Immunotherapy – next challenges of clinical drug development

Immunotherapy of Cancer Conference (ITOC-6)

Vienna, Austria

April 11, 2019

Spectrum of PD-1/PD-L1 Antagonist Activity

Approved

- **Melanoma**
- **Renal cancer (clear cell)**
- **NSCLC – adenocarcinoma and squamous cell**
- **Head and neck cancer**
- **MMR-repair deficient tumors (colon, cholangiocarcinoma)**
- **Bladder**
- **Hodgkin lymphoma**
- **Merkel cell**
- **Gastric and gastroesophageal junction**
- **Hepatocellular carcinoma**
- **Squamous Cell Ca of Skin**
- **Small cell lung cancer**
- **Refractory primary mediastinal large B-cell lymphoma (PMBCL)**
- **Triple negative breast cancer**

Approved Anti-PD-1 agents

- Nivolumab, Pembrolizumab, Cemiplimab

Approved Anti-PD-L1 agents

- Atezolizumab, Durvalumab, Avelumab

Active:

- Basal Cell Carcinoma
- Renal (non-clear cell)
- Ovarian
- Thymoma
- Mesothelioma
- Cervical Cancer
- Diffuse large cell lymphoma
- Follicular lymphoma
- T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)
- Prostate cancer (with ipilimumab)
- Subsets of sarcoma

Minimal to no activity

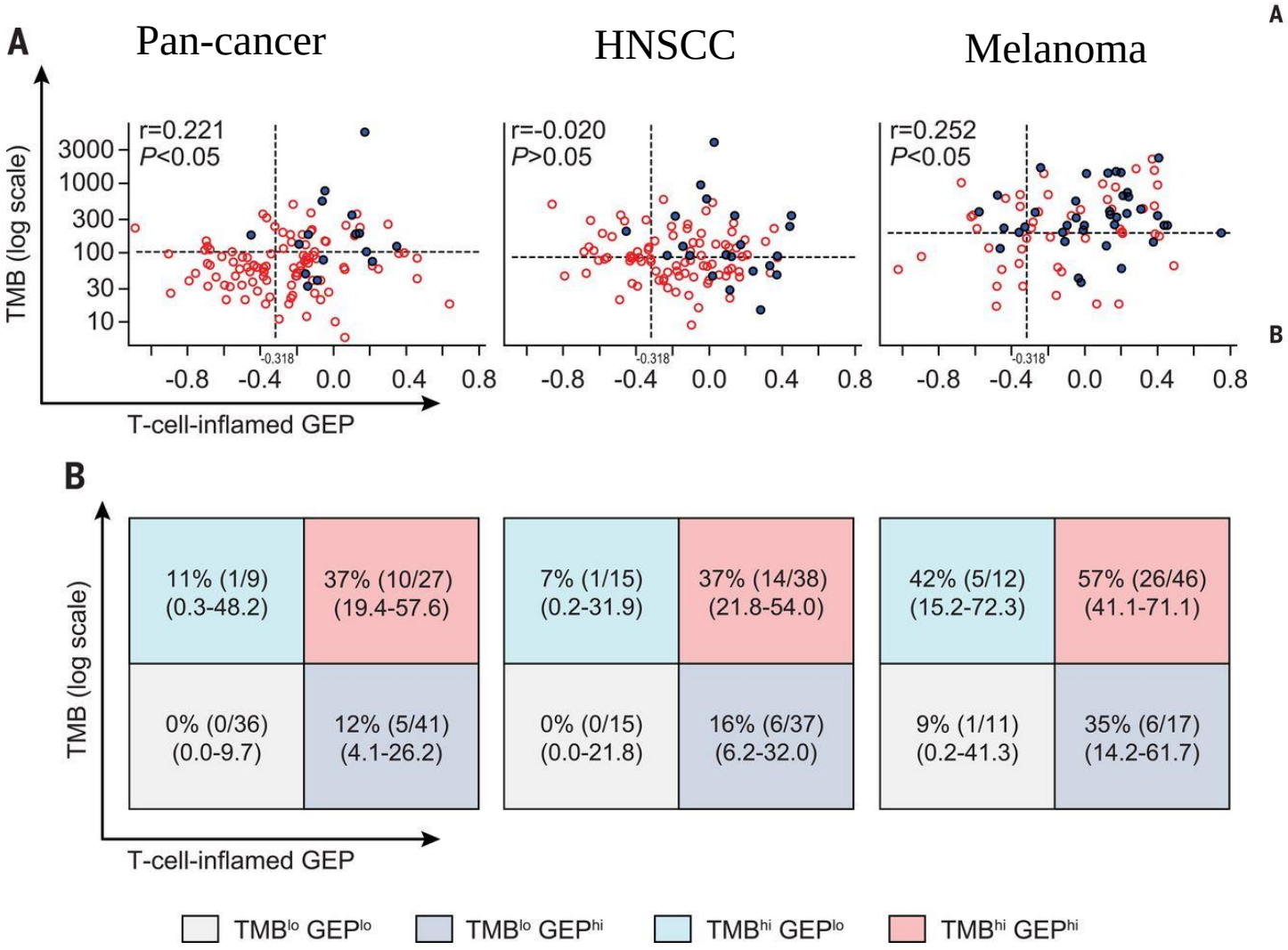
- MMR+ (MSS) colon cancer
- Myeloma
- Pancreatic cancer

