in bTMB; probably due to a nearby homopolymer sequence





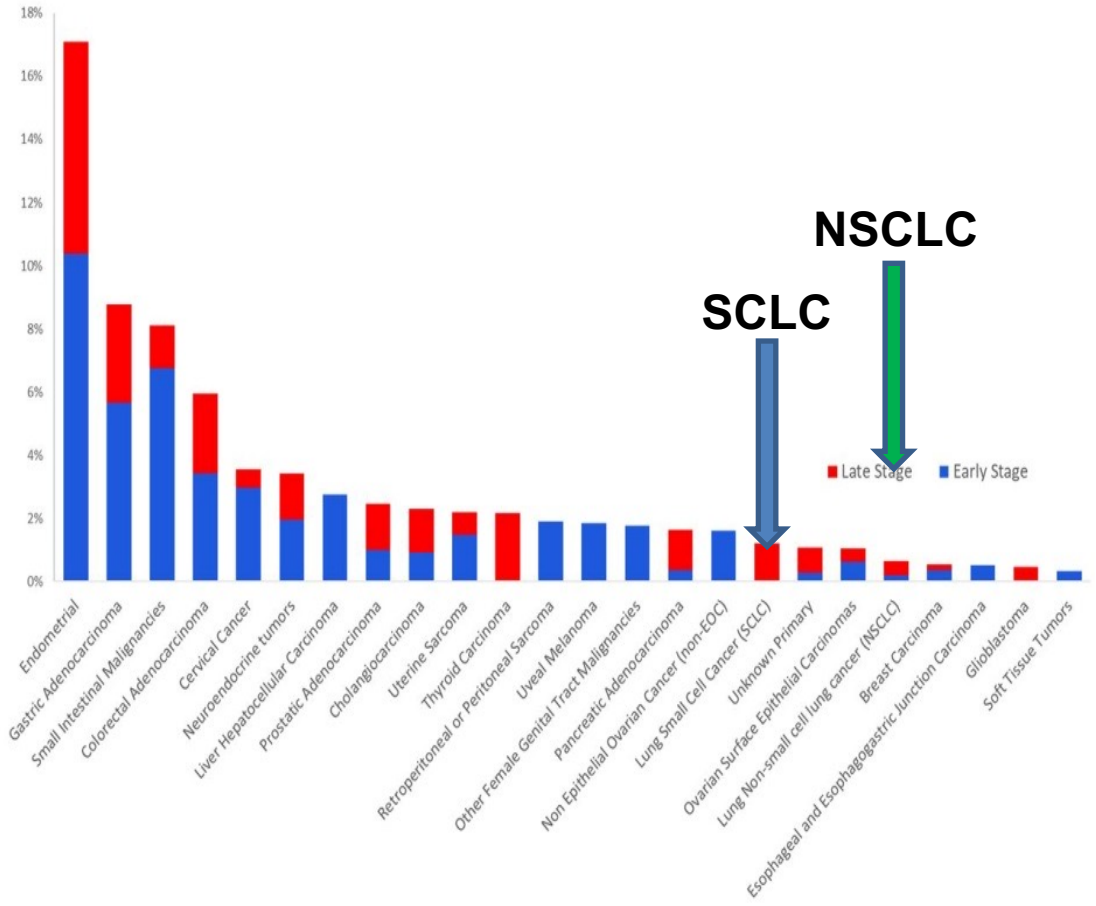


Nat Med. 2018 Sep;24(9):1441-1448

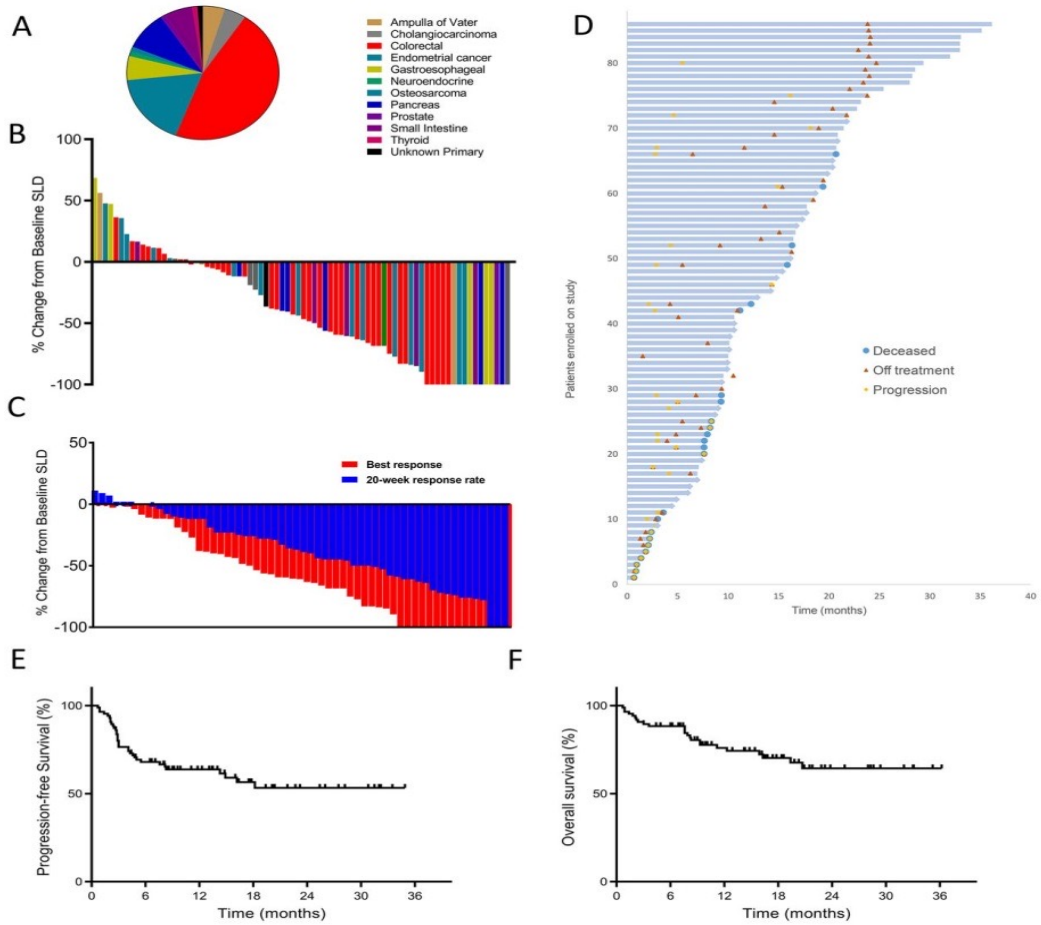
Microsatellite Instability

Mismatch Repair Deficiency

Incidence across 12,019 tumors



Patient survival and clinical response to Pembrolizumab across 12 different tumor types with mismatch repair deficiency.



