ESMO ADVANCED COURSE ON LUNG CANCER IN IMMUNOTHERAPY

Biomarkers

John Haanen MD PhD

Zürich, 3-4 July 2019

Netherlands Cancer Institute, Amsterdam
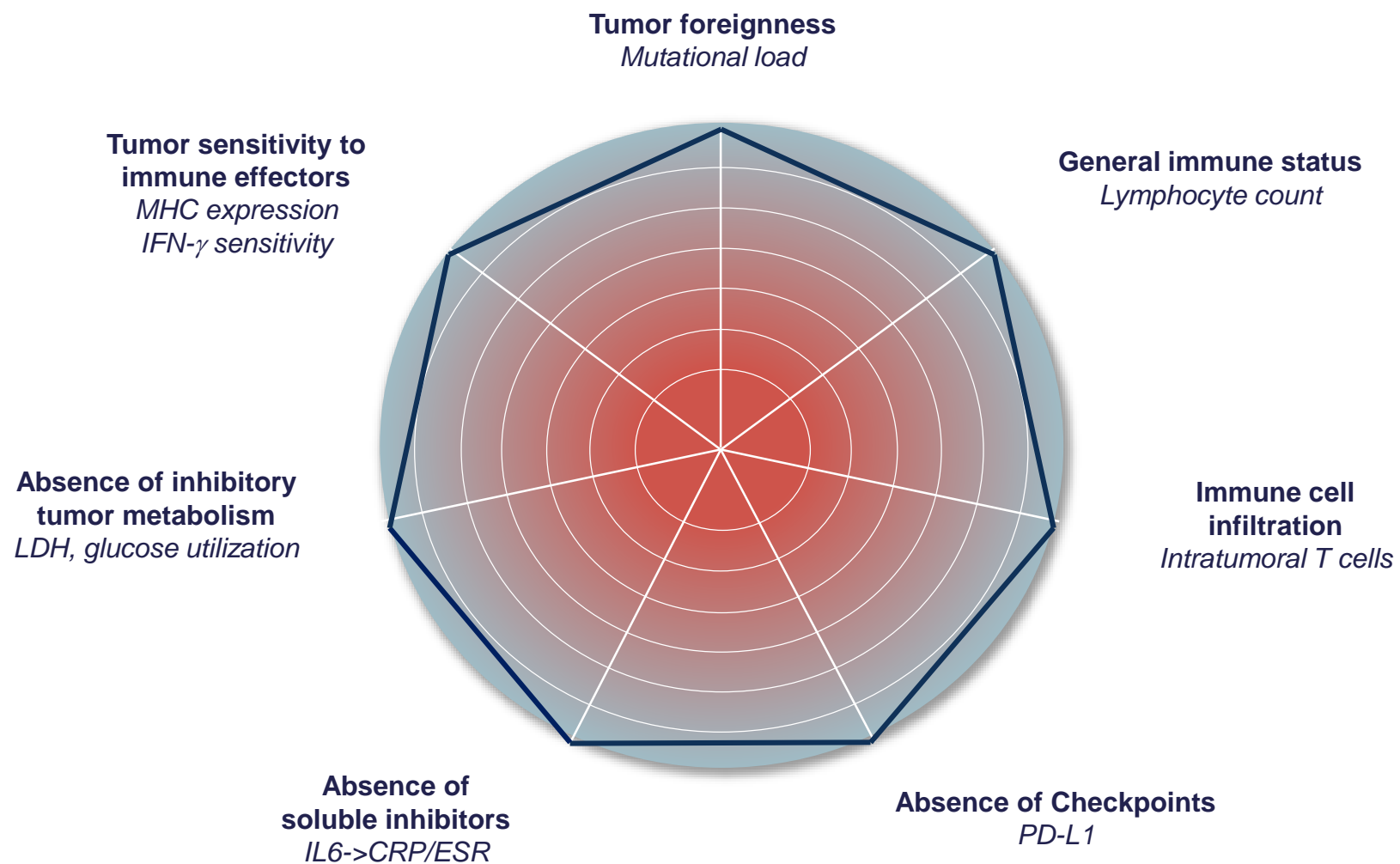
DISCLOSURE OF INTEREST

I have provided consultation, attended advisory boards, and/or provided lectures for: **Amgen, AZ/Medimmune, Bayer, BMS, GSK, Ipsen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics** for which NKI received honoraria

I am on the **SAB** of **AIMM, Celsius Therapeutics, Immunocore and Neon Therapeutics.** Financial compensation goes to NKI

Through my work NKI received grant support from **Bayer**, **BMS, MSD, Novartis, Neon Therapeutics, Pfizer**

The Cancer Immunogram

Describing the state of Cancer - Immune interaction

- **Tumor foreignness** — *Mutational load*
- **General immune status** — *Lymphocyte count*
- **Immune cell infiltration** — *Intratumoral T cells*
- **Absence of Checkpoints** — *PD-L1*
- **Absence of soluble inhibitors** — *IL6->CRP/ESR*
- **Absence of inhibitory tumor metabolism** — *LDH, glucose utilization*
- **Tumor sensitivity to immune effectors** — *MHC expression / IFN-γ sensitivity*

Blank et al. Science 2016

Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

- PD-L1 IHC
- Tumor Mutational Burden (WES/WGS or targeted panel seq)
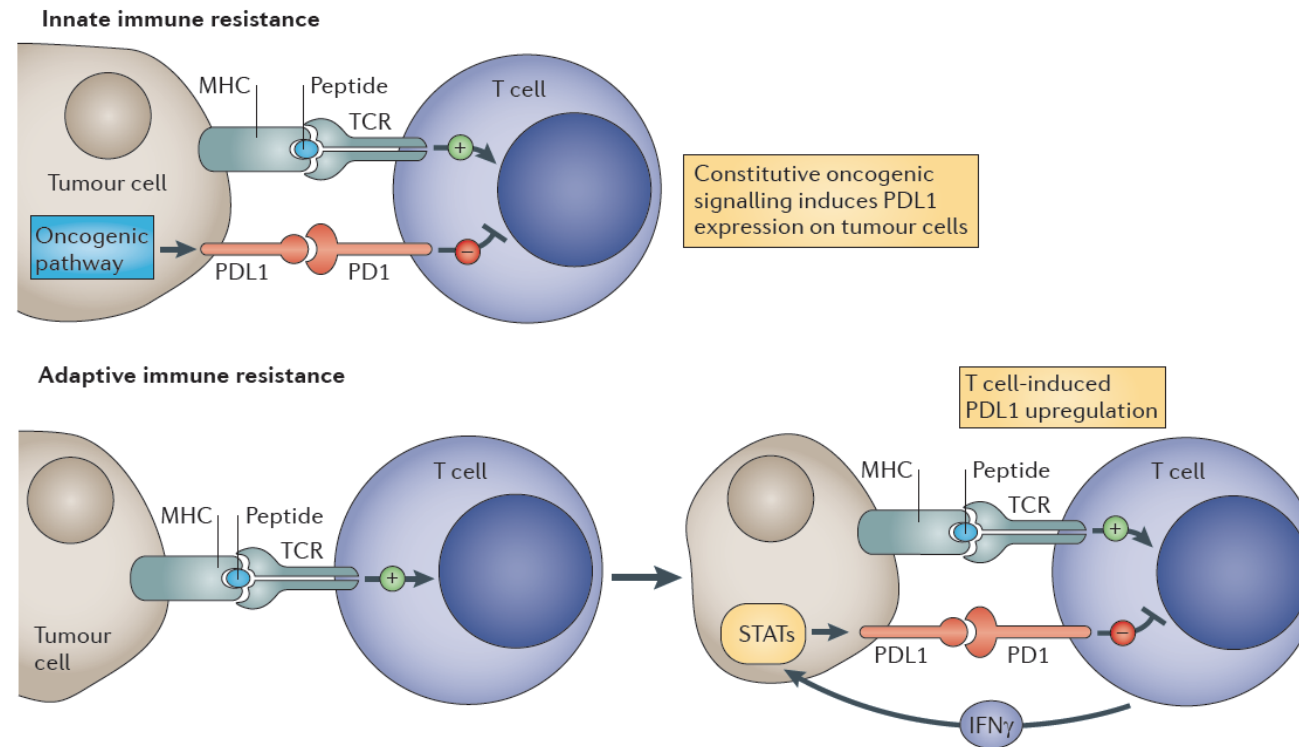- TIL (IHC)
- Gene Expression Profiling

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# Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

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- Gene Expression Profiling

PD-L1 on human tumor cells mediates T cell inhibition

Categorical vs continuous biological variable as predictive biomarker of therapeutic benefit

Ross Camidge et al., Nat Rev Clin Oncol 2019
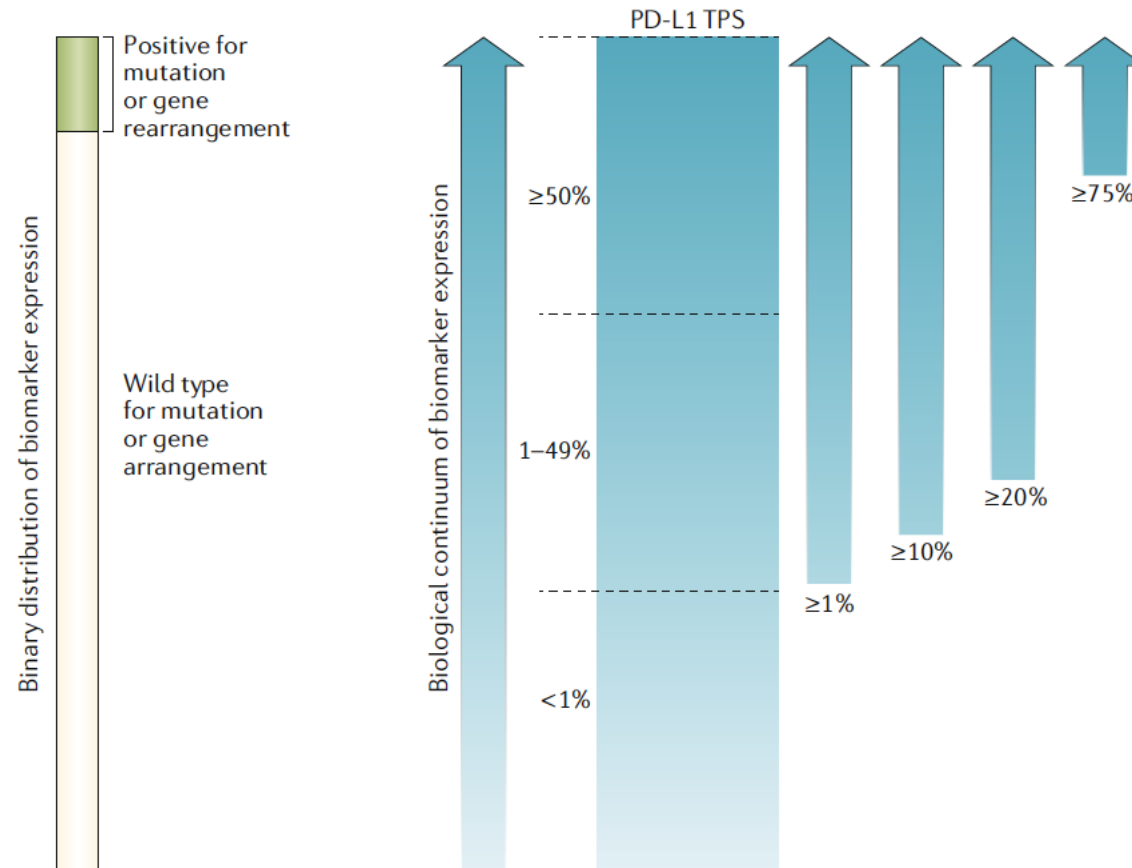
Five-drug PD-L1 assay trial-validated combinations

Drug	Company	PD-L1 Diagnostic Ab Clone	Staining Platform	Clinically Relevant Cutoffs[a]
Nivolumab	Bristol-Myers Squibb	28-8 (Dako)	Dako Link 48	TC $\geq$ 1%, 5%, and 10%
Pembrolizumab	Merck/Merck Sharp and Dohme	22C3 (Dako)	Dako Link 48	TC $\geq$ 1% and 50%
Atezolizumab	Genentech/Roche	SP142 (Ventana)	Ventana BenchMark ULTRA	TC $\geq$ 1%, 10%, and 50% IC $\geq$ 1%, 5%, and 10%
Durvalumab	AstraZeneca	SP263 (Ventana)	Ventana Benchmark	TC $\geq$ 25%
Avelumab	Pfizer/Merck Serono	73-10 (Dako)	Dako Link 48	TC $\geq$ 1%, 50%, and 80%

[a]Variable according to trials and line of therapy.
Modified with permission from Tsao et al.[9]

PD-L1, programmed death ligand 1; Ab, antibody; TC, tumor cells by percentage staining for PD-L1; IC, percentage of tumor area infiltrated by PD-L1-positive immune cells.

PD-L1 as a Biomarker: A summum in complexity

- Inter and intratumor heterogeneity
- Inducible and dynamic (IFN, post-treatment)
- Cell type (immune cell versus tumor versus both)
- Location (membrane versus cytoplasm)

Expression of PD-L1 is heterogeneous[1]

- Epitope stability
- Distribution (patchy versus diffuse)
- Different antibodies and platforms
- Different thresholds for expression
- Interobserver readability

Abs are not identical: >25% discordant[1,6,7]

Challenges Surrounding Biomarker

Logistics: Tissue[1,8,9]

- Interval between tissue and treatment (archived versus fresh)
- Primary versus metastatic disease
- Some circumstances not amenable to obtaining any tissue

IFN = interferon; PD-L1 = programmed death ligand 1.
1. McLaughlin J et al. *JAMA Oncol.* 2015 doi: 10.1001/jamaoncol.2015.3638. [Epub ahead of print]. 2. Heskamp S et al. *Cancer Res.* 2015. [Epub ahead of print]. 3. Pardoll DM. *Nat Rev Cancer*. 2012;12:252-264. 4. Wilson BE et al. *J Immunol Methods*. 1991;139:55-64. 5. Phillips T et al. *Appl Immunohistochem Mol Morphol*. 2015;23(8):541-549. 6. Rimm D et al. *Breast Cancer Res Treat.* 2014;147(2):457-458. 7. Velcheti V et al. *Lab Invest*. 2014;94(1):107-116. 8. Check W. *Cap Today*. 2010. 9. Warth A et al. *Recent Results Cancer Res.* 2015;199:71-84. Courtesy of Martin reck

PD-L1 Immunohistochemistry:
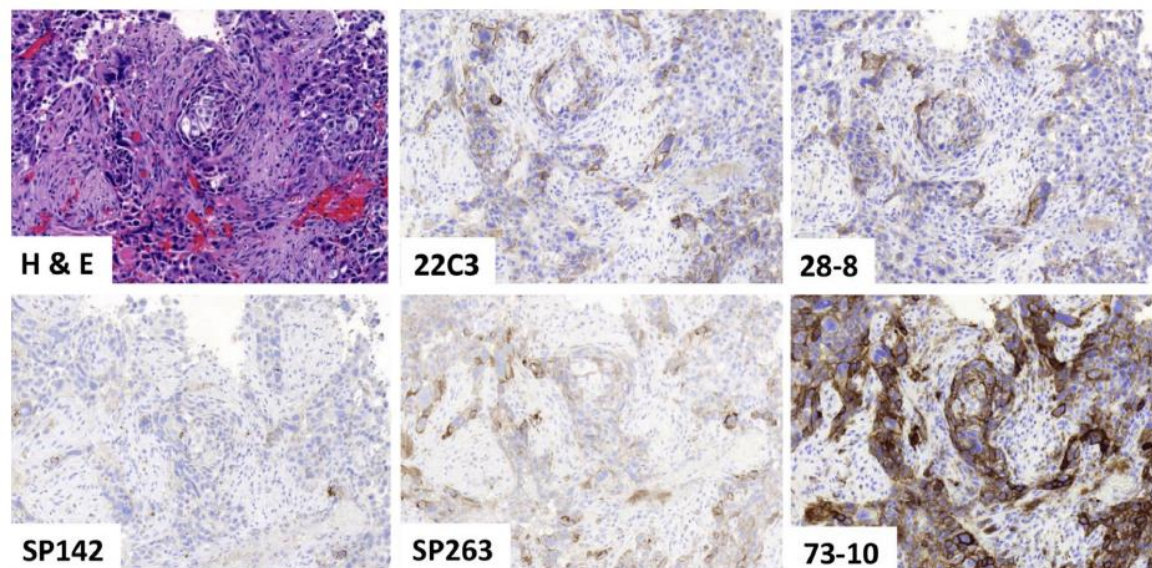# Results from the Blueprint 2 project

Table 1. Reliability (Intraclass Correlation Coefficient) of Scoring PD-L1 Expression on Tumor Cells among All Pathologists (Excluding the Trainer) for All Cases and NSCLC Biopsy Samples/Resected Cases

Assay	Glass Slide Scoring		Digital Scoring	
	All Cases	NSCLC Tissue Only	All Cases	NSCLC Tissue Only
22C3	0.89	0.88	0.91	0.91
28-8	0.92	0.94	0.86	0.88
SP-142	0.88	0.86	0.80	0.84
SP-263	0.89	0.92	0.90	0.93
73-10	0.93	0.95	0.91	0.93
All assays	0.86	0.89	0.91	0.93

PD-L1, programmed death ligand 1.