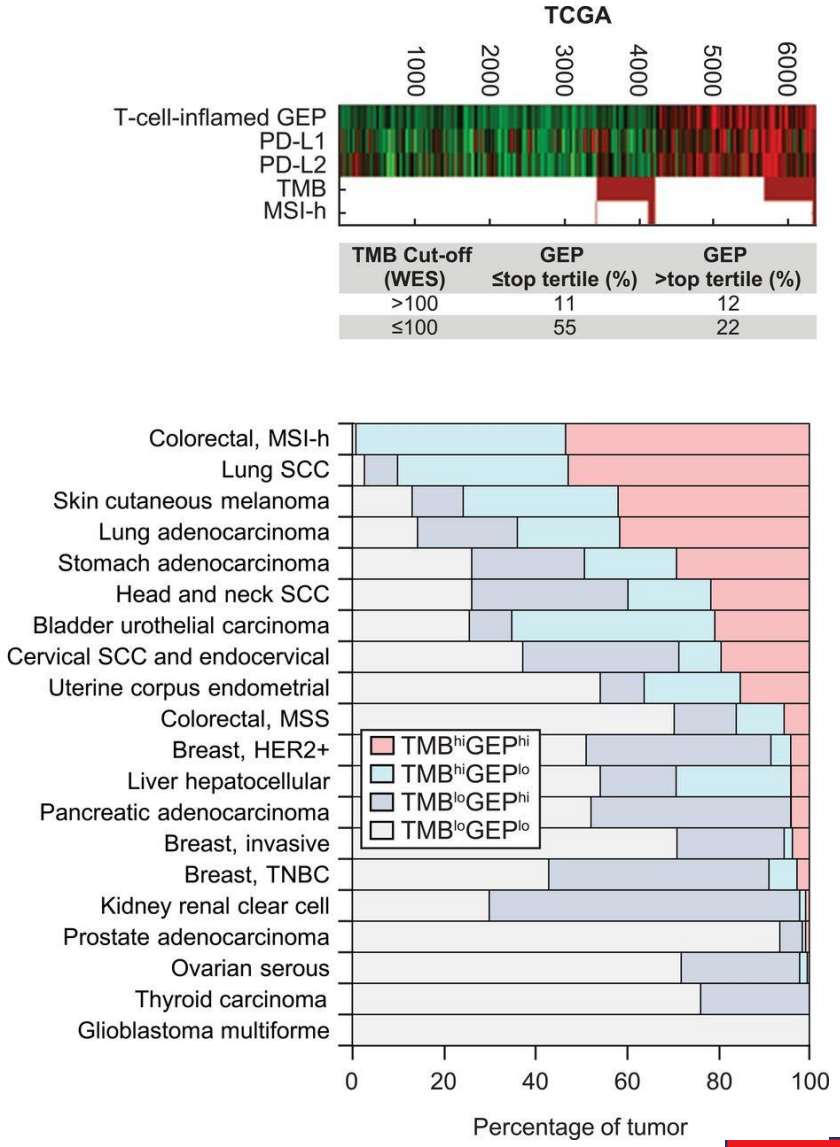
Predictors for Clinical Response to Anti-PD-1/PD-L1 pathway blockade