Dung T. Le et al. Science 2017;science.aan6733

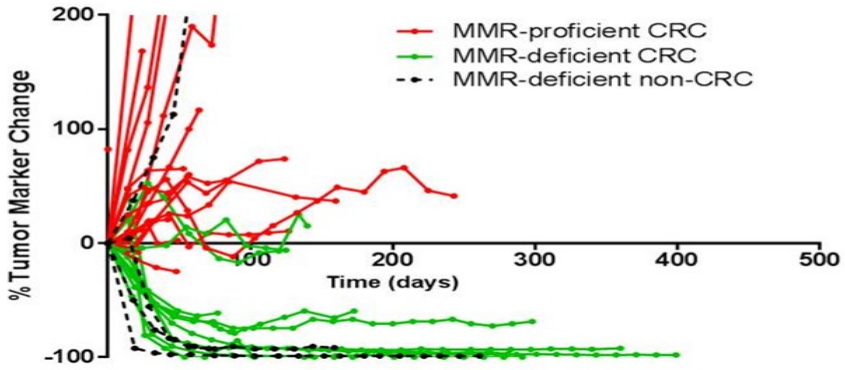
Biomarkers

Microsatellite Instability (MSI)

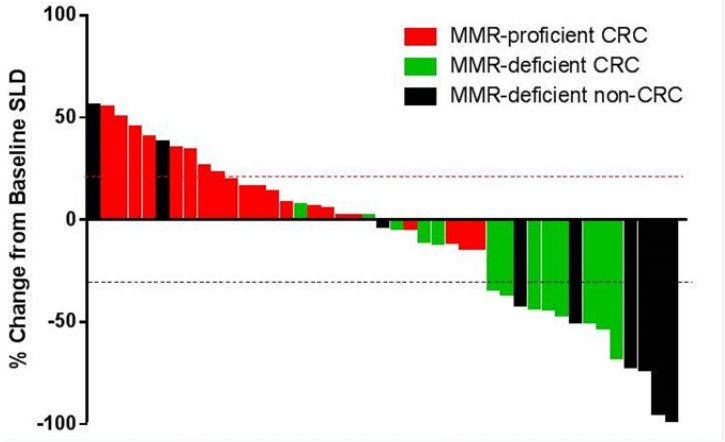
Objective Responses

	MMR-deficient CRC	MMR-proficient CRC	MMR-deficient non-CRC
N	13	25	10
Objective Response Rate	62%	0%	60%
Disease Control Rate	92%	16%	70%

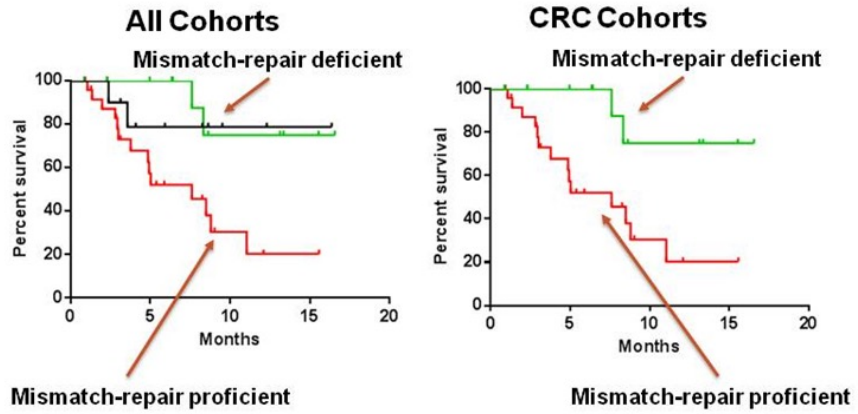
Biochemical Responses



Target Lesions



Overall Survival



Dung T. Le 2015 J Clin Oncol 33, 2015 (suppl; abstr LBA100)

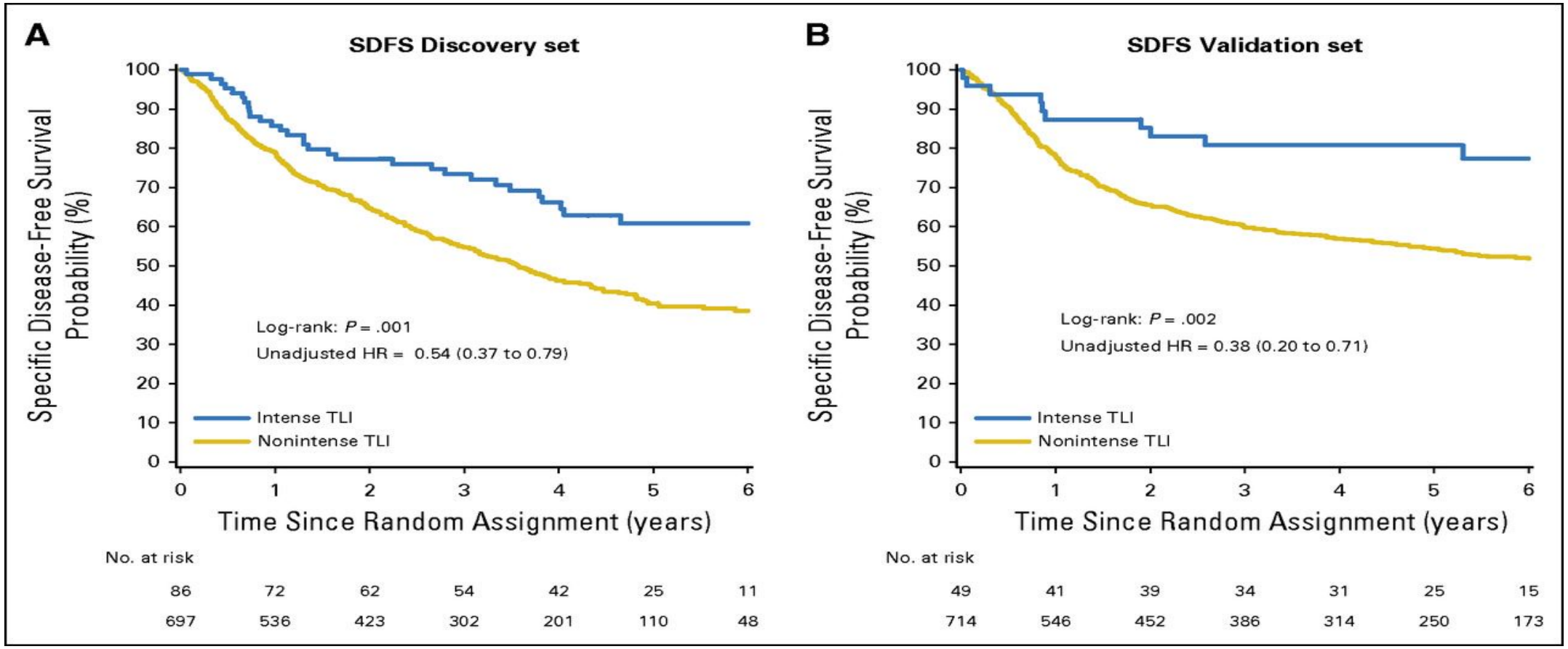
Biomarkers

Microsatellite Instability (MSI)

Presented By Kurt Schalper at 2017 ASCO Annual Meeting

Biomarkers

TIL - Prognostic



Survival curves for tumor lymphocytic infiltration (TLI; intense and nonintense) for specific disease-free survival (SDFS) on discovery (A) and validation (B) sets.