Tsao et al., JTO 2018
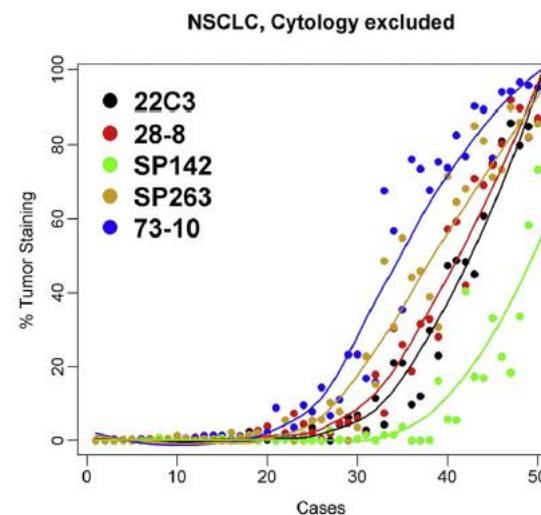
Potential explanations why PD-L1 expression might not predict benefit from PD-1/PD-L1 inhibition

Hypothesis	Evidence	Potential explanations
PD-L1 expression apparently not necessary	PD-L1 absent by IHC but clinical benefit seen from inhibition of PD-1 or PD-L1	• Spatial and/or temporal variability in PD-L1 expression within tumour (sampling error) • Incomplete sensitivity of IHC in the detection of PD-L1, with variation between assays (false-negative result) • PD-L2, the alternative ligand for PD-1, could provide a bypass mechanism for immunosuppression, leading to responses of PD-L1⁻ tumours to anti-PD-1 antibodies, although in theory, not to anti-PD-L1 antibodies
PD-L1 expression apparently not sufficient	PD-L1 present by IHC but no clinical benefit from inhibition of PD-1 or PD-L1	• Elevation in PD-L1 expression for reasons other than in response to a primed immune attack (for example, intrinsic induction in some oncogene-addicted NSCLCs) • Engagement of other immune checkpoints in addition to the PD-1–PD-L1 axis and/or immune suppression or deficiencies with different causes • The measured extent of PD-L1 positivity (a continuous variable) might be insufficient for a response to PD-1 or PD-L1 inhibition, reflecting substantial heterogeneity in the underlying tumour biology (including neoantigen profiles and mechanisms of immune escape)

- PD-L1 IHC is a validated and approved biomarker
- It is the only 'drug specific' biomarker
- Clinically it can be reliably delivered
- It does enrich for treatment benefit

- But it is not perfect
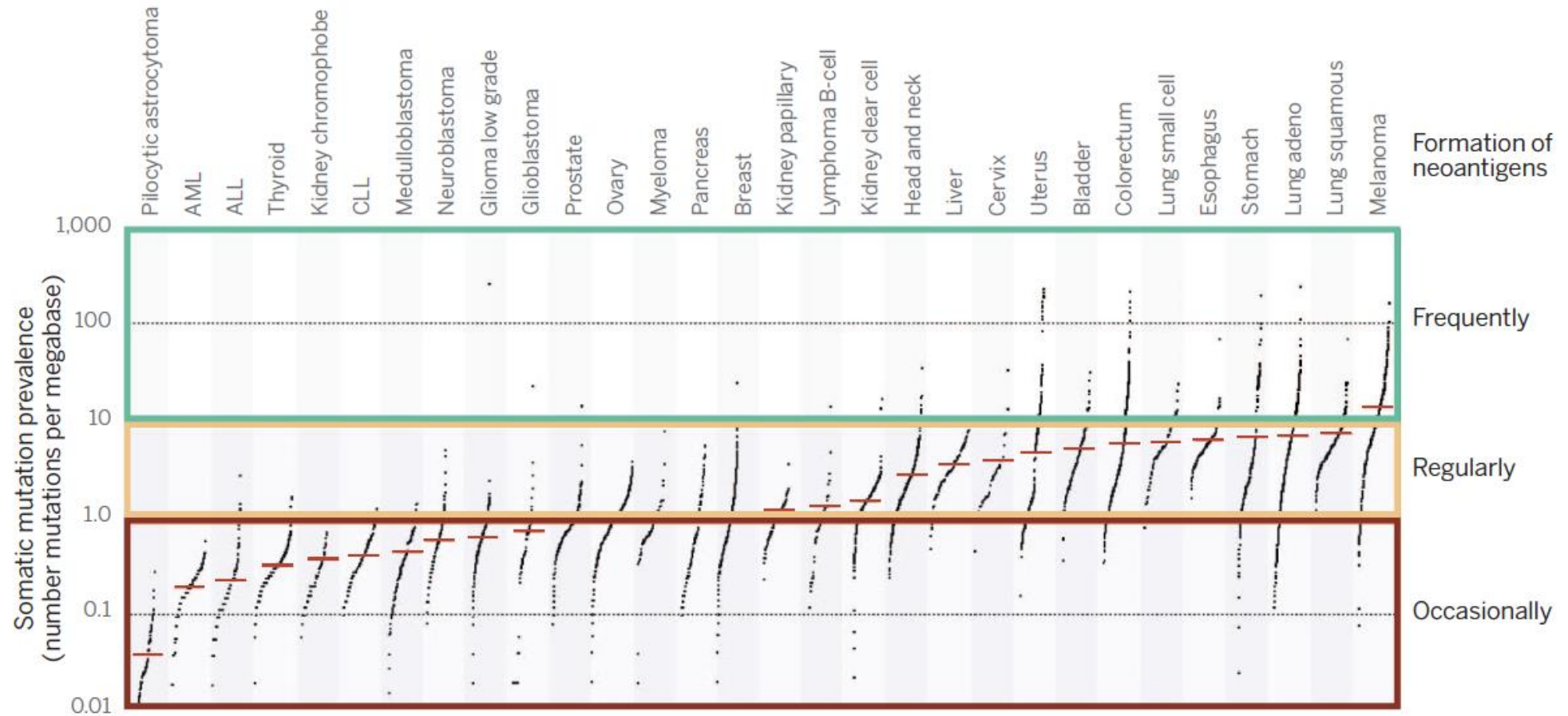- Implementation can be complicated

Solid predictive biomarkers of response or resistance to IO treatment are lacking
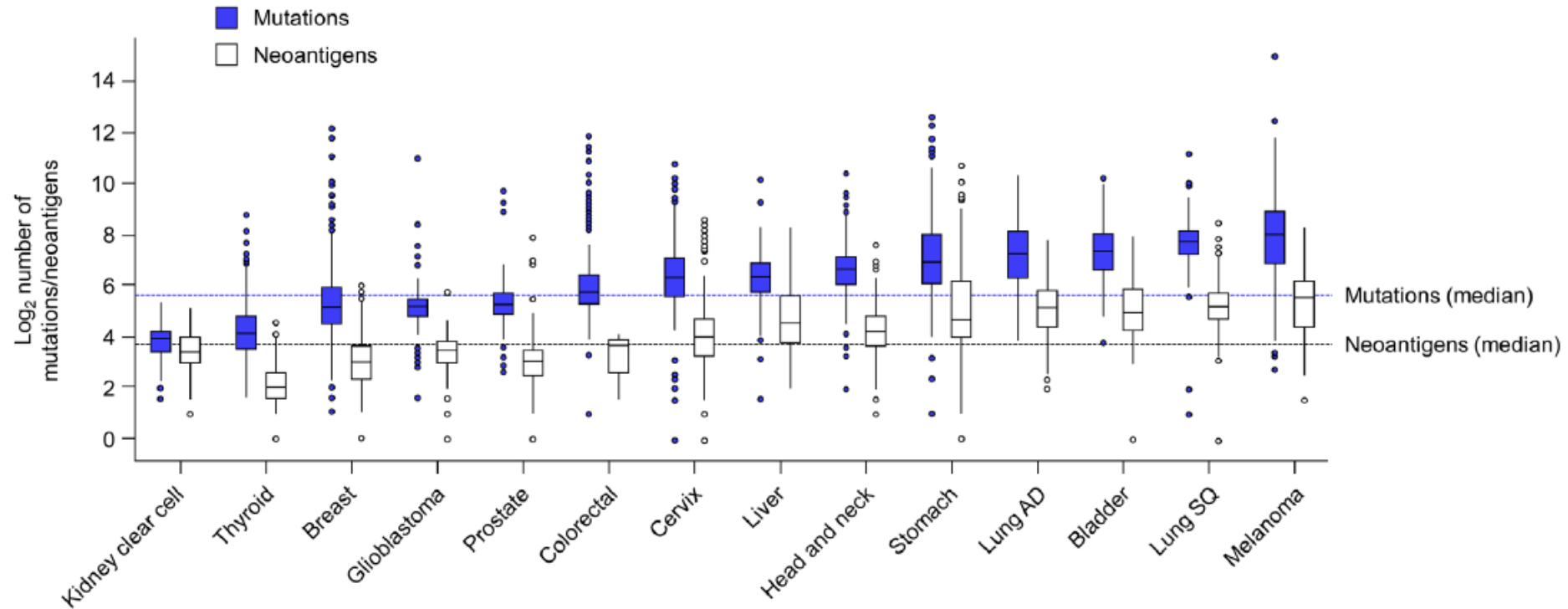
What do we have so far?

- PD-L1 IHC
- **Tumor Mutational Burden (WES/WGS or targeted panel seq)**
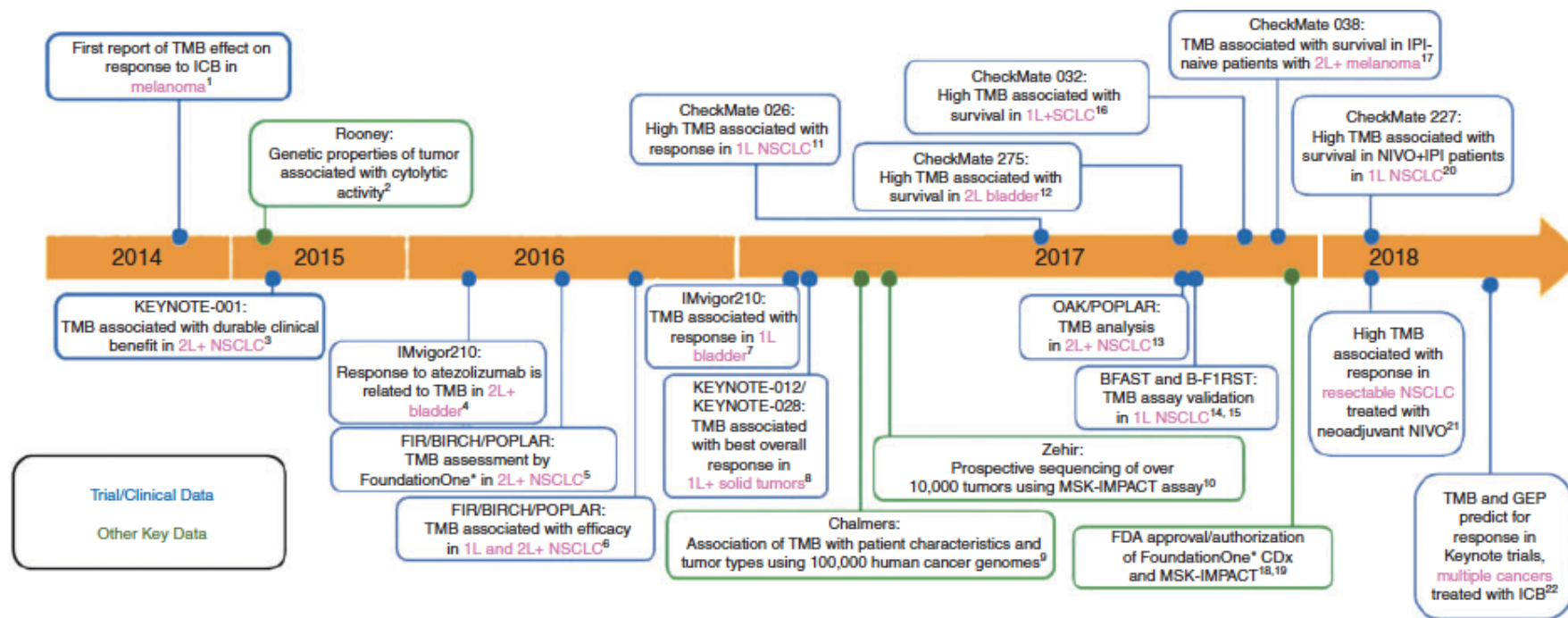- TIL (IHC)
- Gene Expression Profiling

# Estimate of the neoantigen repertoire in human cancers

Alexandrov, Nature 2013; Schumacher & Schreiber Science 2016

Comparison between mutational load and neoantigen load:
Not a 1:1 relationship per se

Evolvement of TMB over time as a potential biomarker of response to immunotherapy

Connection between TMB, neoantigens and immunotherapy

Chan et al. Ann Oncol 2019
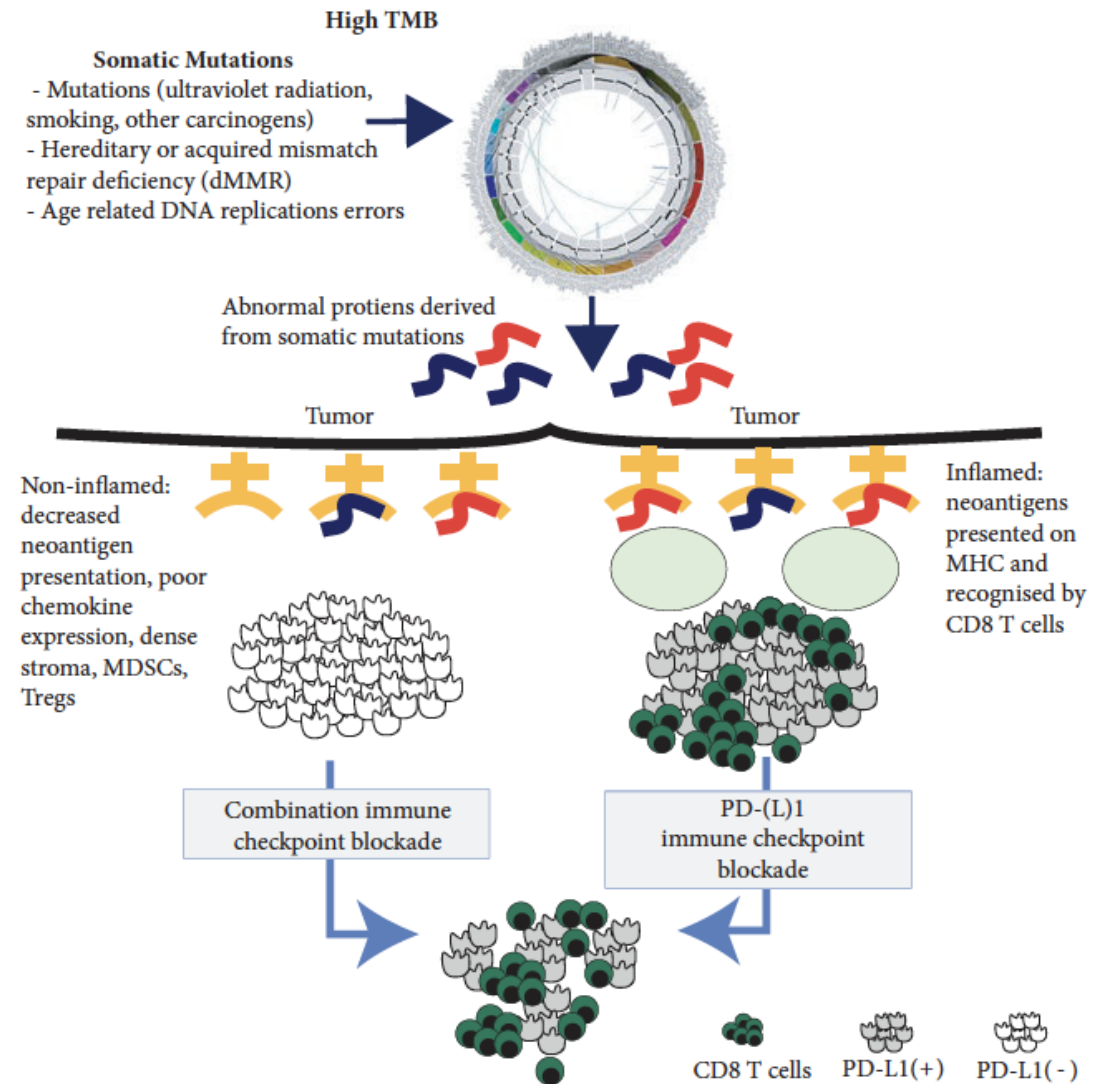
Clinical evidence demonstrating TMB as a biomarker for response to immunotherapy

Immunotherapy agent and tumour type	Study/trial*	TMB assay used	Type of benefit
Nivolumab			
NSCLC (1 L)	CheckMate 026[52]	WES	ORR, PFS
NSCLC	Flatiron Health[117]	Foundation CGP panel	OS
Melanoma (1 L or 2 L)	CheckMate 038[22]	WES	ORR, OS, PFS
Melanoma	CheckMate 064[23]	WES	ORR, OS
Bladder	CheckMate 275[83]	WES	ORR, OS, PFS
GBM	Bouffet et al, 2016[118]	WES	DRR
Ipilimumab			
Melanoma	Van Allen et al, 2015[119]	WES	CBR
	Snyder et al, 2014[86]	WES	CBR, OS
Nivolumab and ipilimumab in combination			
NSCLC (1 L)	CheckMate 012[35]	WES	ORR, DCB, PFS
NSCLC (1 L)	CheckMate 227†[8]	FoundationOne CDx	ORR, PFS
NSCLC (1 L)	CheckMate 568[9]	FoundationOne CDx	ORR
SCLC (2 L)	CheckMate 032[84]	WES	ORR, OS, PFS
Pembrolizumab			
NSCLC (1 L)	KEYNOTE-001[36]	WES	ORR, DCB, PFS
CRC	Le et al, 2015[44]	WES	ORR, PFS
Multiple solid tumours	KEYNOTE-012/KEYNOTE-028[120][121]	WES	ORR