- PD-L1 expression – (tumor, tumor-infiltrating immune cells)
  - Proximity of PD-L1 to PD-1 (IDO/HLA-DR) – (Clin Cancer Res. 2018 Nov 1;24(21):5250-5260)
- Presence of interferon-gamma (or T-effector) gene signature
- High tumor mutation burden (DNA sequencing or RNA-seq, dMMR, MSI-high)

- Assessment of T cells (CD8+) in tumor microenvironment
  - Number of CD8+ T cells at tumor invasive margin (Nature. 2014 Nov 27; 515(7528): 568–571).
  - Presence of stromal CD8+ T cells
  - Increased clonality of intra-tumoral T-cells (Nature. 2014 Nov 27; 515(7528): 568–571).
  - Presence of certain subsets of CD8+ T-cells
    - Percent intra-tumoral CTLA4+PD-1+ CD8+ T-cells (JCI Insight. 2017 Jul 20;2(14))
    - *CD8+ PD-1+TIM3- TCF1+CD28+? (proposed) (Science. 2017 Mar 31;355(6332):1423-1427.)*
    - *Ratio of TCF7+/TCF7- T cells (*Cell. 2019 Jan 10;176(1-2):404.)
  - Functional properties
    - Polyfunctionality (cytokine production) of intra-tumoral T-cells? (Nat Med 2011; 17:738-43)
    - Presence of quiescent T-cells (Ki-67, granzyme negative)

Joint relationship of TMB or T cell–inflamed GEP with anti–PD-1 response across multiple patient cohorts.





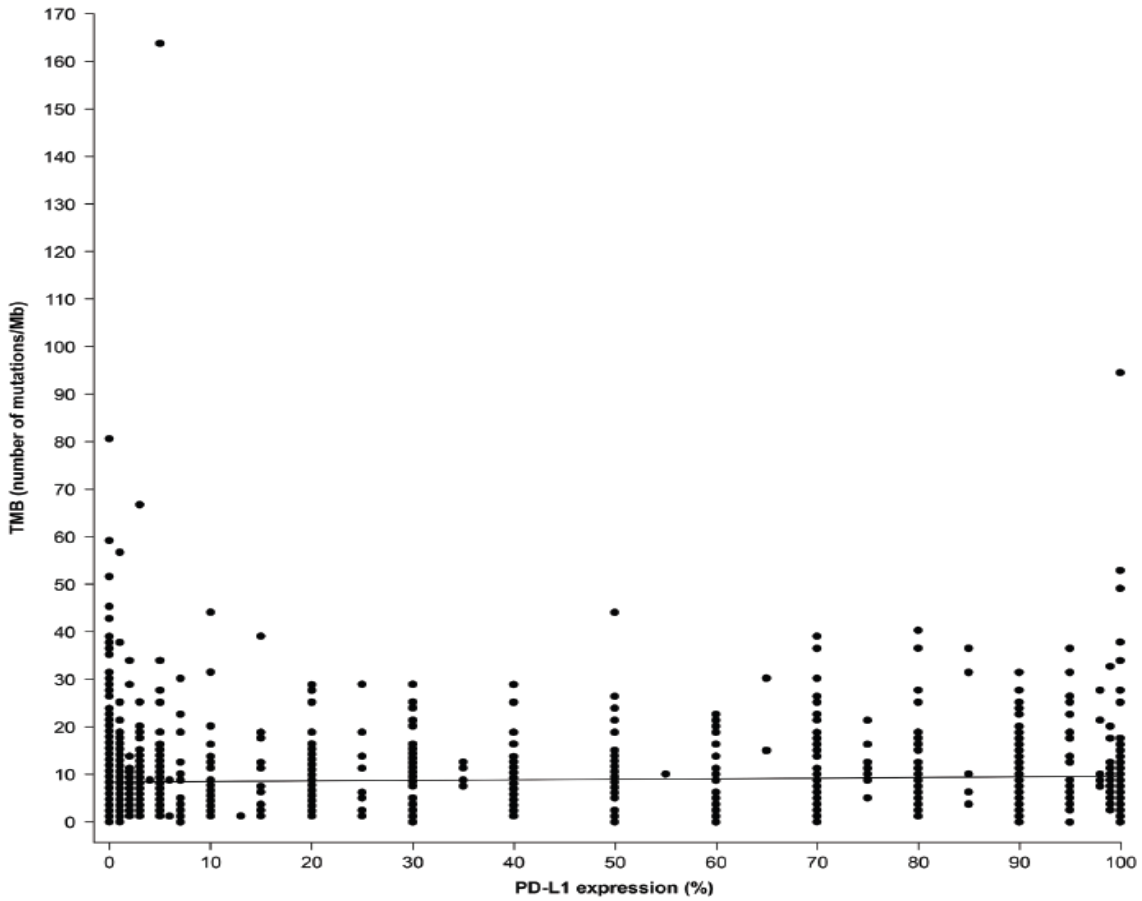
TMB Cut-off (WES)	GEP ≤top tertile (%)	GEP >top tertile (%)
>100	11	12
≤100	55	22