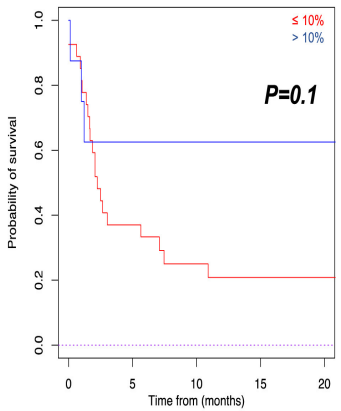
Elisabeth Brambilla et al. JCO 2016;34:1223-1230

Biomarkers

TIL density– Predictive

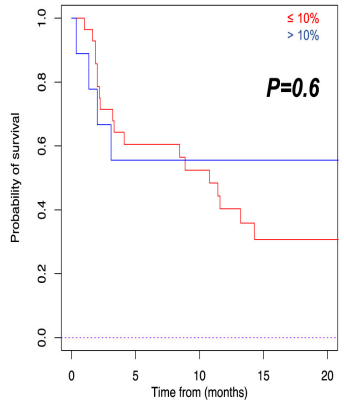
Negative PD-L1 population,

PFS according to >10% TIL



A

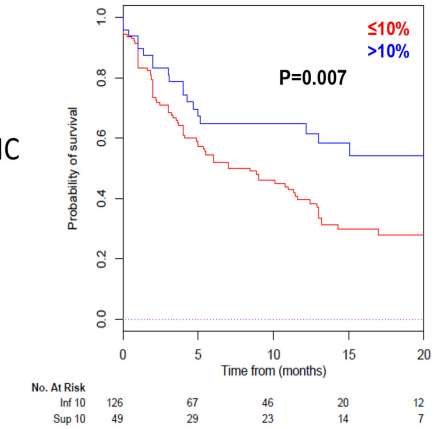
OS according to >10% TIL



B

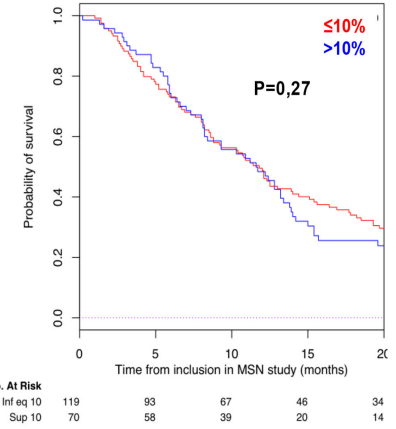
OS in the immunotherapy cohort according to >10% TIL

IC

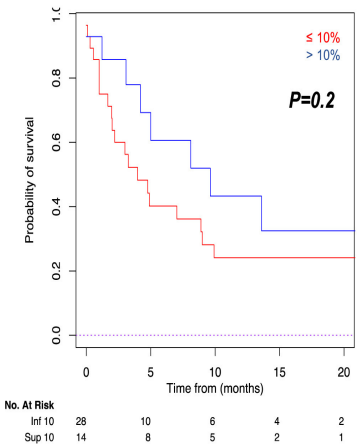


OS in the chemotherapy cohort according to >10% TIL

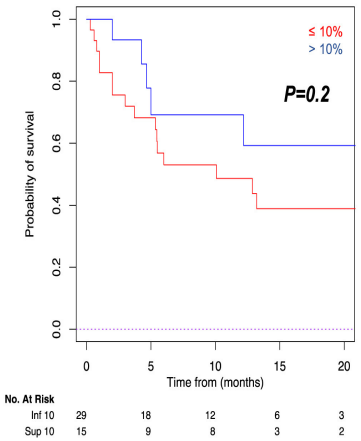
CC



Positive PD-L1 population,



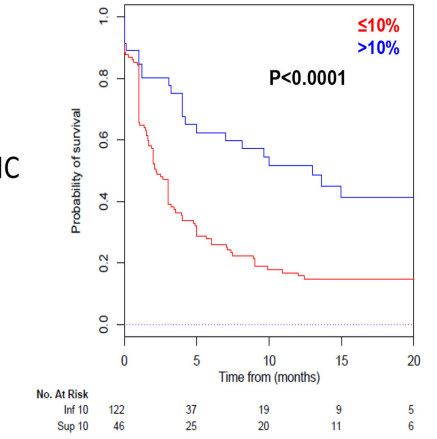
C



D

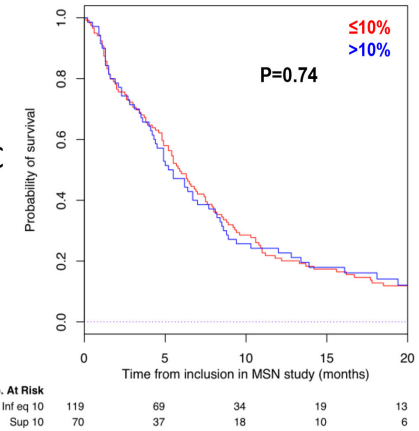
PFS in the immunotherapy cohort according to >10% TIL