Büttner et al., ESMO Open 2019

TMB has predictive predictive value for IO activity across cancer types
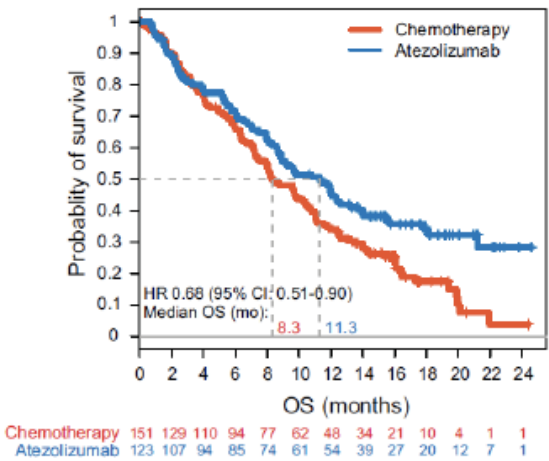
Melanoma and anti-CTLA-4
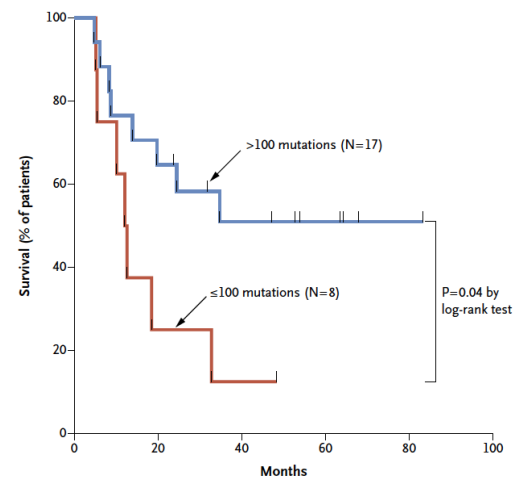
Melanoma and anti-PD-1
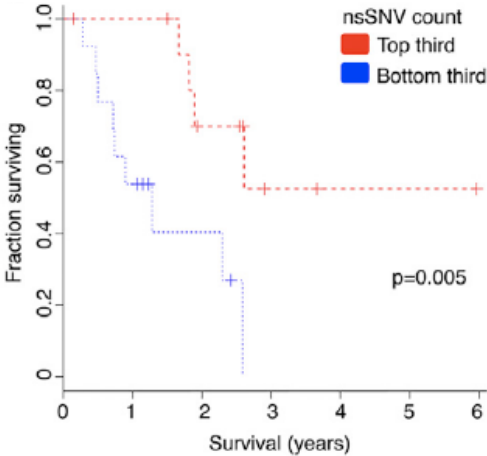
Bladder cancer and anti-PD-L1
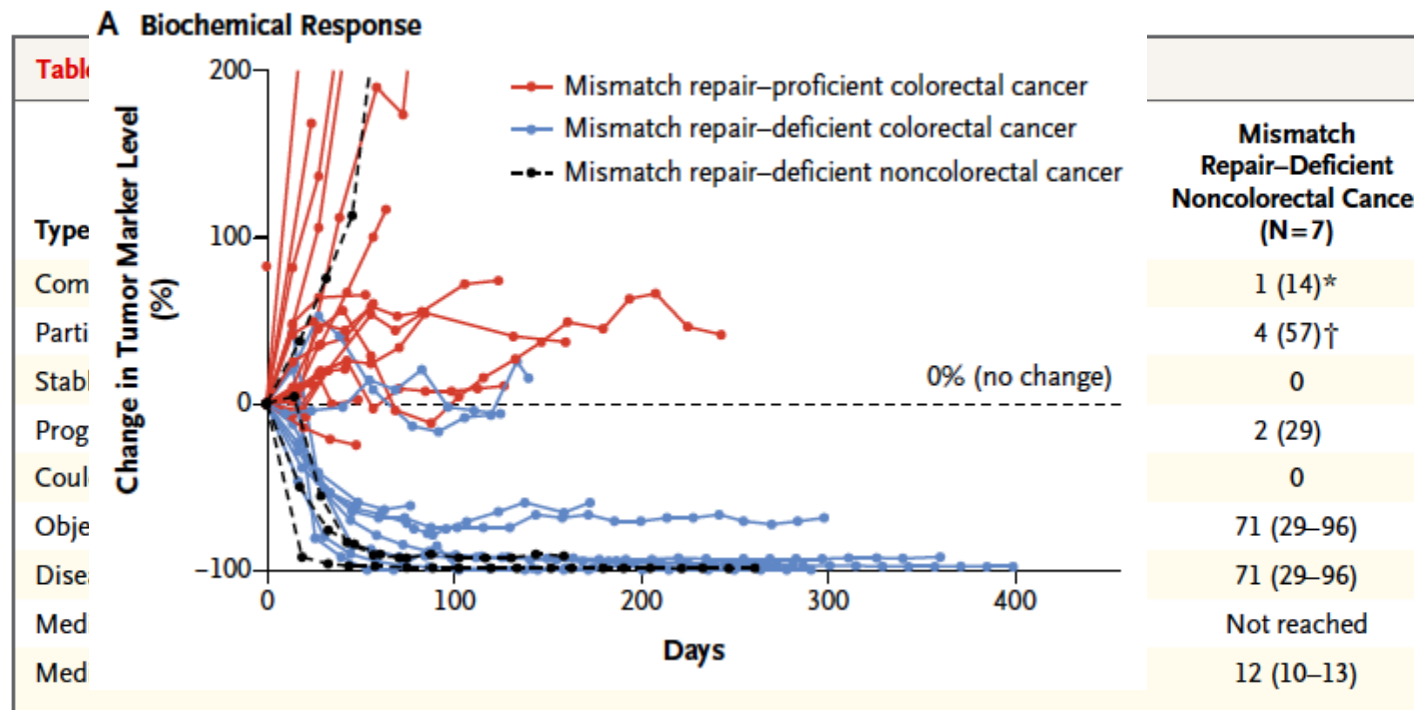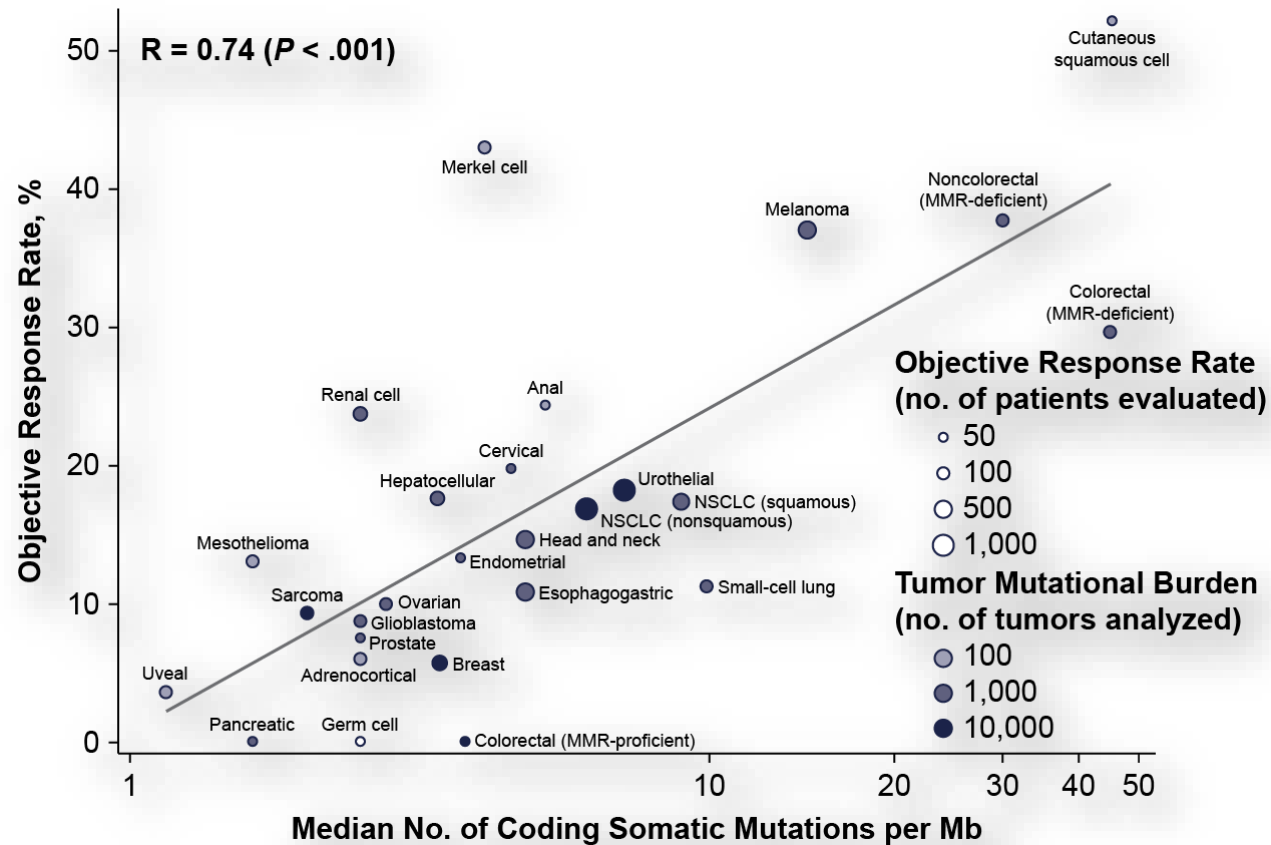

Colon cancer and anti-PD-1
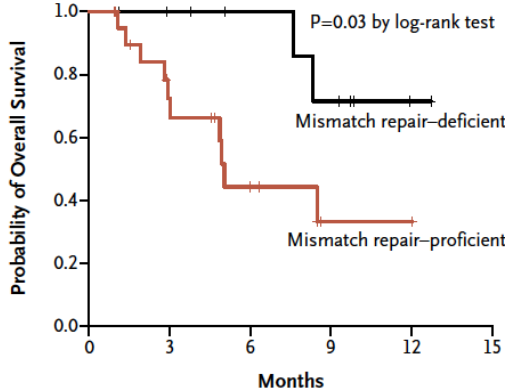
Snyder, N Engl J Med. 2014; Le DT , N Engl J Med. 2015; Van Allen EM , Science. 2015 ; Hugo , Cell. 2016 ; Powles, Lancet 2018

Requirements for a response to anti-PD1: MSI-high tumors

TMB correlates to tumor type specific response rate

NGS gene panels to assess TMB

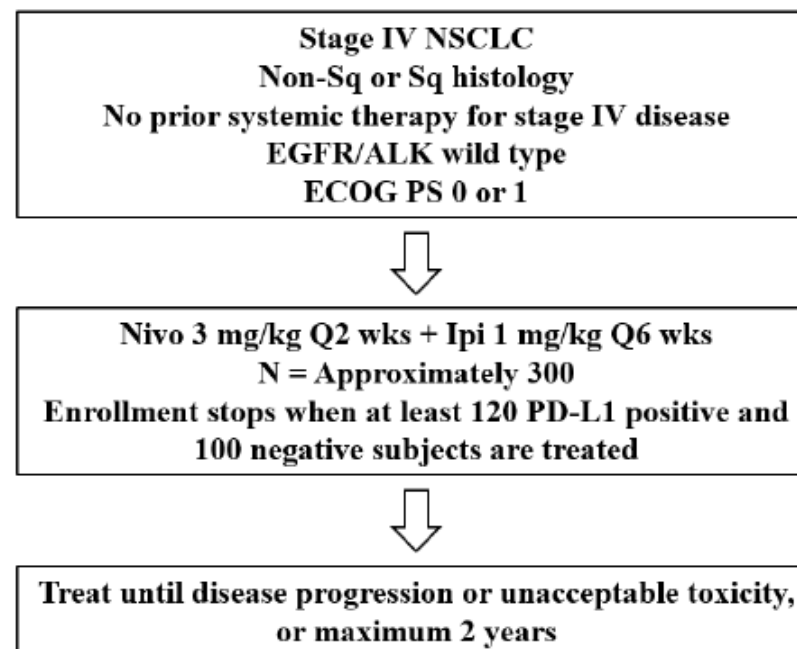| Status	Test name	Number of genes	Coverage (Mb)*	Gene variants	Sample type
FDA-approved or authorised diagnostic assays†	MSK-IMPACT[15][56][68]	468	1.5	SNVs, indels, rearrangements/fusions, CNAs, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
	Foundation Medicine FoundationOne CDx[14][49]	324	0.8	SNVs, indels, CNAs, select rearrangements, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
Commercial assays for research use only	Caris Molecular Intelligence[132]	592	1.4	Somatic missense mutations	FFPE
	Illumina TruSight 500 gene panel[133]	500	2.0	SNVs and indels	FFPE
	Thermo Fisher Scientific Oncomine Tumor Mutation Load Assay[77]	409	1.7	SNVs	FFPE
	NEO New Oncology NEOplus v2 RUO[134]	>340	1.1	SNVs, indels, fusions, CNAs, parallel analysis of TMB, MSI, and driver mutations	FFPE
	Foundation Medicine FoundationOne[50]	315	1.1	SNVs, indels, CNAs, select gene rearrangements, genomic signatures for MSI and TMB	FFPE
	Foundation Medicine bTMB assay[88][122]	394	1.1	SNVs	Blood
	TruSight Tumor 170[135]	170	0.5	Fusions, splice variants, SNVs, indels, amplifications	FFPE
	QIAGEN GeneRead DNAseq Comprehensive Cancer Panel[97]	160	0.7	SNVs, CNAs, indels, and fusions	FFPE
	NEO New Oncology NEOplus[105][136]	94		SNVs, indels, CNAs, rearrangements, and fusions	FFPE

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TMB is not correlated to PD-L1 Expression
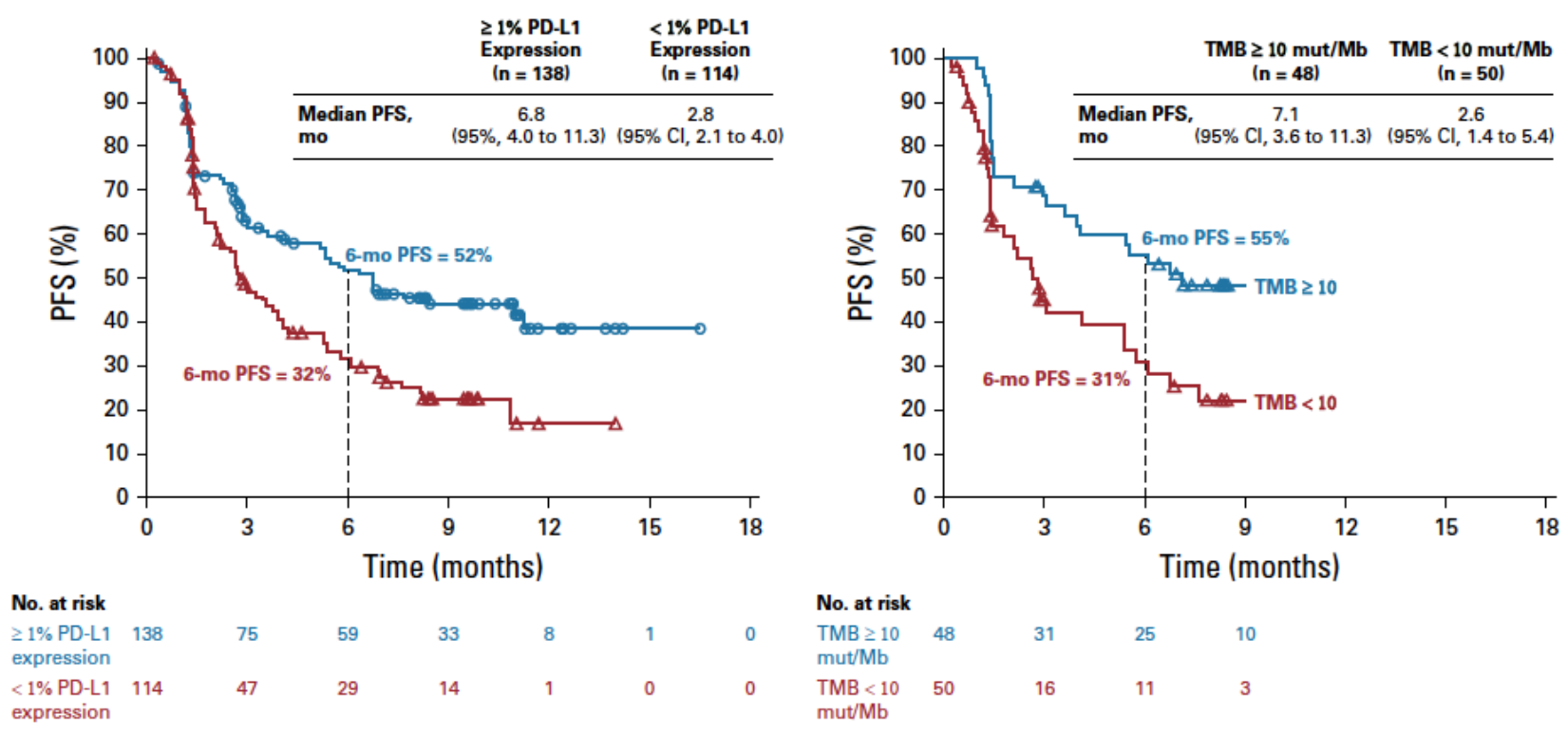## Both biomarkers might be additive
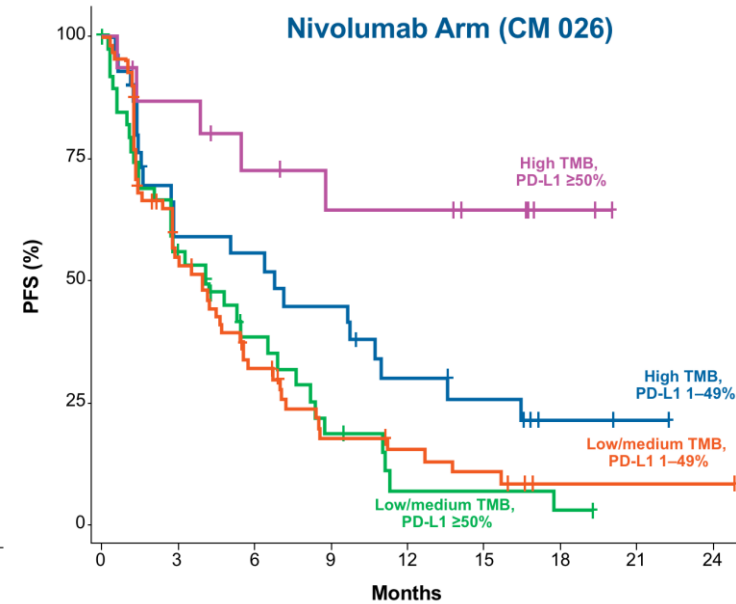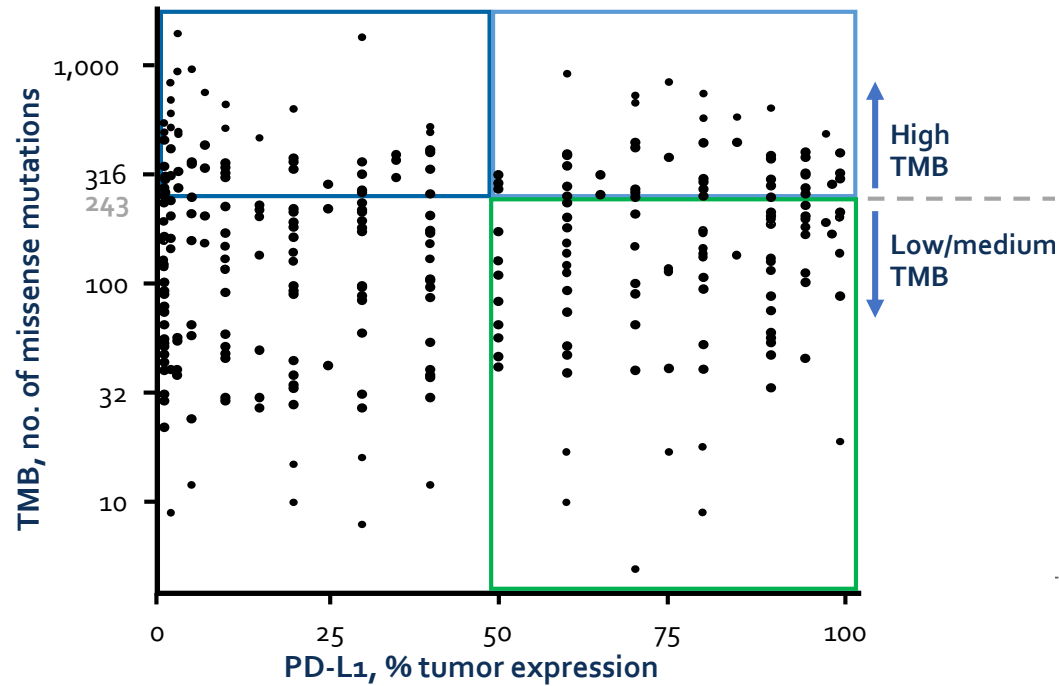
First-Line Nivolumab Plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers

Neal Ready, MD, PhD[1]; Matthew D. Hellmann, MD[2]; Mark M. Awad, MD, PhD[3]; Gregory A. Otterson, MD[4]; Martin Gutierrez, MD[5]; Justin F. Gainor, MD[6]; Hossein Borghaei, DO[7]; Jacques Jolivet, MD[8]; Leora Horn, MD[9]; Mihaela Mates, MD[10]; Julie Brahmer, MD[11]; Ian Rabinowitz, MD[12]; Pavan S. Reddy, MD[13]; Jason Chesney, MD, PhD[14]; James Orcutt, MD[15]; David R. Spigel, MD[16]; Martin Reck, PhD[17]; Kenneth John O'Byrne, MD[18]; Luis Paz-Ares, MD, PhD[19]; Wenhua Hu, PhD[20]; Kim Zerba, PhD[20]; Xuemei Li, MD[20]; Brian Lestini, MD, PhD[20]; William J. Geese, PhD[20]; Joseph D. Szustakowski, PhD[20]; George Green, PhD[20]; Han Chang, PhD[20]; and Suresh S. Ramalingam, MD[21]

original report

Stage IV NSCLC
Non-Sq or Sq histology
No prior systemic therapy for stage IV disease
EGFR/ALK wild type
ECOG PS 0 or 1

⬇

Nivo 3 mg/kg Q2 wks + Ipi 1 mg/kg Q6 wks
N = Approximately 300
Enrollment stops when at least 120 PD-L1 positive and 100 negative subjects are treated

⬇

Treat until disease progression or unacceptable toxicity, or maximum 2 years

# Progression free survival according to PD-L1 expression or TMB

ORR following ipilimumab + nivolumab according to PD-L1 or TMB

Variable	All Treated (N = 288)	< 1% PD-L1 Expression (n = 114)	≥ 1% PD-L1 Expression (n = 138)	≥ 50% PD-L1 Expression (n = 68)	PD-L1 Expression Not Quantifiable (n = 36)
Objective response					
No. of patients	86	17	57	34	12
Percentage of patients (95% CI)	29.9 (24.6 to 35.5)	14.9 (8.9 to 22.8)	41.3 (33.0 to 50.0)	50.0 (37.6 to 62.4)	33.3 (18.6 to 51.0)
Best overall response, No. (%)					
Complete response	7 (2.4)	3 (2.6)	4 (2.9)	3 (4.4)	0
Partial response	79 (27.4)	14 (12.3)	53 (38.4)	31 (45.6)	12 (33.3)
Stable disease	104 (36.1)	53 (46.5)	40 (29.0)	17 (25.0)	11 (30.6)
Progressive disease	76 (26.4)	36 (31.6)	31 (22.5)	13 (19.1)	9 (25.0)
Could not be determined	22 (7.6)	8 (7.0)	10 (7.2)	4 (5.9)	4 (11.1)

	TMB (mut/Mb)				
Variable	< 5 (n = 23)	≥ 5 to < 10 (n = 27)	≥ 10 (n = 48)	≥ 10 to < 15 (n = 20)	≥ 15 (n = 28)
Objective response					
No. of patients	2	4	21	10	11
Percentage of patients (95% CI)	8.7 (1.1 to 28.0)	14.8 (4.2 to 33.7)	43.8 (29.5 to 58.8)	50.0 (27.2 to 72.8)	39.3 (21.5 to 59.4)
Best overall response, No. (%)					
Complete response	0	1 (3.7)	4 (8.3)	2 (10.0)	2 (7.1)
Partial response	2 (8.7)	3 (11.1)	17 (35.4)	8 (40.0)	9 (32.1)
Stable disease	11 (47.8)	12 (44.4)	14 (29.2)	5 (25.0)	9 (32.1)
Progressive disease	6 (26.1)	7 (25.9)	13 (27.1)	5 (25.0)	8 (28.6)
Could not be determined	4 (17.4)	4 (14.8)	0	0	0

Receiver operating characteristic curves (ROC) of objective response rate by tumor programmed death li-gand 1 (PD-L1) expression and tumor mutational burden (TMB)
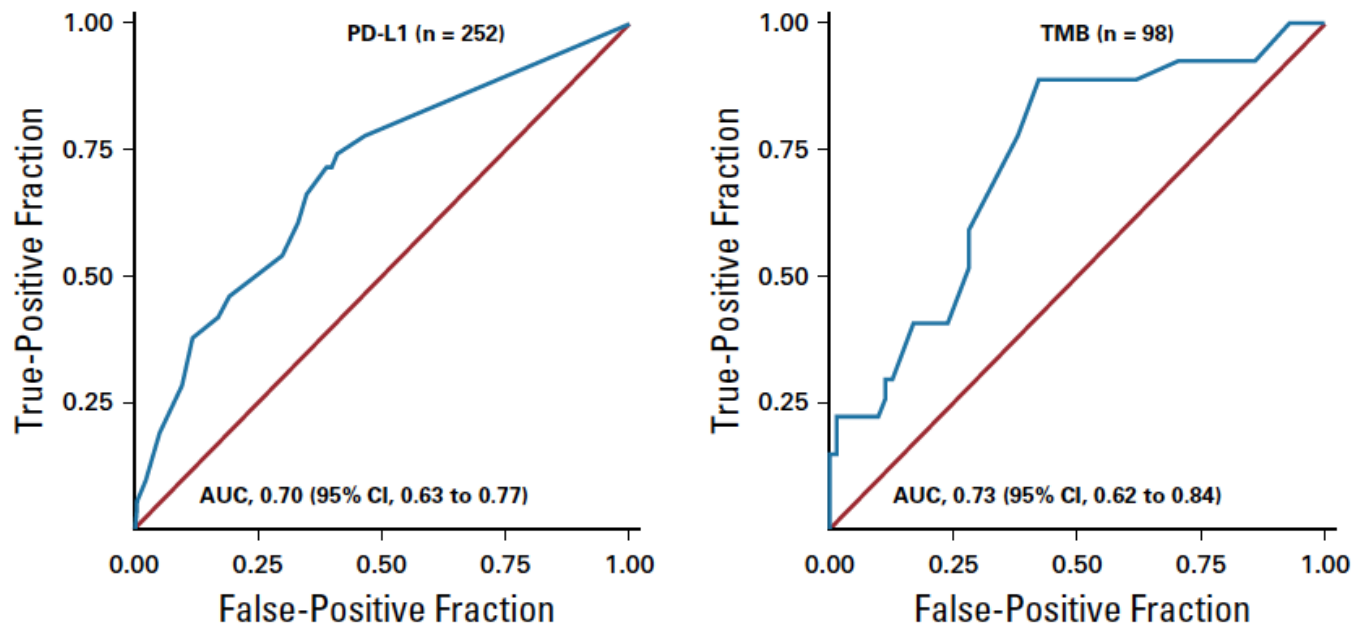
No data were provided on using the combination of both biomarkers

Ready et al. J Clin Oncol 2019
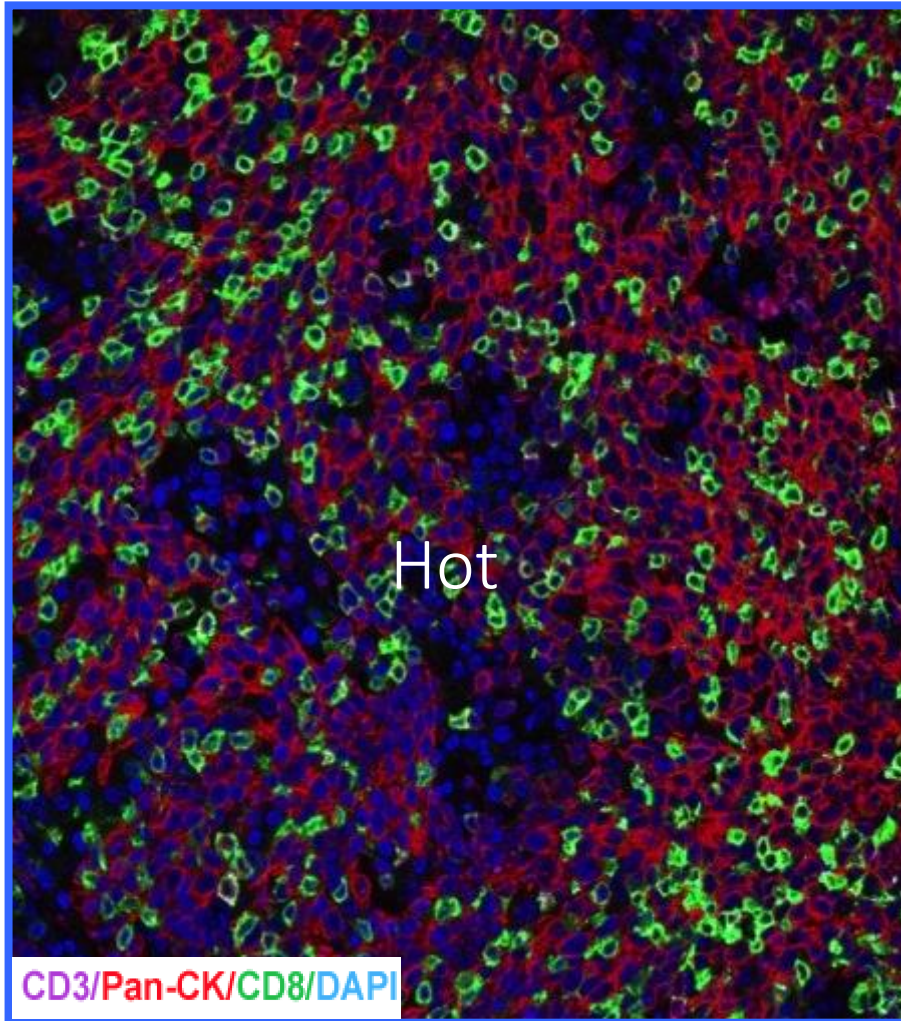
Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

- PD-L1 IHC
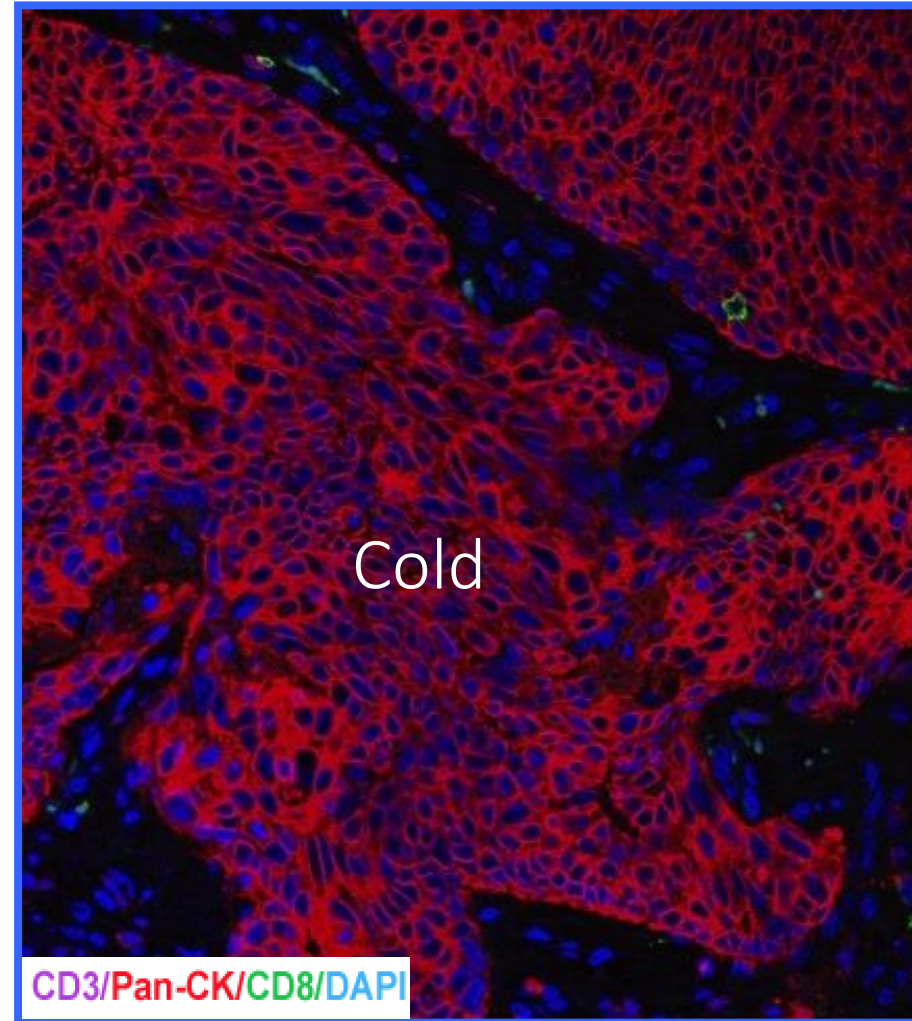- Tumor Mutational Burden (WES/WGS or targeted panel seq)
- **TIL (IHC)**
- **Gene Expression Profiling**

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Tumor Infiltrating Lymphocyten (TIL)

Diffuse infiltration with CD8+ TILs

Absence of TILs

Keck et al., Clin Canc Res 2014

Role for T cells in cancer

ORIGINAL ARTICLE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D.,
Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D.,
Marco Massobrio, M.D., Giorgia Regnani, M.D.,
Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D.,
Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D.,
Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

OXFORD

ARTICLE

Objective Measurement and Clinical Significance of TILs in Non–Small Cell Lung Cancer

Kurt A. Schalper, Jason Brown, Daniel Carvajal-Hausdorf, Joseph McLaughlin, Vamsidhar Velcheti, Konstantinos N. Syrigos, Roy S. Herbst, David L. Rimm

Science
AAAS

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Cancer Research

AACR

Immunotype and Immunohistologic Characteristics of Tumor-Infiltrating Immune Cells Are Associated with Clinical Outcome in Metastatic Melanoma