IC



PFS in the chemotherapy cohort according to >10% TIL

CC

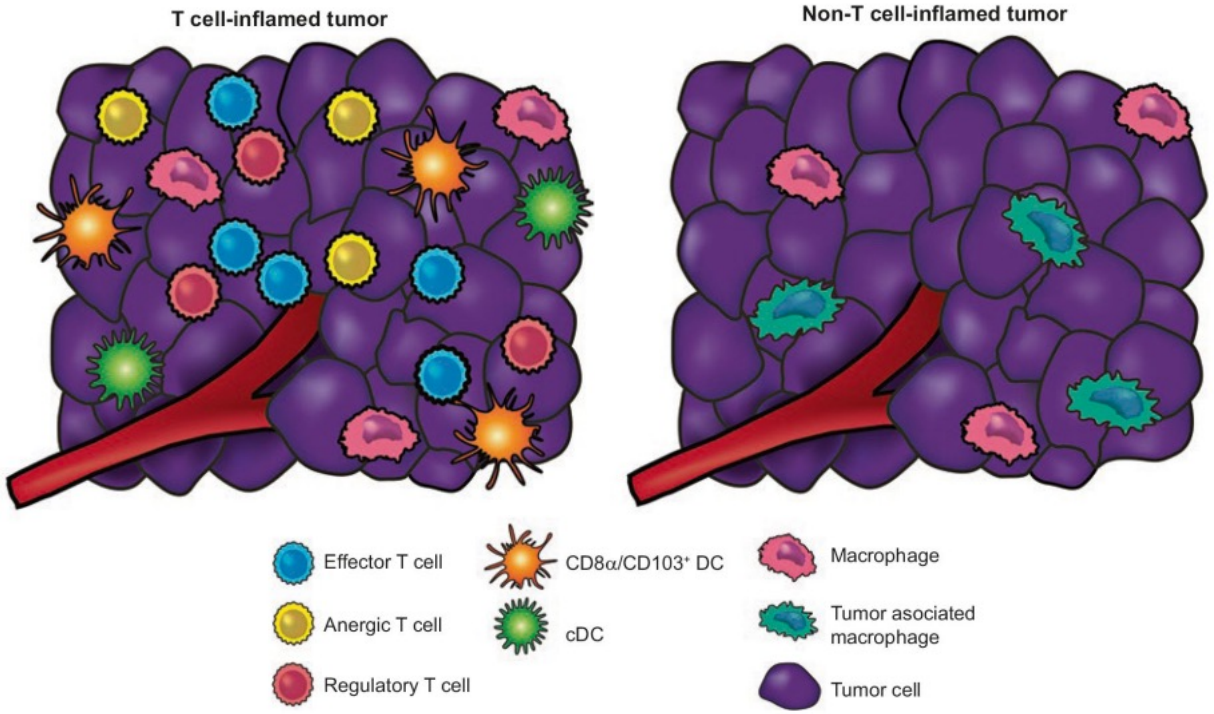


I. Gataa et al. European Journal of Cancer 145 (2021) 221e229

Biomarkers

TIL – Inflamed vs. Non-Inflamed Phenotype

Inflamed



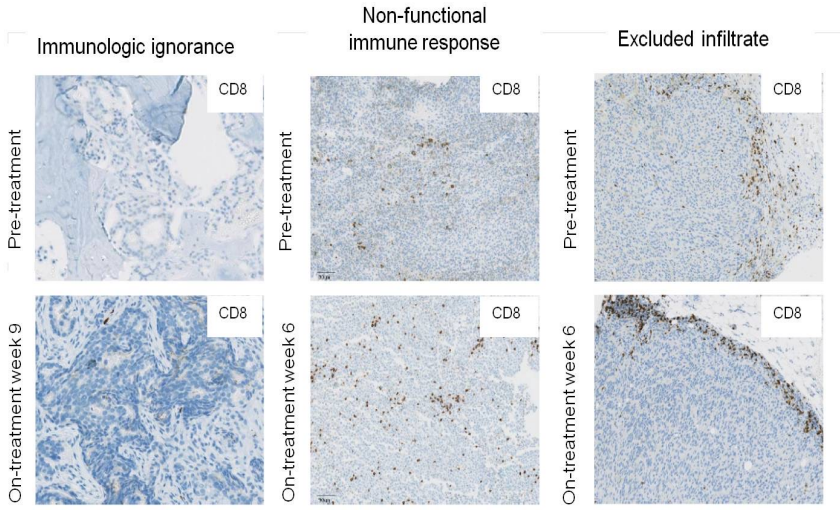
Immunologic composition of the T cell-inflamed versus non-T cell-inflamed tumor microenvironments. The T cell-inflamed tumors contain variable numbers of $CD8^+$ T cells and CD8α/CD103-lineage DCs, but also possess the highest density of $FoxP3^+$ Tregs. In addition, many of the conventional T cells have a dysfunctional anergic phenotype. In contrast, the non-T cell-inflamed tumors lack these elements but still contain blood vessels, fibroblasts, and macrophages that help support tumor growth. Recruitment of $CD8^+$ effector cells is largely dependent on the chemokines CXCL9 and CXCL10, which engage the receptor CXCR3. Treg recruitment is primarily driven by CCL22, which is in part produced by activated $CD8^+$ T cells

Adv Exp Med Biol. 2017 ; 1036: 19–31

Non-Inflamed

Biomarker Analyses

Defining the Profile of Non-responders



- Three distinct patterns of nonresponse were observed
- Most patients who progressed failed to show up-regulation of PD-L1 or evidence of activated T cells
- These results provide evidence for the "inflamed tumor" hypothesis

Herbst RS et al. *Nature* 2014;515: 563-567;

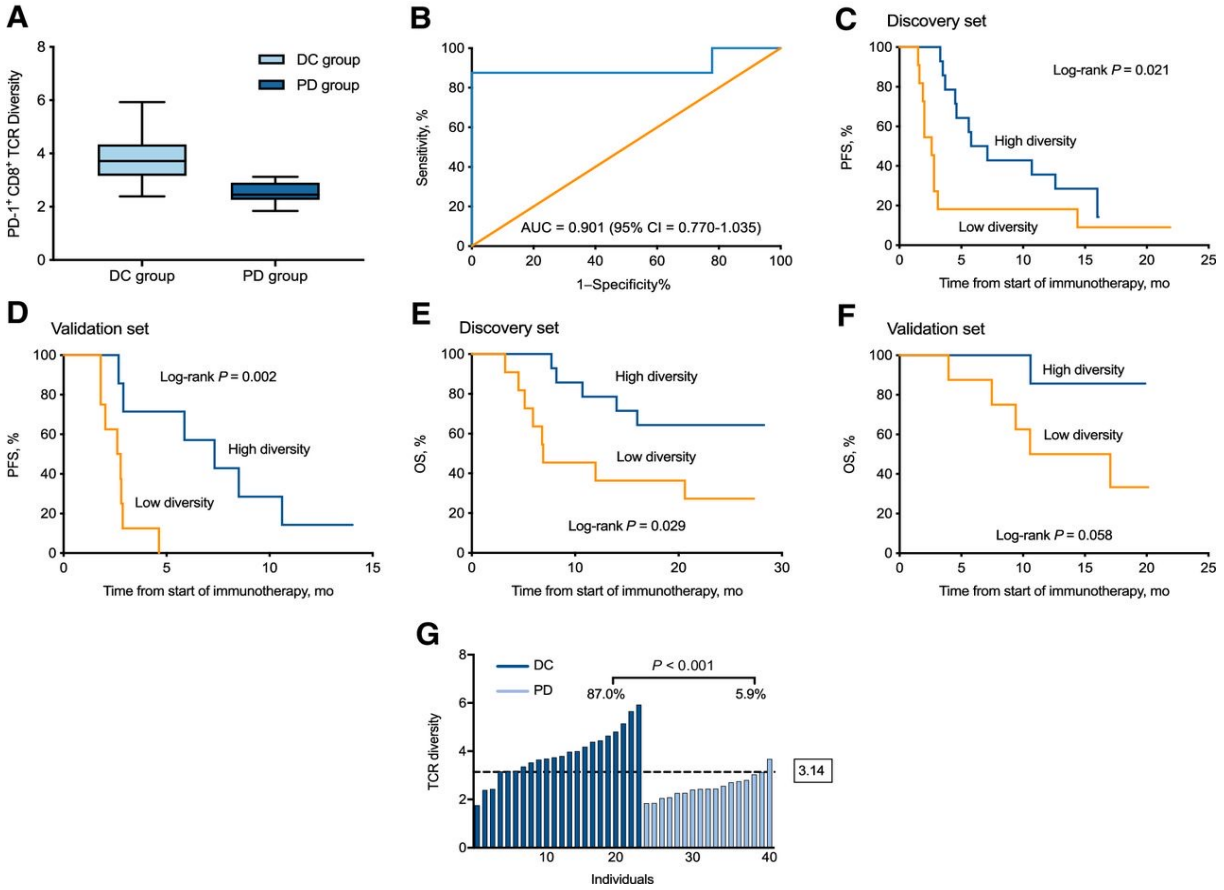
TCR Clonality – TCR Repertoire

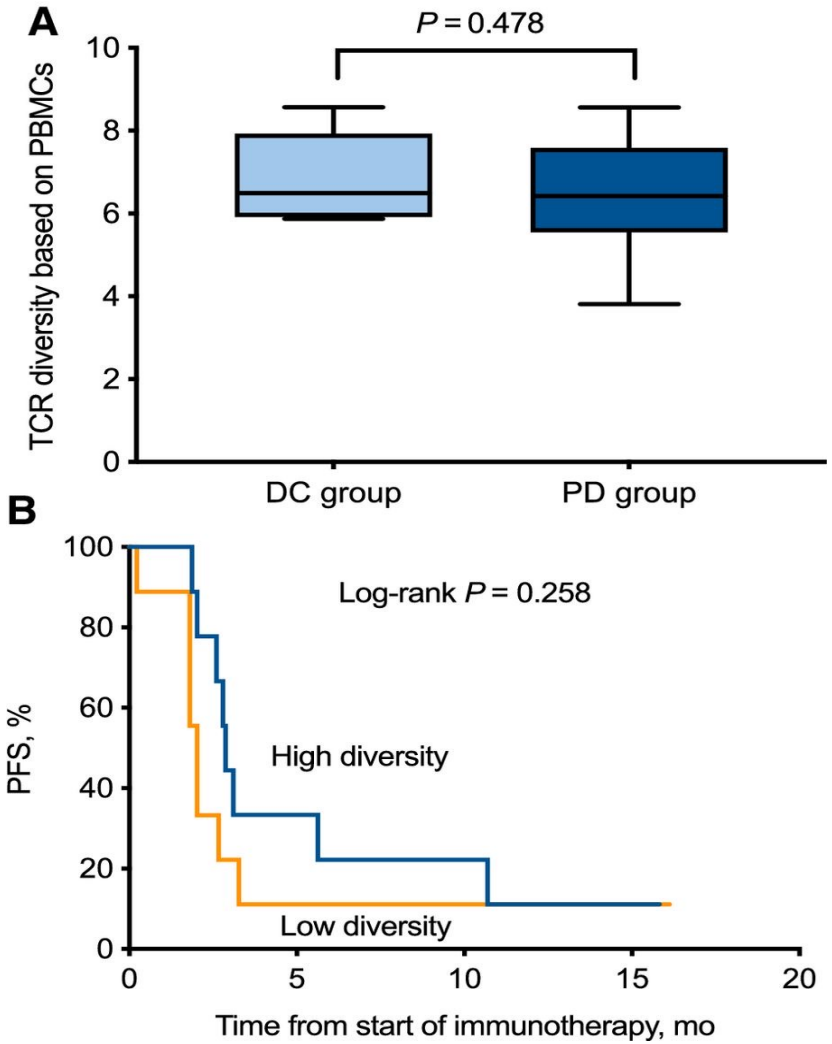
NGS to capture uniquely rearranged variable T-cell Receptor (TCR) beta-chain