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TIL and cancer outcome

The association of immune cell infiltrates with prognosis in cancer
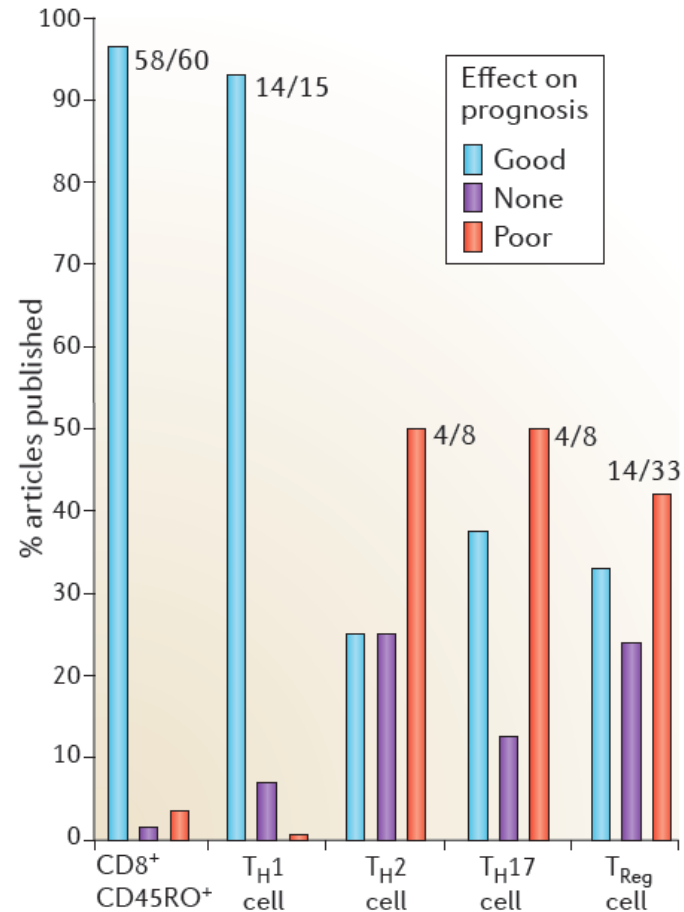
Patients with a pre-existing immune response derive the most benefit from checkpoint inhibitors

Teff/IFNγ: CD8A, GZMA, GZMB, CXCL9, EOMES, IFNg, CXCL10, T-bet

Fridman et al., NRC 2012; Fehrenbacher et al., Lancet 2016

Early pathological response to neoadjuvant anti-PD-1 or anti-CTLA-4 + anti-PD-1is correlated with improved survival

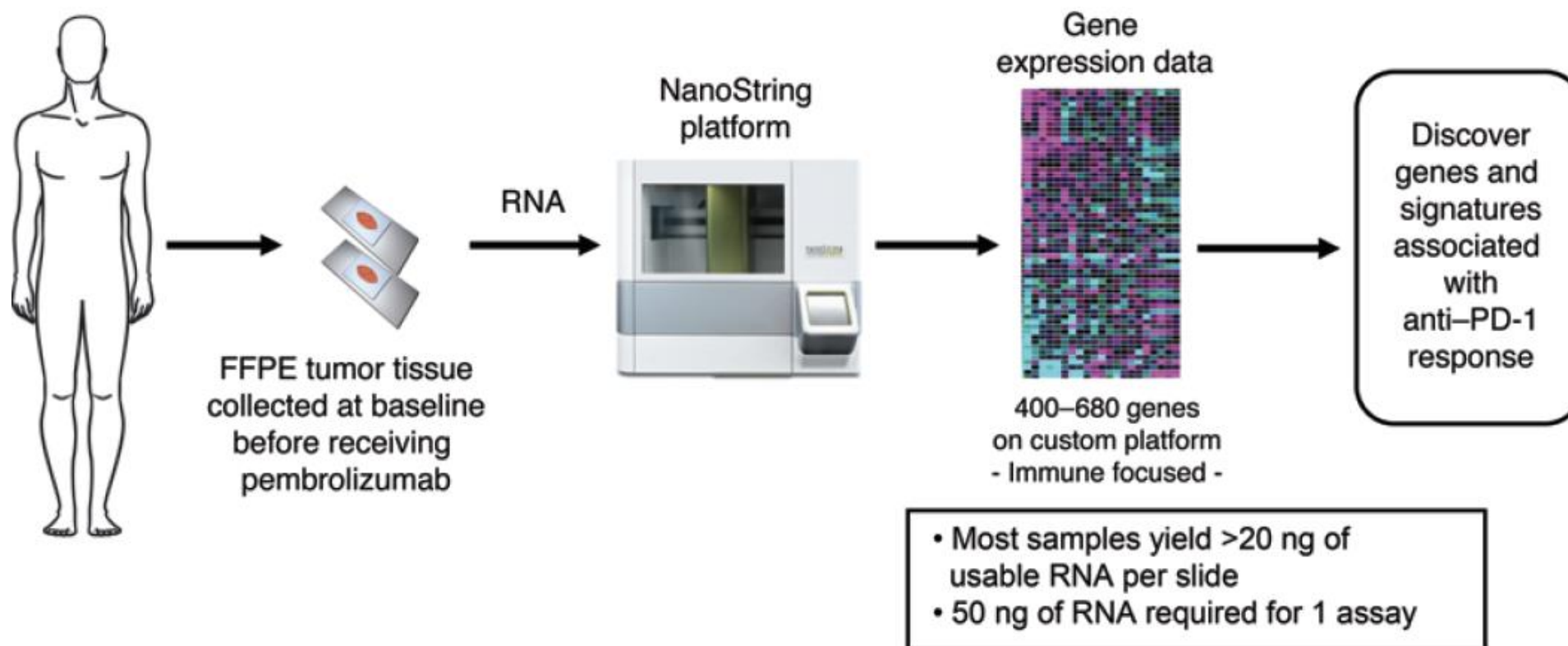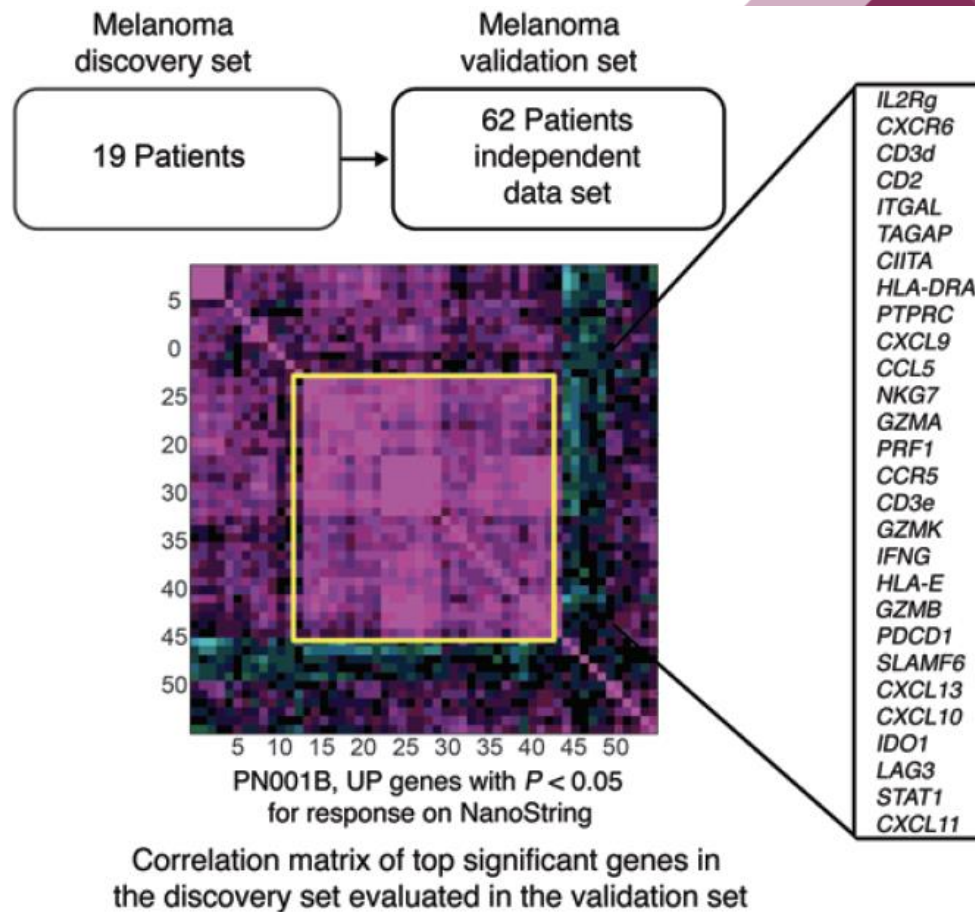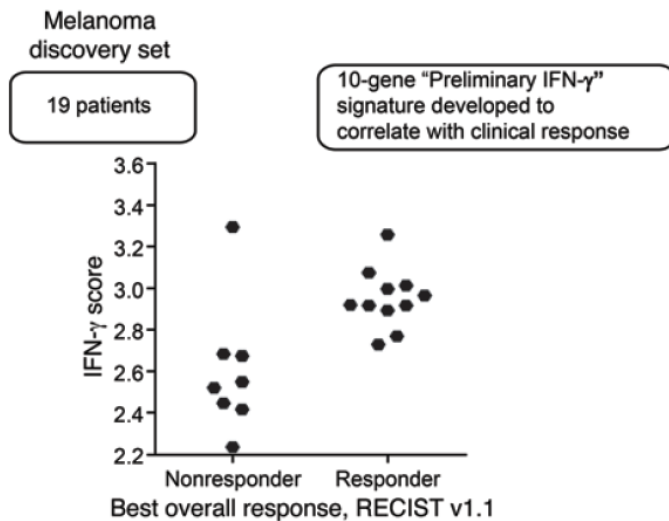Huang et al., Nat Med 2019, Blank et al., Nat Med 2018; Amaria et al., Nat Med 2018
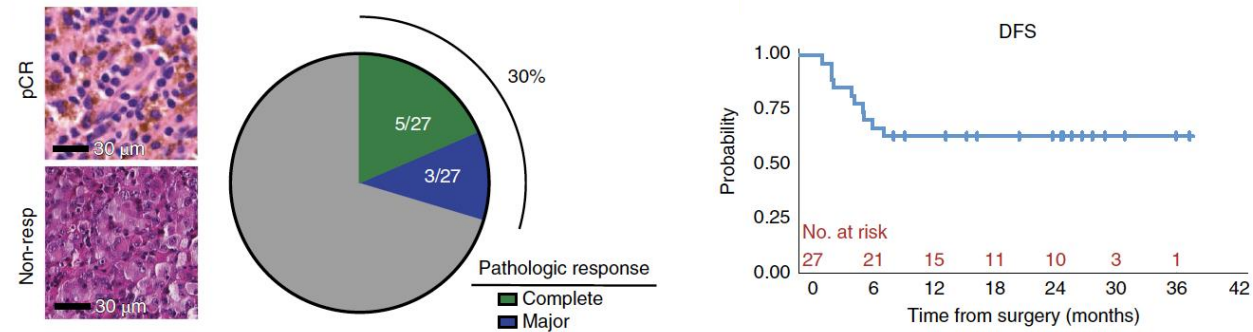
Early TIL infiltration following anti-PD-1 correlated with improved survival
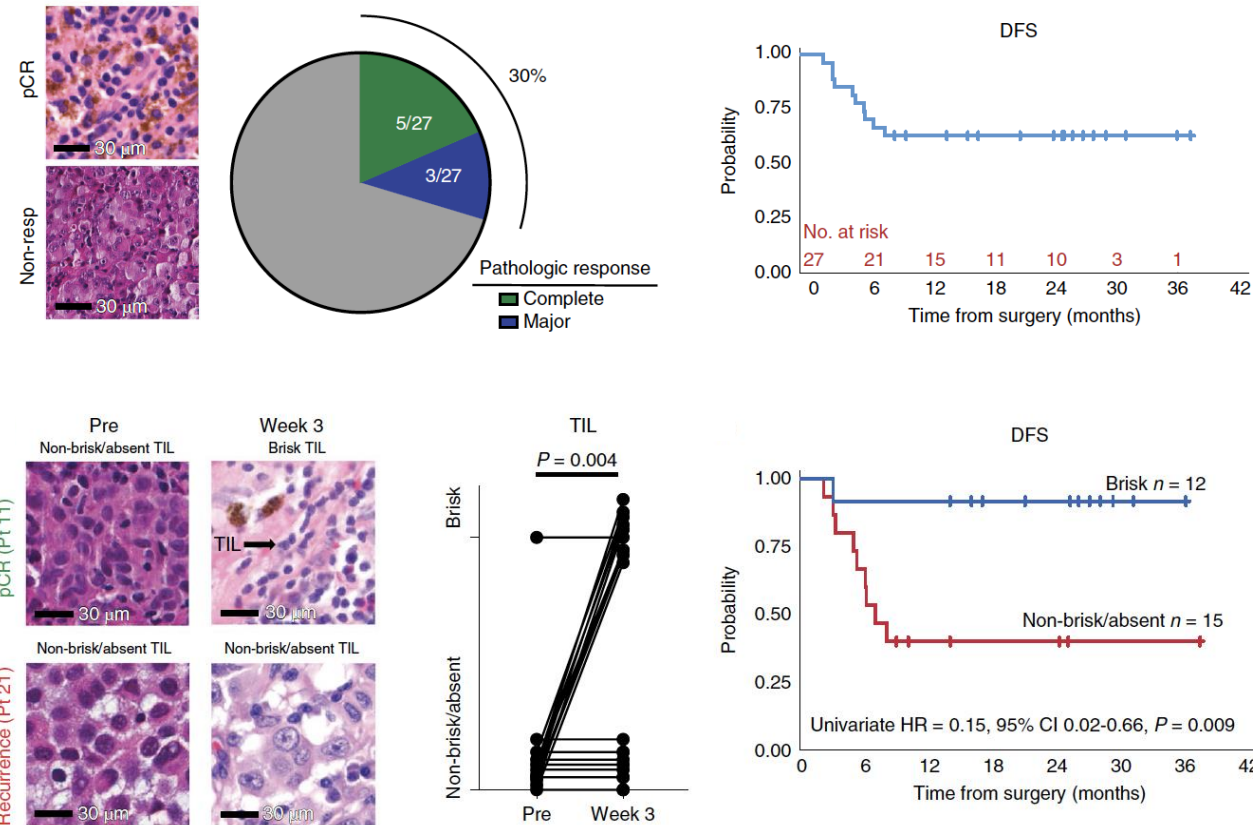
IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade
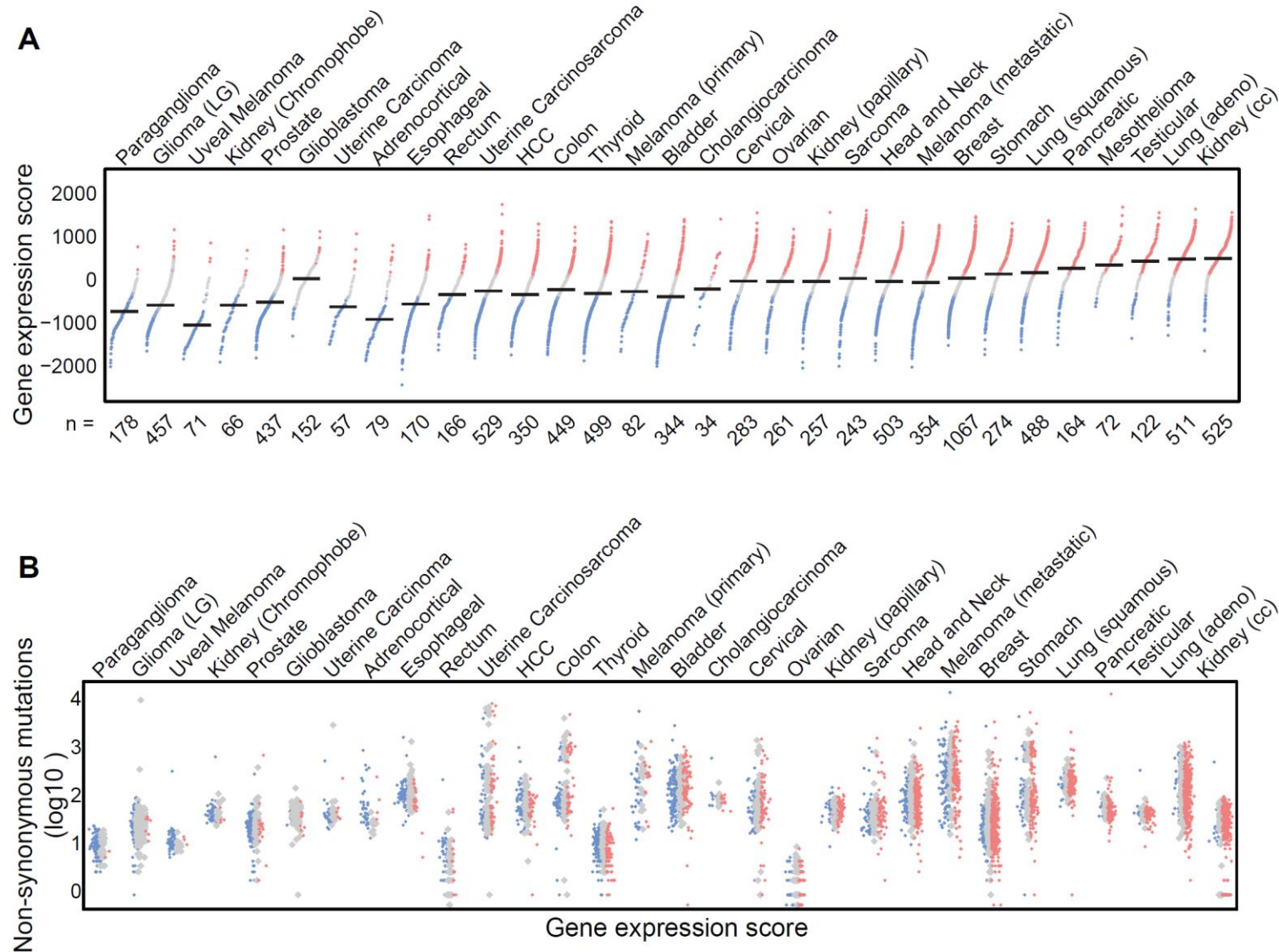
Mark Ayers,[1] Jared Lunceford,[1] Michael Nebozhyn,[1] Erin Murphy,[1] Andrey Loboda,[1] David R. Kaufman,[1] Andrew Albright,[1] Jonathan D. Cheng,[1] S. Peter Kang,[1] Veena Shankaran,[2] Sarina A. Piha-Paul,[3] Jennifer Yearley,[1] Tanguy Y. Seiwert,[4] Antoni Ribas,[5] and Terrill K. McClanahan[1]