TCR beta-chain more clonal (less diverse) in (anti-PD-1) responders

Increased TCR clonality in (anti-PD-1) responders

TCR Clonality – TCR Repertoire





TCR Repertoire Diversity of Peripheral PD-1⁺CD8⁺ T Cells Predicts Clinical Outcomes after Immunotherapy in Patients with NSCLC

Cancer Immunol Res. 2020;8(1):146-154. doi:10.1158/2326-6066.CIR-19-0398

Point Mutations and Co-mutations

EGFR, ALK, KRAS, STK11, POLE, Co-mutations

Gene Expression Profiles

INF- gamma Signature

Immunoscore

Antibodies Profile

Microbiome

Clostridiales in Responders

Bacteroidiales in Non-responders

Combinations of Biomarkers

TMB and PD-L1 (essentially) independent variables

TMB and INF-gamma sig. are minimally associated

TMB high and MSI, minimal overlap

PD-L1 plus TILs

Complex (multifactorial) bio-markers necessary (?)

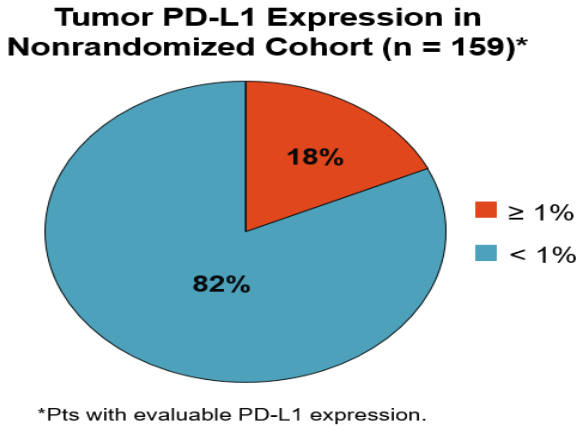
More Predictive (?)

SCLC

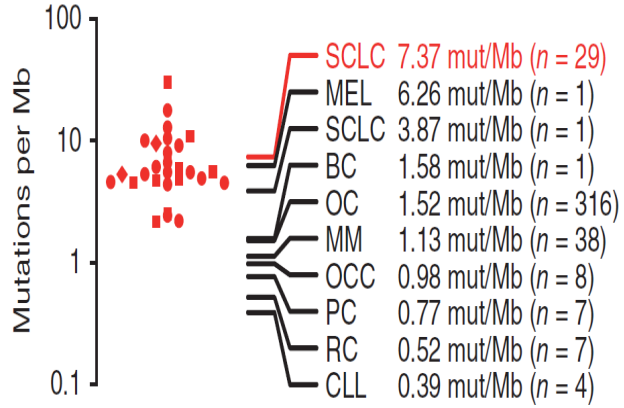
Potential Predictive Biomarkers

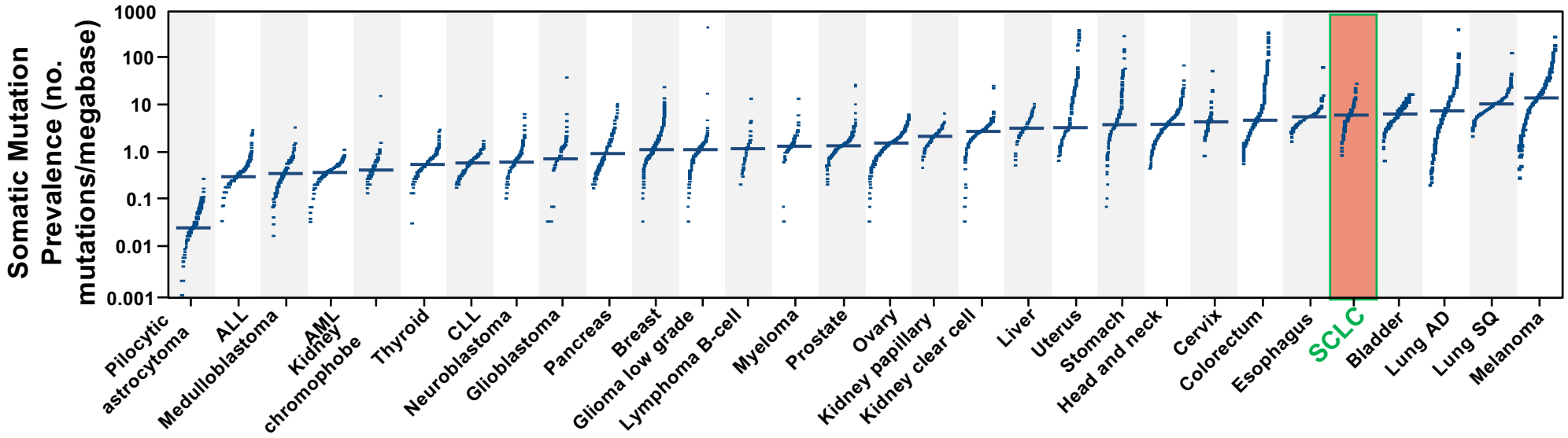
Immunotherapy

PD-L1 expression and TMB



PD-L1 Expression	ORR, % (n/N)	
	Nivolumab (n = 98)	Nivolumab + Ipilimumab (n = 61)
< 1%	14 (9/64)	32 (10/31)
≥ 1%	9 (1/11)	10 (1/10)

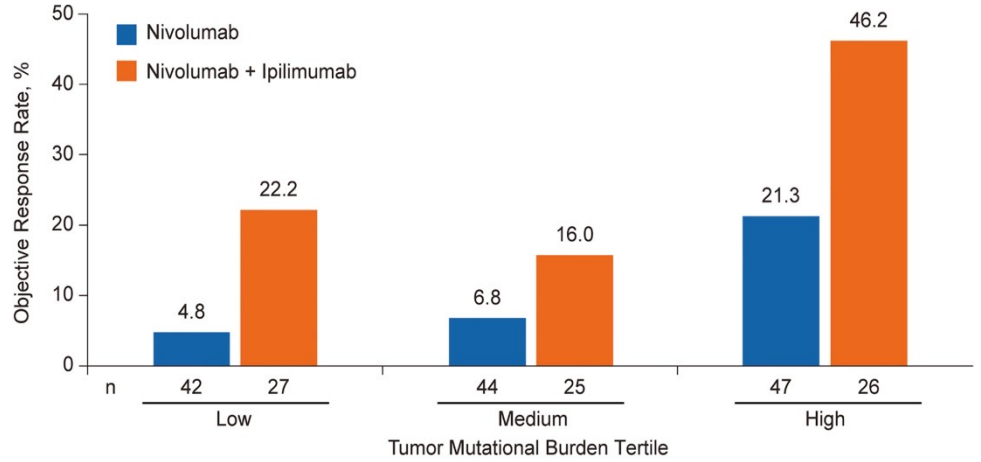


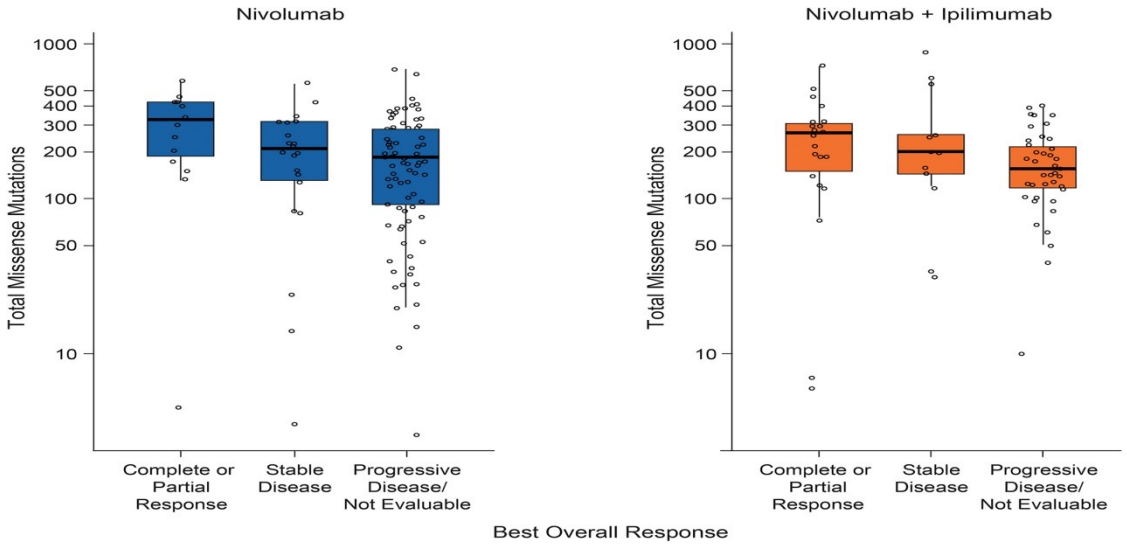


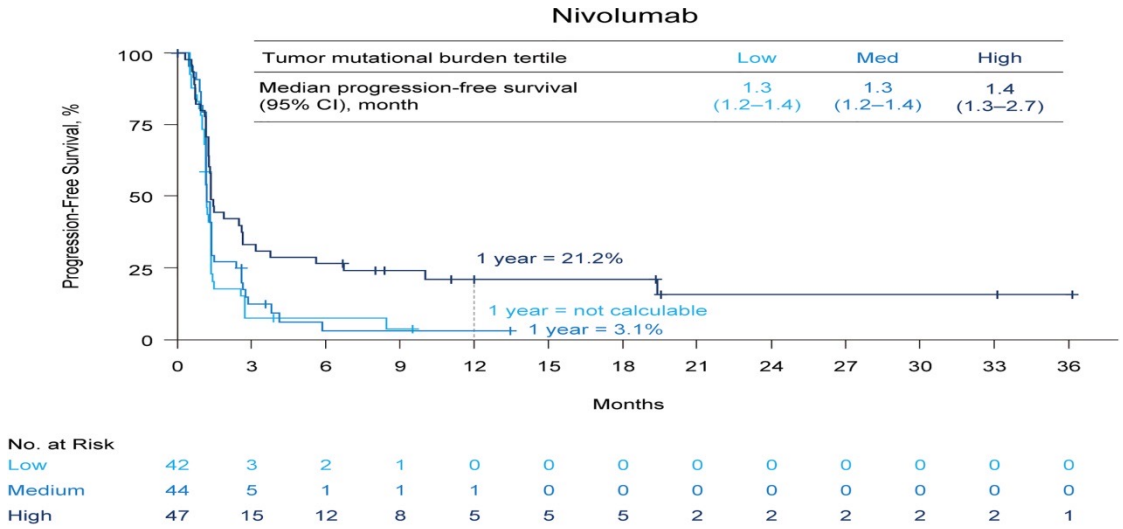
High mutational burden seen in SCLC

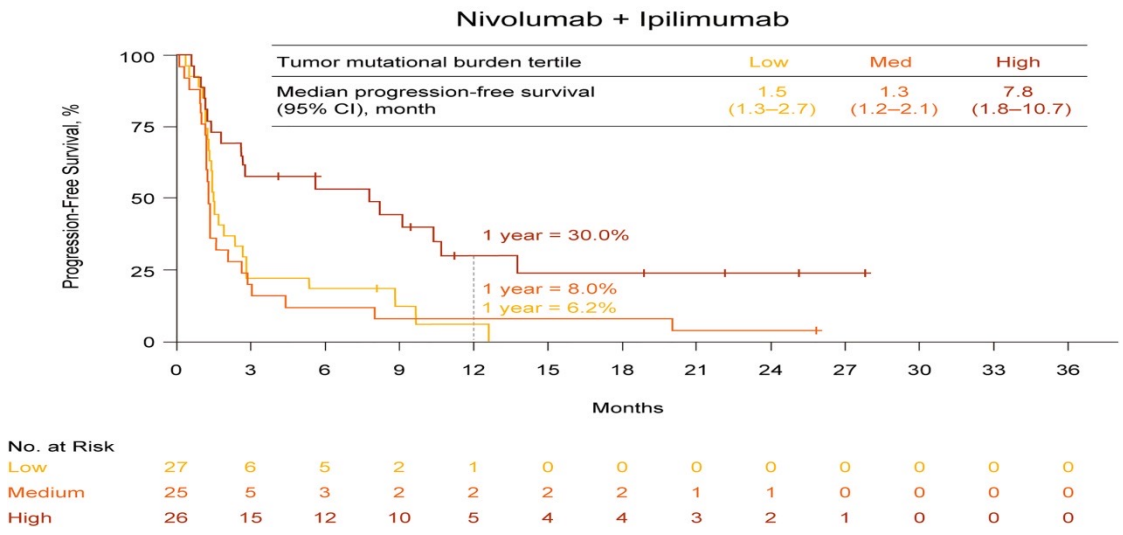
Hellmann MD, et al. ASCO 2017. Abstract 8503. Peifer. Nat Genet. 2012;44:1104. Alexandrov. Nature. 2013;500:415.