Melanoma discovery set

19 patients

10-gene "Preliminary IFN-γ" signature developed to correlate with clinical response

Melanoma discovery set

19 Patients

Melanoma validation set

62 Patients independent data set

IL2Rg
CXCR6
CD3d
CD2
ITGAL
TAGAP
CIITA
HLA-DRA
PTPRC
CXCL9
CCL5
NKG7
GZMA
PRF1
CCR5
CD3e
GZMK
IFNG
HLA-E
GZMB
PDCD1
SLAMF6
CXCL13
CXCL10
IDO1
LAG3
STAT1
CXCL11

PN001B, UP genes with $P < 0.05$ for response on NanoString

Correlation matrix of top significant genes in the discovery set evaluated in the validation set

"Preliminary expanded immune" (28-gene) signature: coherent set correlated with the 10-gene "preliminary IFN-γ" signature genes (bolded text)

Best overall response, RECIST v1.1

IFN-γ score

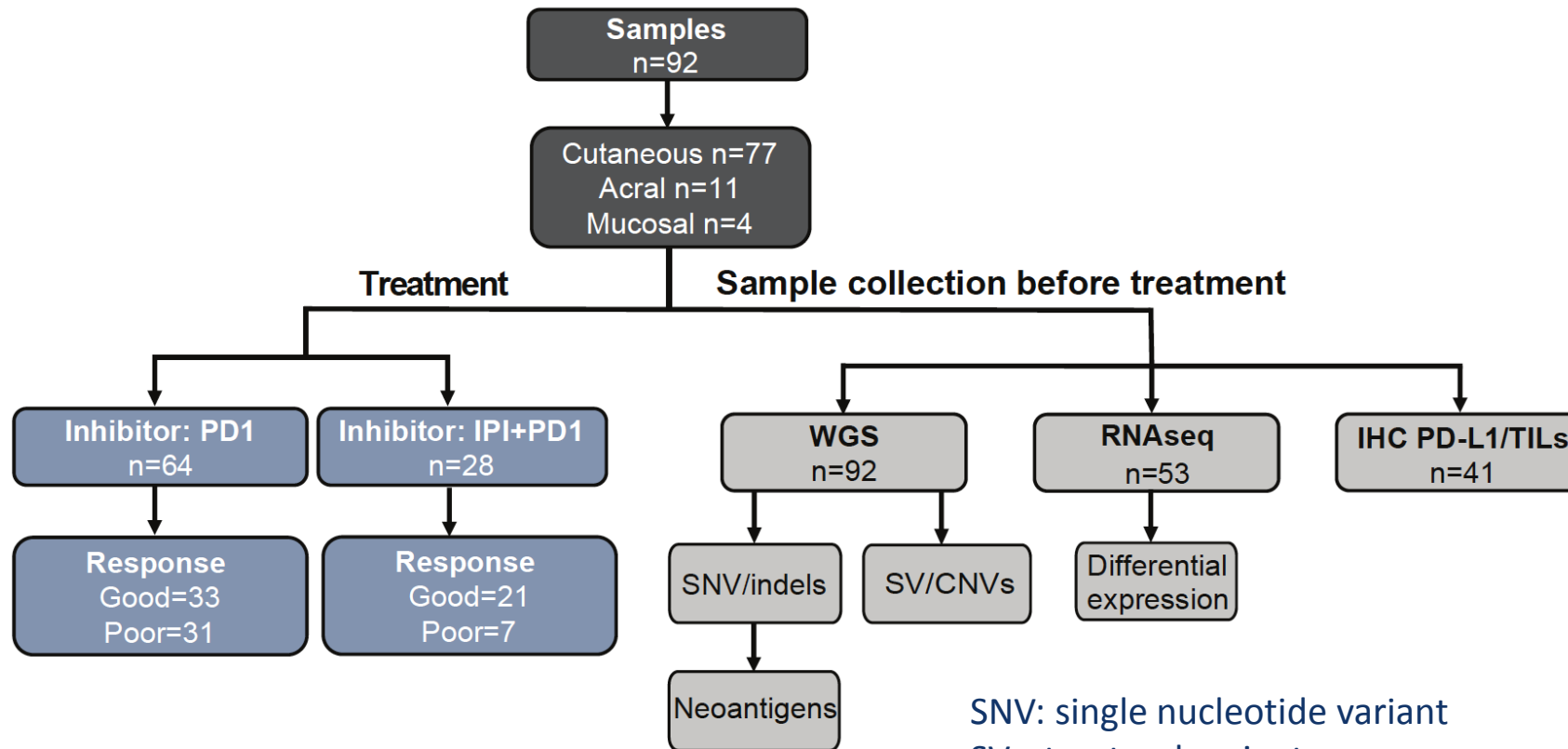Nonresponder Responder

Ayers et al. JCI 2017

Tumor mutational burden does not correlate with T cell gene signature in any cancers among TCGA

Integrated analysis of immune gene signature + TMB enriches for responders
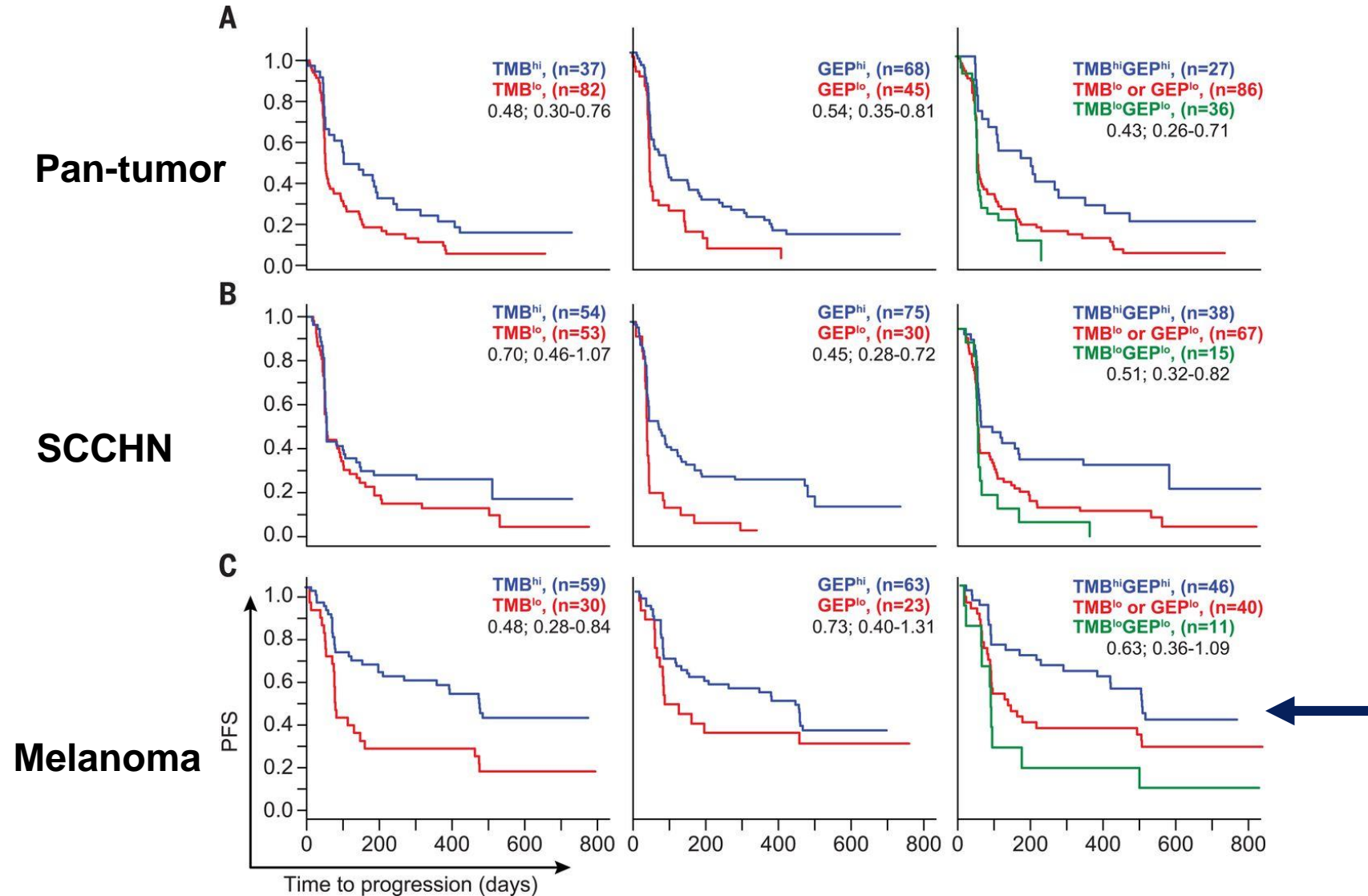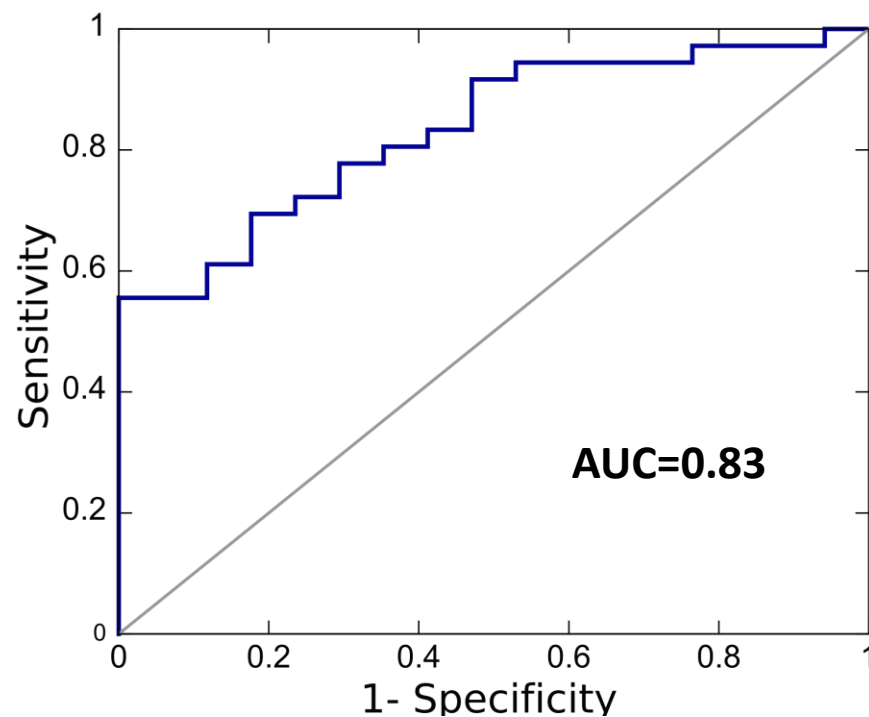
Ines Pires da Silva et al: samples analyzed

SNV: single nucleotide variant
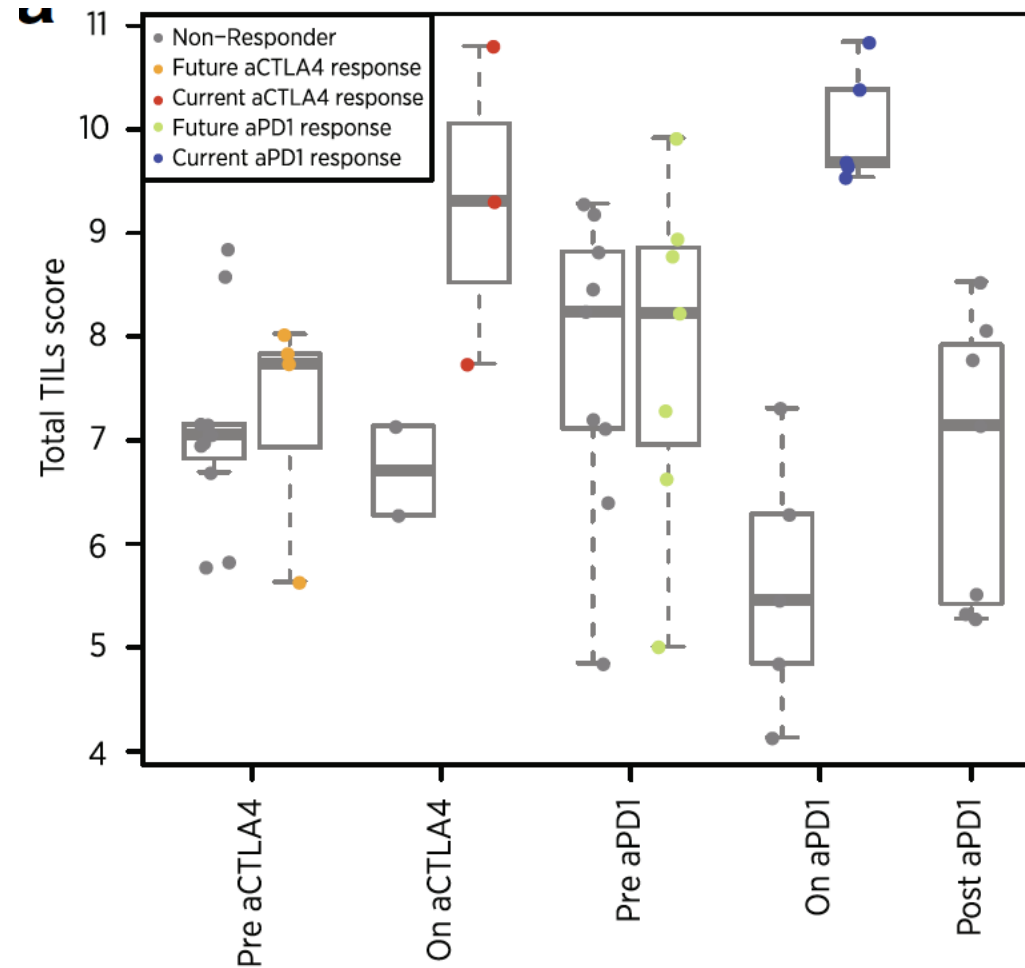SV: structural variant
CNV: copy number variation

Combined immune gene signature and TMB was best predictive biomarker

TMB and IFNg.6 score predictive model

AUC=0.83
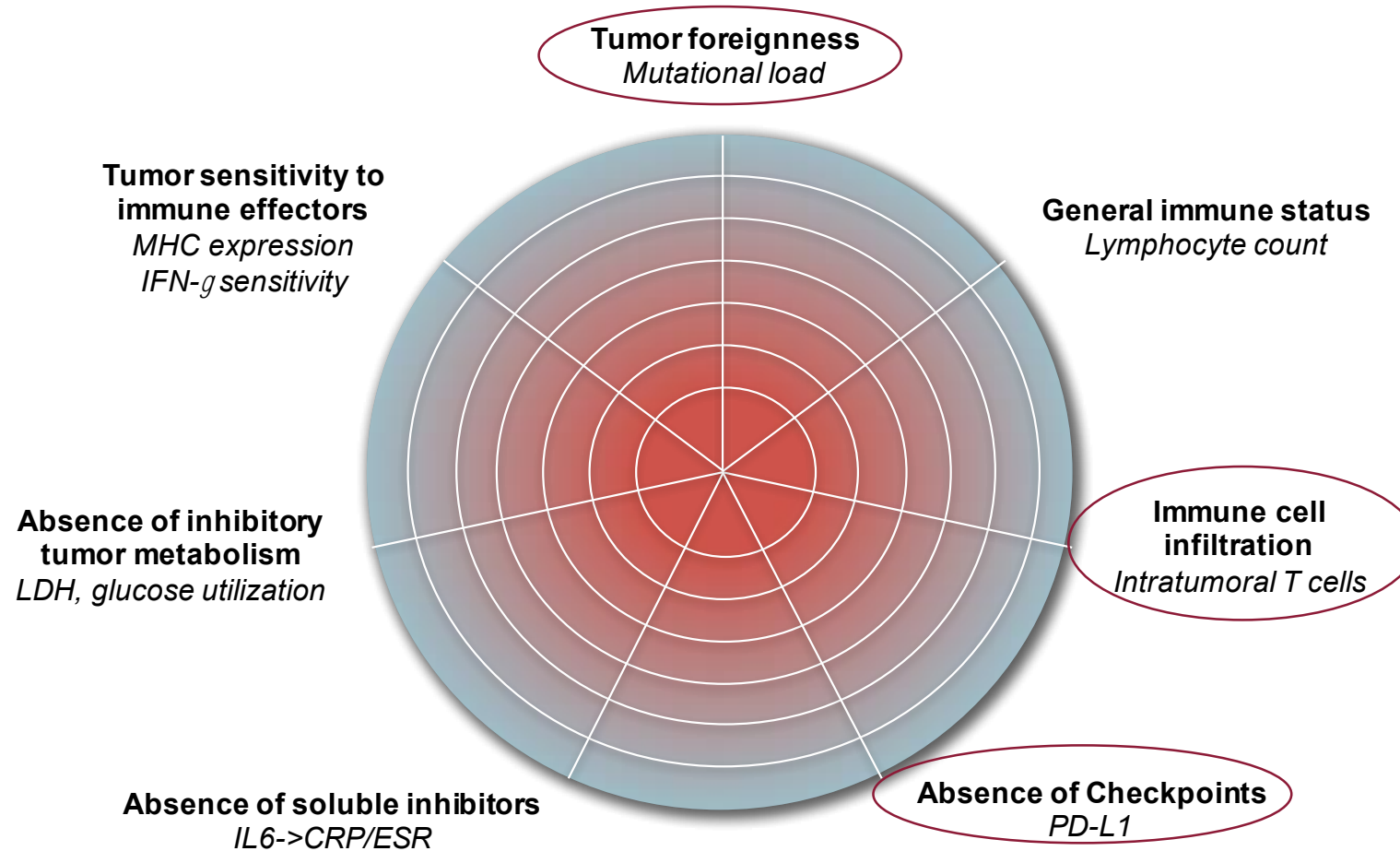
Plan to study outliers and to identify mechanisms of resistance