TMB
CheckMate 032: Results





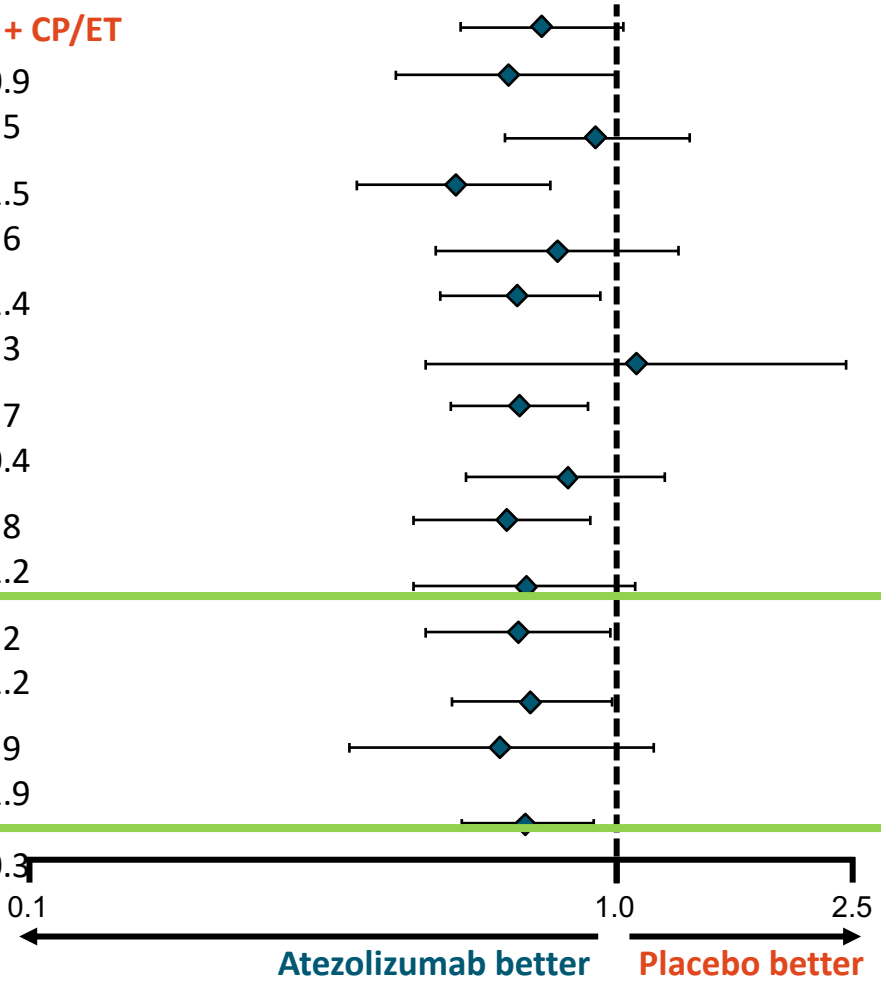




Hellmann, M. Cancer Cell 2018; 33(5):853-861

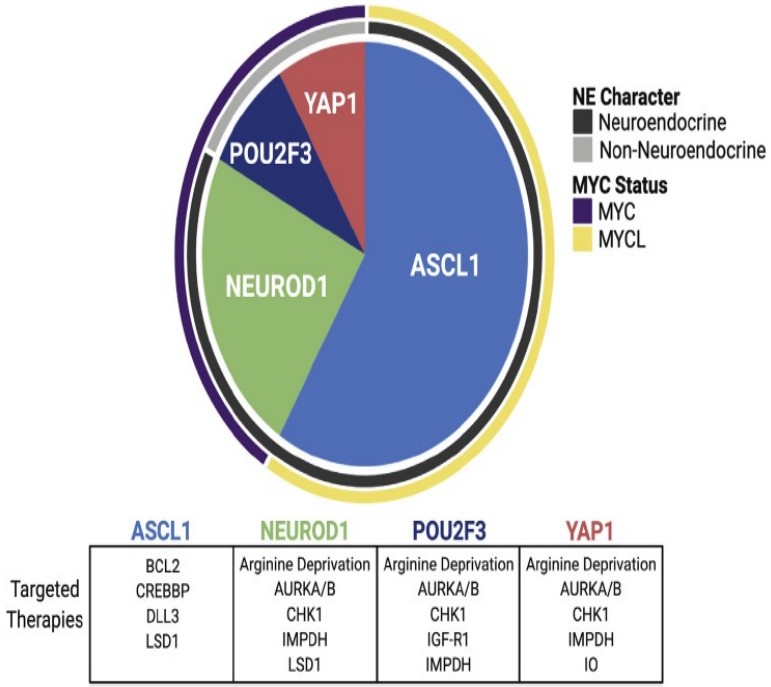
TMB
IMpower133 - OS by Subgroup

Population	Median OS, Mos: Atezolizumab + CP/ET	Median OS, Mos: Placebo + CP/ET	OS HR (95% CI)
Male (n = 261)	12.3	10.9	0.74 (0.54-1.02)
Female (n = 142)	12.5	9.5	0.65 (0.42-1.00)
< 65 yrs (n = 217)	12.1	11.5	0.92 (0.64-1.32)
≥ 65 yrs (n = 186)	12.5	9.6	0.53 (0.36, 0.77)
ECOG PS 0 (n = 140)	16.6	12.4	0.79 (0.49-1.27)
ECOG PS 1 (n = 263)	11.4	9.3	0.68 (0.50, 0.93)
Brain metastases (n = 35)	8.5	9.7	1.07 (0.47-2.43)
No brain metastases (n = 368)	12.6	10.4	0.68 (0.52-0.89)
Liver metastases (n = 149)	9.3	7.8	0.81 (0.55-1.20)
No liver metastases (n = 254)	16.8	11.2	0.64 (0.45-0.90)
bTMB < 10 mut/mb (n = 139)	11.8	9.2	0.70 (0.45-1.07)
bTMB ≥ 10 mut/mb (n = 212)	14.6	11.2	0.68 (0.47, 0.97)
bTMB < 16 mut/mb (n = 271)	12.5	9.9	0.71 (0.52-0.98)
bTMB ≥ 16 mut/mb (n = 80)	17.8	11.9	0.63 (0.35-1.15)
ITT (N = 403)	12.3	10.3	0.70 (0.54-0.91)



Liu SV, et al. WCLC 2018. Abstract PL02.07. Horn L, et al. N Engl J Med. 2018;379:2220.

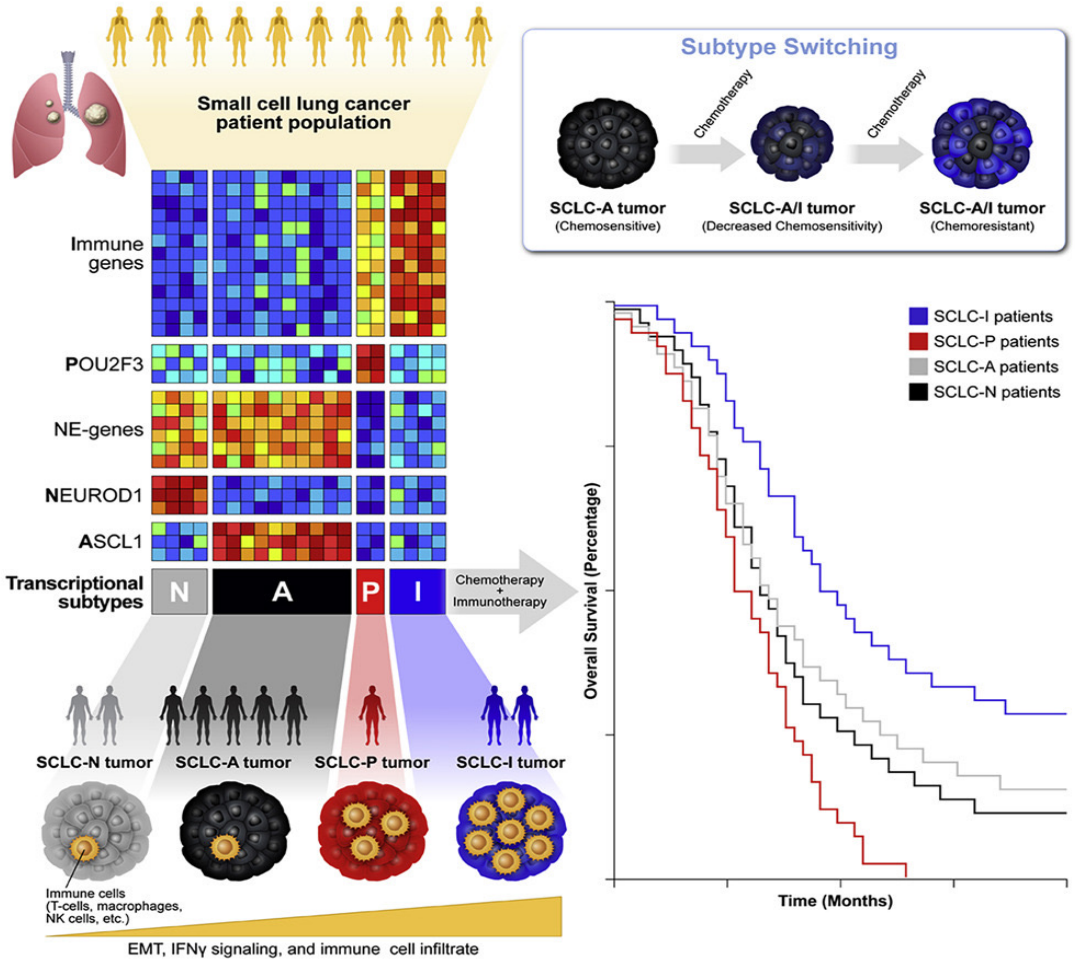
Molecular subtypes of SCLC
Potential Biomarkers