Changes in TIL score upon anti-CTLA4 or anti-PD-1 treatment in metastatic melanoma

Danaher et al. J Immunoth Cancer 2017

The Cancer Immunogram

Tumor foreignness
Mutational load

General immune status
Lymphocyte count

Tumor sensitivity to immune effectors
MHC expression
IFN-g sensitivity

Immune cell infiltration
Intratumoral T cells

Absence of inhibitory tumor metabolism
LDH, glucose utilization

Absence of Checkpoints
PD-L1

Absence of soluble inhibitors
IL6->CRP/ESR

An evolving immunogram : increasing complexity requiring big data

Tumor foreignness
Mutational load
Neoantigen load

Tumor sensitivity to immune effectors
MHC expression
IFN-g sensitivity
Heterozygocity at all MHC loci

General immune status
Lymphocyte count; LAG3, TIGIT, CD27
Ki67 PD1+ CD8; NLR

Microbiome
Diversity of microbiome

Absence of inhibitory tumor metabolism
LDH, glucose utilization
IDO, TDO, tumor acidity
Argenase, glutamase
CD39 → CD73 → adenosine

Immune cell infiltration
Intratumoral T cells
T cell clonality
B7-H3/CD276 macrophages
CCL5 attracting T cells
B-cells, CXCL13 Tertiary Lymphoid Structures
CCR5 expressing BATF3 DC
PD-1, LAG3, TIM3, TIGIT, CTLA-4, CD39
GEP: IFN-γ, TIL, TIS
β-catenin, p53, Myc, PTEN, LKB1

Absence of soluble inhibitors
IL-6 → CRP/ESR
IL-8, IL-1b

Absence of checkpoints
PD-L1
PD-L2

Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients
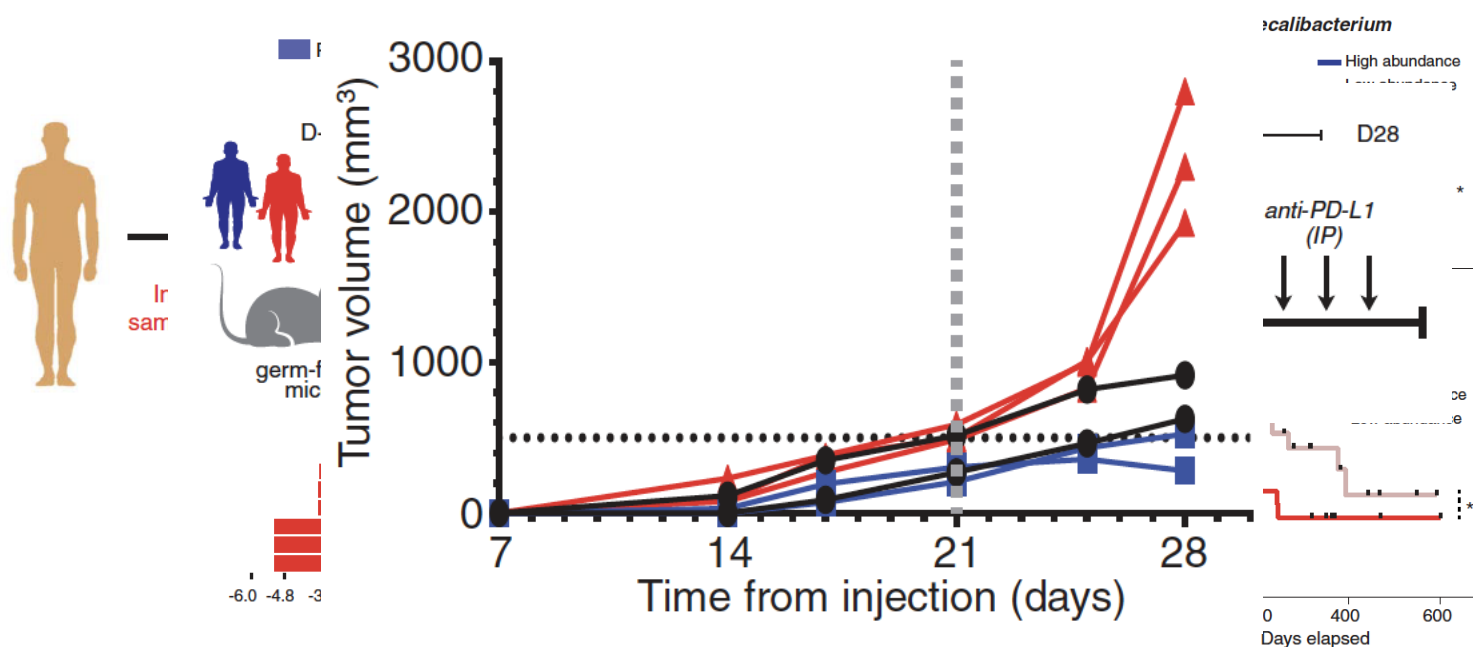
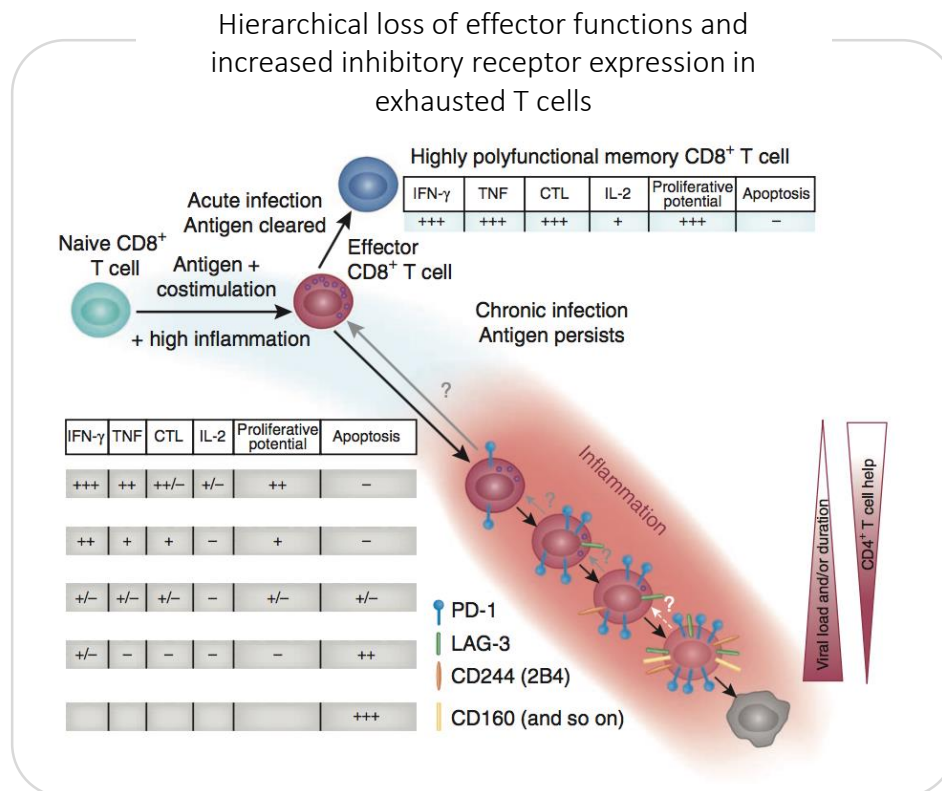| Trial Number	Patient Population	Intervention	Outcome(s)	Status
NCT02843425	all cancer patients treated at MDACC	addition of ½ cup beans per day to regular diet in a crossover design	**primary:** change in fecal microbiome profile from baseline (via 16S profiling)	open and recruiting (MDACC)
NCT02079662	stages II and III breast cancer patients treated at MDACC ages 18+	randomized intensive lifestyle change (diet, exercise, psychosocial)	**primary:** disease-free survival (DFS) **secondary:** change in fecal and oral microbiome (via 16S profiling)	open and recruiting (MDACC)
NCT01895530	CRC patients ages 18+ undergoing elective CRC resection	randomized probiotic (*S. Boulardii*) administration	**primary:** cytokine expression in colonic mucosa (via qPCR) **secondary:** post-operative complications	completed (Consoli et al., 2016)
NCT03072641	CRC patients ages 18+	randomized probiotic (ProBion Clinica *B. lactis* BI-04, *L. acidophilus* NCFM + Inulin) administration	**primary:** change in fecal and tumor microbiota from baseline **secondary:** changes in epigenetic patterns of tumor tissue from baseline	completed (Hibberd et al., 2017)
NCT03358511	post-menopausal breast cancer patients stages I–III	single-arm probiotic (Primal Defense Ultra multi-strain probiotic formula) administration	**primary:** change in mean number of CD8+ cells from baseline	open and recruiting (Mayo Clinic)
NCT02928523	acute myeloid leukemia patients ages 18–65 treated with intensive chemo and antibiotics	single-arm autologous FMT (frozen inoculum)	**primary:** diversity of the gut microbiome, multi-drug-resistant bacteria eradication **secondary:** signature of dysbiosis of gut microbiome	ongoing, closed to recruiting (France)
NCT03353402	metastatic melanoma patients ages 18+ who previously failed standard therapies	single-arm FMT (colonoscopy or gastroscopy) from patient donors who responded to immunotherapy	**primary:** safety (AEs associated with FMT), engraftment of FMT **secondary:** changes in immune cell populations and activity, objective response rate	open and recruiting (Israel)

Daniela Thommen

Hierarchical loss of effector functions and increased inhibitory receptor expression in exhausted T cells

Testing the progressive exhaustion model in human NSCLC

Hierarchical loss of effector functions and increased inhibitory receptor expression in exhausted T cells

Would the presence of cells with low expression of dysfunction-associated molecules be the best biomarker?
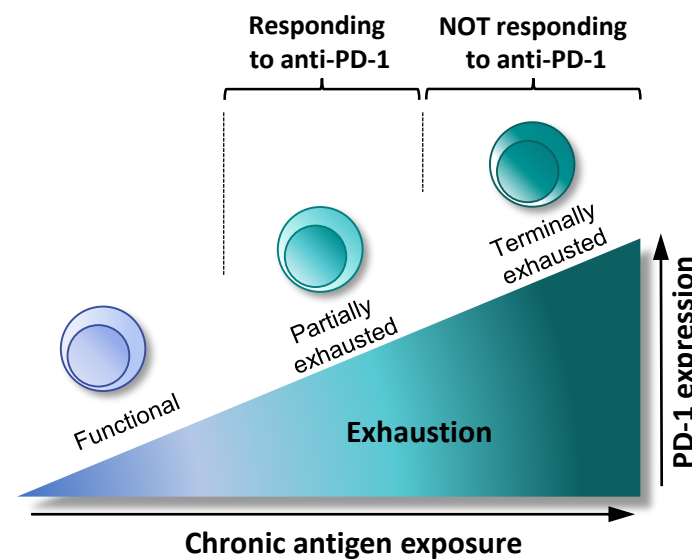
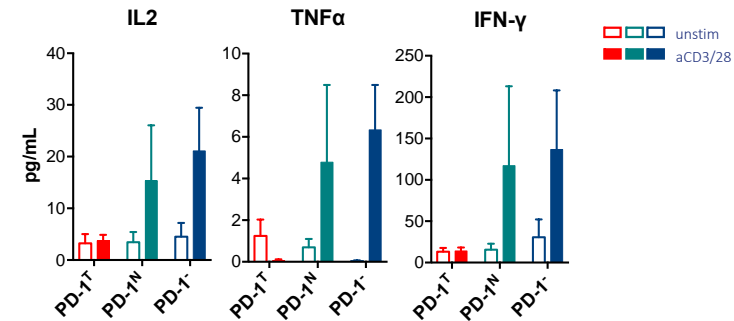# Standardized identification of T cells with different PD-1 expression levels in NSCLC

Thommen et al. Nat Med, 2018
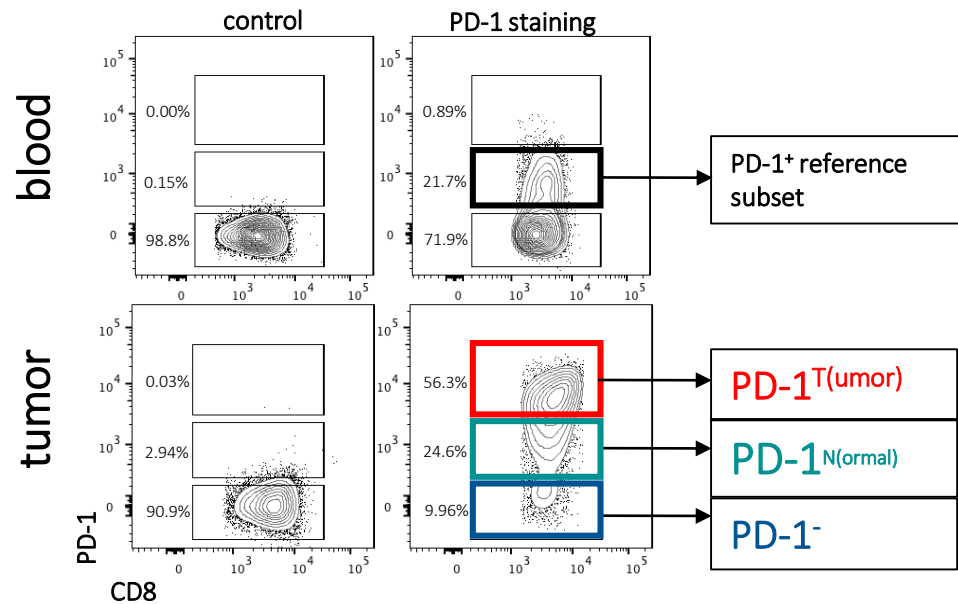
PD-1^T TILs show loss of effector function

Sorting of TIL subsets from NSCLC specimens
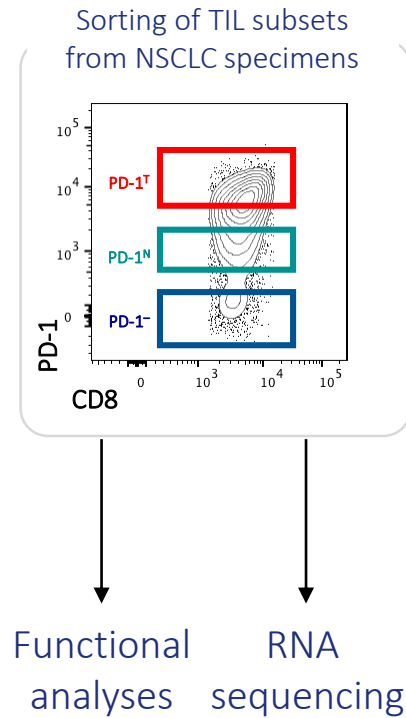
Impaired secretion of classical effector cytokines by PD-1^T TILs

Functional analyses   RNA sequencing
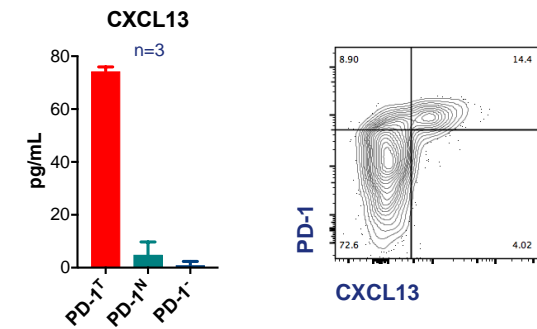
Thommen et al. Nat Med, 2018

A novel function of PD-1^T CD8 T cells in the TME

Expression of CXCL13 mRNA in PD-1^T TILs
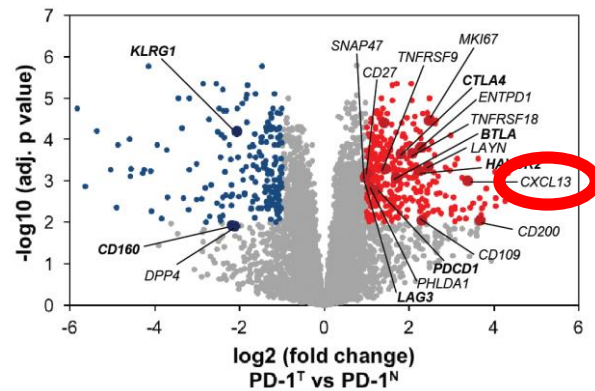
PD-1^T TILs produce and secrete CXCL13 protein