	ASCL1	NEUROD1	POU2F3	YAP1
Targeted Therapies	BCL2 CREBBP DLL3 LSD1	Arginine Deprivation AURKA/B CHK1 IMPDH LSD1	Arginine Deprivation AURKA/B CHK1 IGF-R1 IMPDH	Arginine Deprivation AURKA/B CHK1 IMPDH IO

Figure 3. Diagram of the relative abundance, MYC status, and NE character of the four molecular subtypes of SCLC, each identified by their key transcriptional regulator. These subtypes may exhibit distinct targetable vulnerabilities, which are represented in the table beneath the pie chart. Proportions of each subtype are as follows: ASCL1 (0.70, 95% CI: 0.60-0.79), NEUROD1 (0.11, 95% CI: 0.06-0.20), YAP1 (0.02, 95% CI: 0.01-0.09), POU2F3 (0.16, 95% CI: 0.10-0.26). ASCL1, achaete-scute homolog 1; AURKA/B, Aurora kinase A/B; BCL2, B-cell lymphoma 2; CREBBP, CREB-binding protein; CHK1, checkpoint kinase 1; DLL3, delta-like ligand 3; IMPDH, inosine-5' monophosphate dehydrogenase; IGF-R1, insulin-like growth factor 1 receptor; IO, immuno-oncology; LSD1, lysine-specific histone demethylase 1; NE, neuroendocrine; NEUROD1, neurogenic differentiation factor 1; POU2F3, POU class 2 homeobox 3; YAP1, yes-associated protein 1.

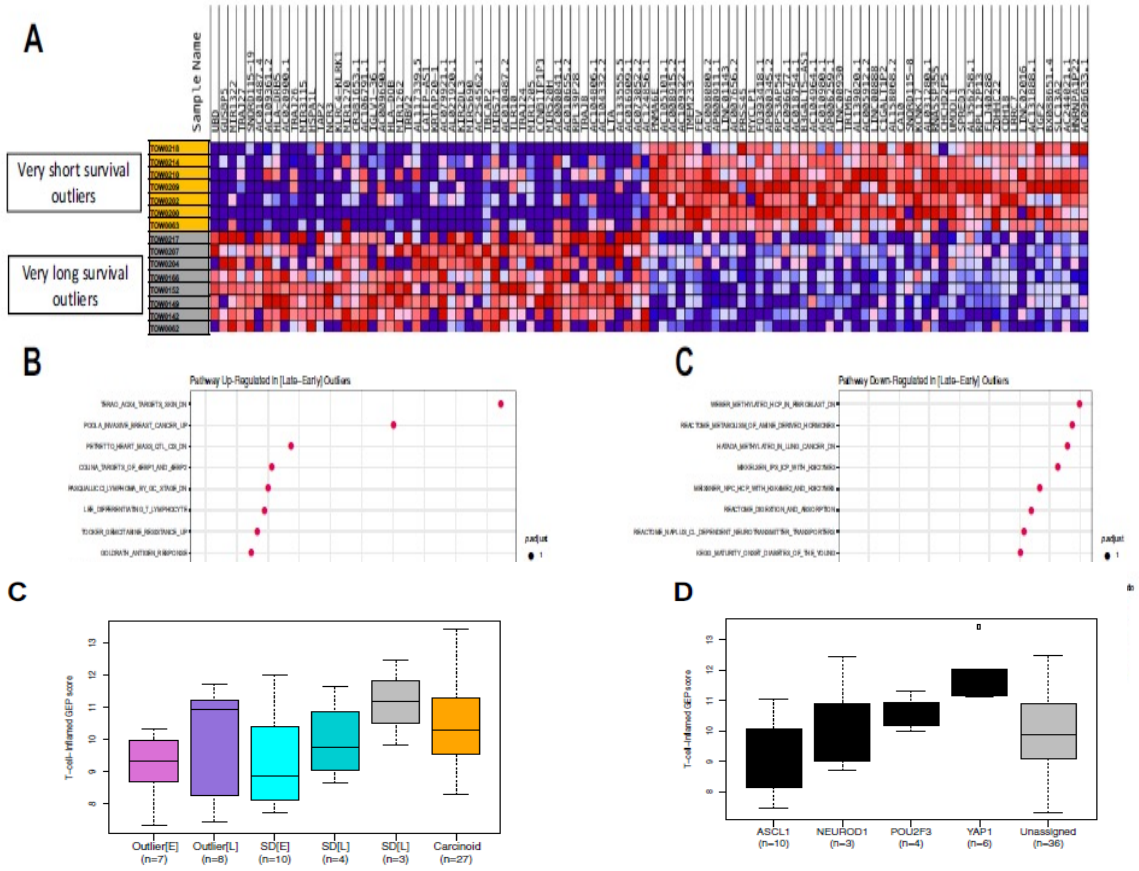
JTO 2020; 15(4):520-540



Gay et al. Cancer Cell 2021; 39:346–360

Molecular subtypes of SCLC
Potential Biomarkers – YAP1 and NOTCH

YAP1 Expression in SCLC Defines a Distinct Subtype With T-cell-Inflamed Phenotype



JTO 2021; 16(3):464-476

Table 2 Notch signaling gene set is the most significant predictor of clinical benefit to immune checkpoint blockade across relapsed SCLC cohorts.

Variable	Estimate[a]	*t* value[a]	*p* value[a]	FDR[b]
Hallmark Notch signaling	0.25	4.31	9.8×10^{-4}	5.9×10^{-4}
Immune signature	0.13	2.06	0.047	0.14
NE score	−0.07	−1.82	0.08	0.16
MYC expression	−0.04	−0.83	0.41	0.62
EZH2 expression	−0.03	−0.56	0.58	0.62

Outcome dependent variable = clinical benefit to immune checkpoint blockade.
[a]Estimates, *t* and *p* values calculated using multivariable logistic regression.
[b]False discovery rate was calculated using the Benjamini–Hochberg procedure.

- Tumors deriving clinical benefit (CB) from ICI exhibited cytotoxic T-cell infiltration, high expression of antigen processing and presentation machinery genes, and low neuroendocrine (NE) differentiation.
- Notch signaling, (correlates positively with low NE differentiation), most significantly predicts CB to ICI.
- Mechanistic link between Notch activation, low NE differentiation and increased intrinsic tumor immunity.

Nat Commun. 2021 Jun 23;12(1):3880.