Thommen et al. Nat Med, 2018
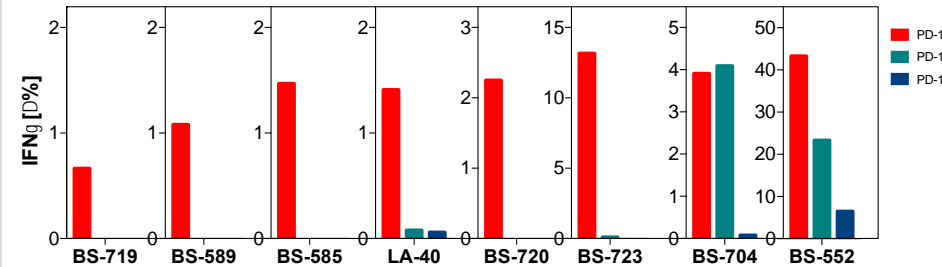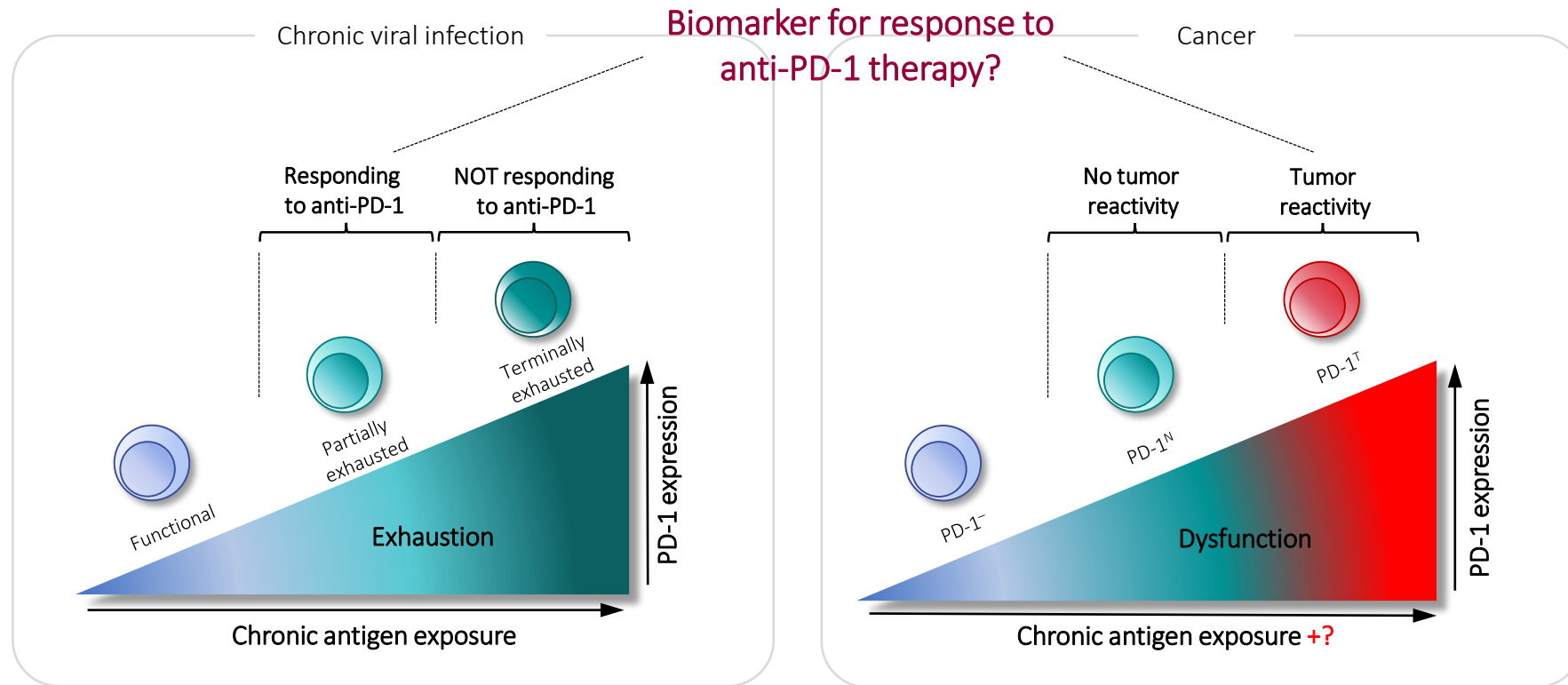
PD-1^T TILs have an increased capacity for tumor recognition

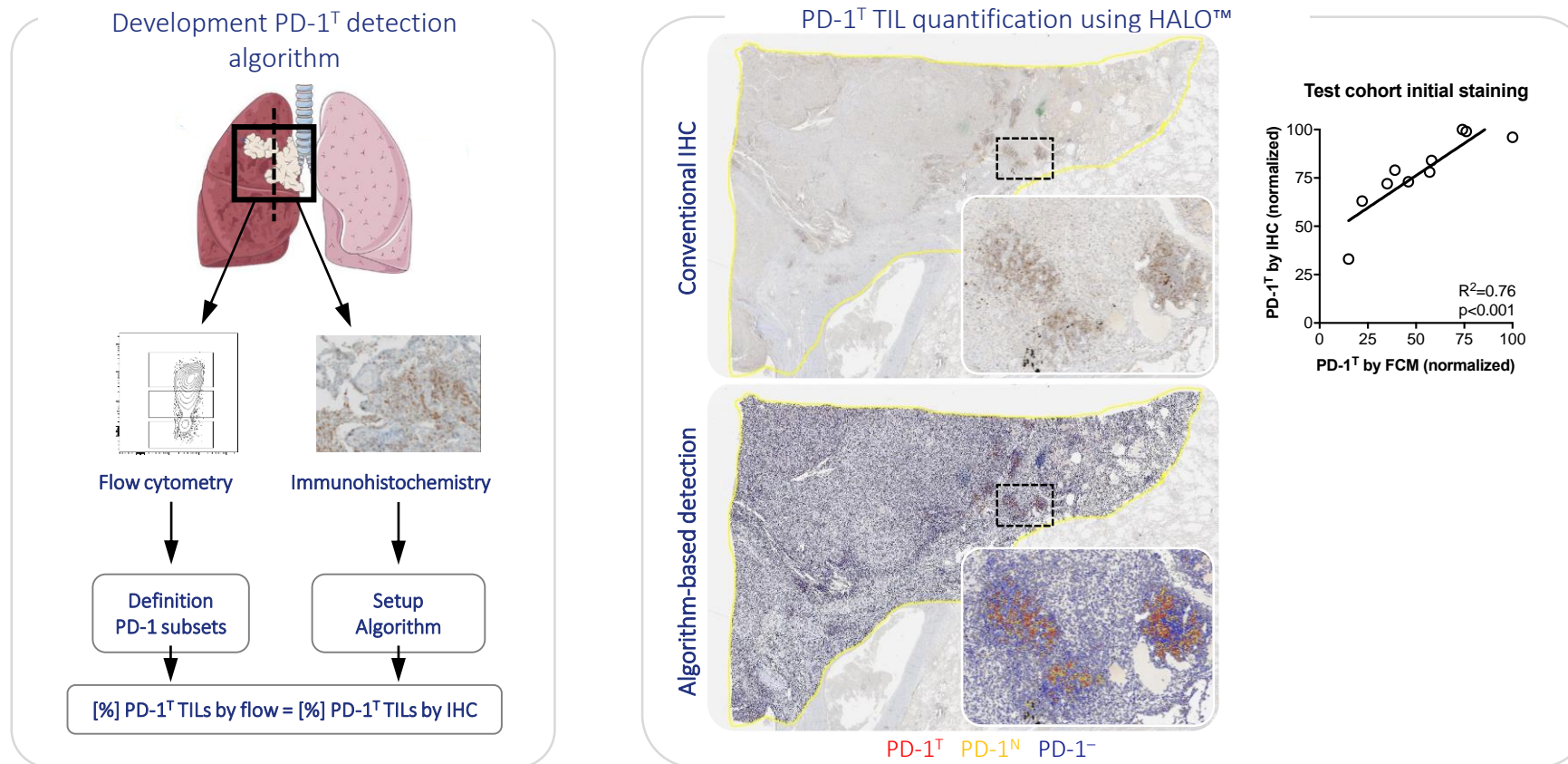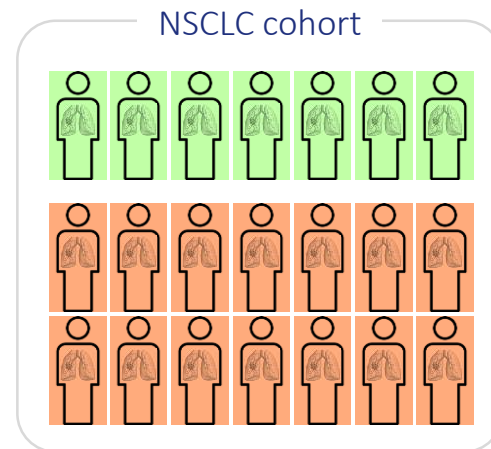PD-1^T TILs upregulate genes involved in proliferation and T cell activation

Antigen-driven expansion of PD-1^T TILs?
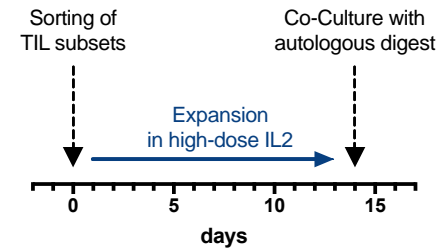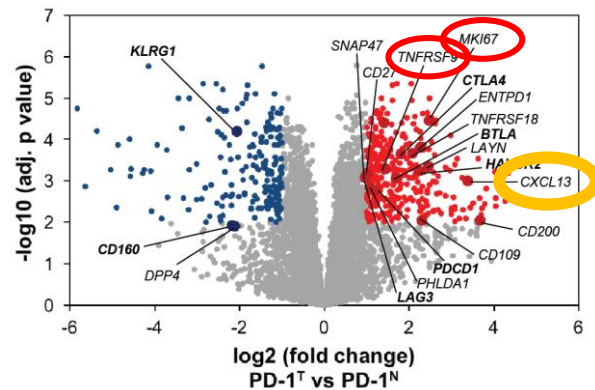
PD-1^T TILs are highly tumor reactive

Thommen et al. Nat Med, 2018

Algorithm-based digital image analysis for quantification of PD-1 expression levels

Development PD-1^T detection algorithm

Flow cytometry   Immunohistochemistry

Definition PD-1 subsets   Setup Algorithm

[%] PD-1^T TILs by flow = [%] PD-1^T TILs by IHC

PD-1^T TIL quantification using HALO™

Conventional IHC

Algorithm-based detection

PD-1^T PD-1^N PD-1$^-$

Test cohort initial staining

PD-1^T by IHC (normalized)

PD-1^T by FCM (normalized)

$R^2 = 0.76$
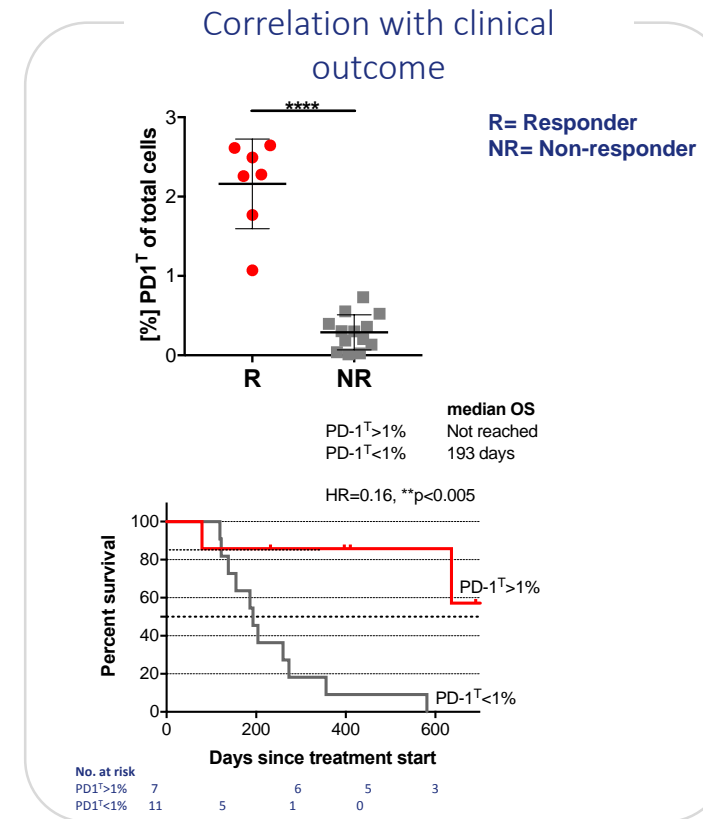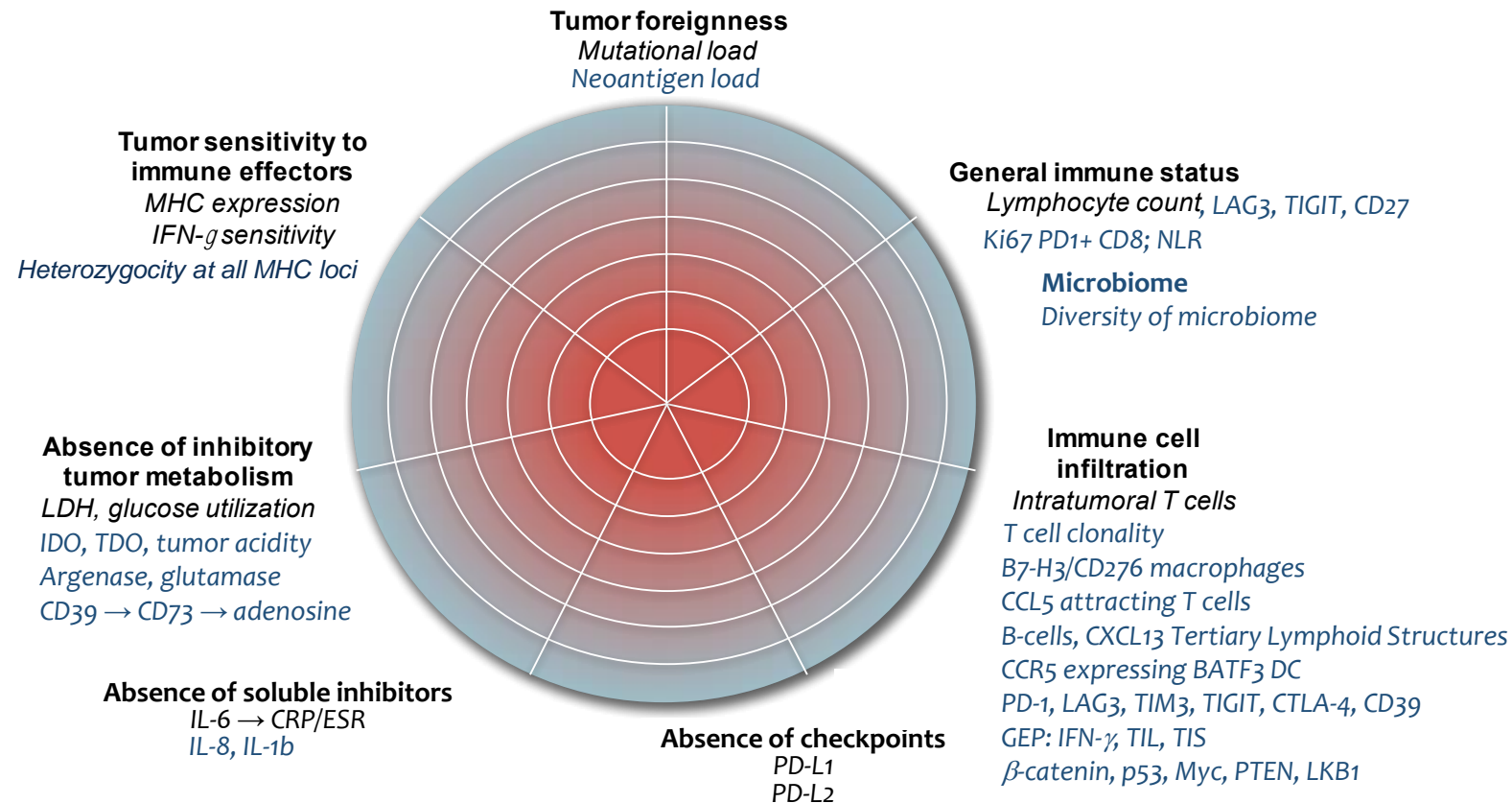$p < 0.001$

Thommen et al. Nat Med, 2018

Presence of PD-1^T TILs correlates with response and survival upon PD-1 blockade

NSCLC cohort

- 21 patients treated with anti-PD-1 therapy
- Stage IV NSCLC
- No previous IT treatment
- Pathologists were blinded to treatment outcome

Correlation with clinical outcome

R= Responder
NR= Non-responder

	median OS
PD-1^T>1%	Not reached
PD-1^T<1%	193 days

HR=0.16, **p<0.005

Cancer Immunogram: a multiparameter framework to check cancer-immune interactions

Working towards a complex multifaceted biomarker to predict response to IO

Take home messages

Unlike predictive biomarkers for targeted therapy, biomarkers for response to IT will be complex:

- Not one single marker predicts response/resistance
- Often no good cut off (PD-L1, TMB, TIL, GEP) as these are continuous biomarkers
- Combination of markers is being examined and appears better than single biomarkers
- New potential biomarkers are emerging:
    - Microbiome
    - PD-1
    - TLS, CXCL13
    - Myeloid markers
    - Metabolic biomarkers
    - TCR clonality
- We are in need of liquid biomarkers!
- We should start looking at early time points following IT (change timing of liquid and tumor biopsies)