Razvan Cristescu et al. Science 2018;362:eaar3593

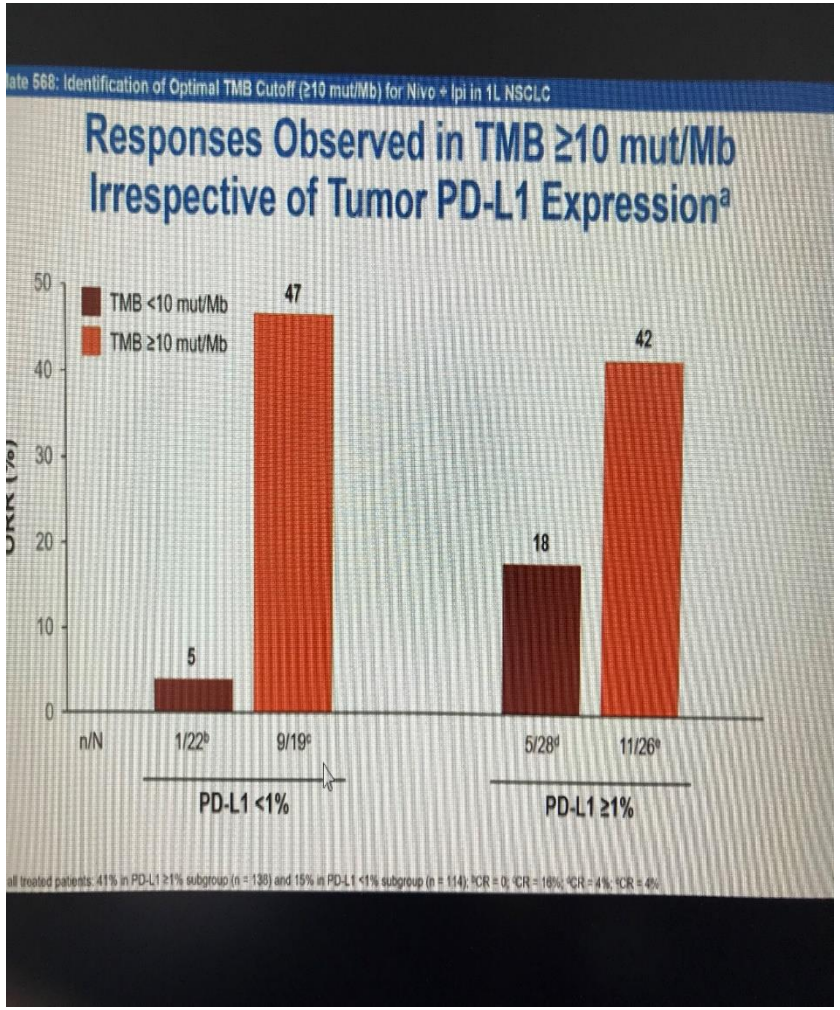
Published by AAAS

For Ipilimumab/Nivolumab in NSCLC, TMB and PD-L1 Identify Different Populations

Figure S2. Scatterplot of TMB and PD-L1 Expression in All TMB-Evaluable Patients[a]



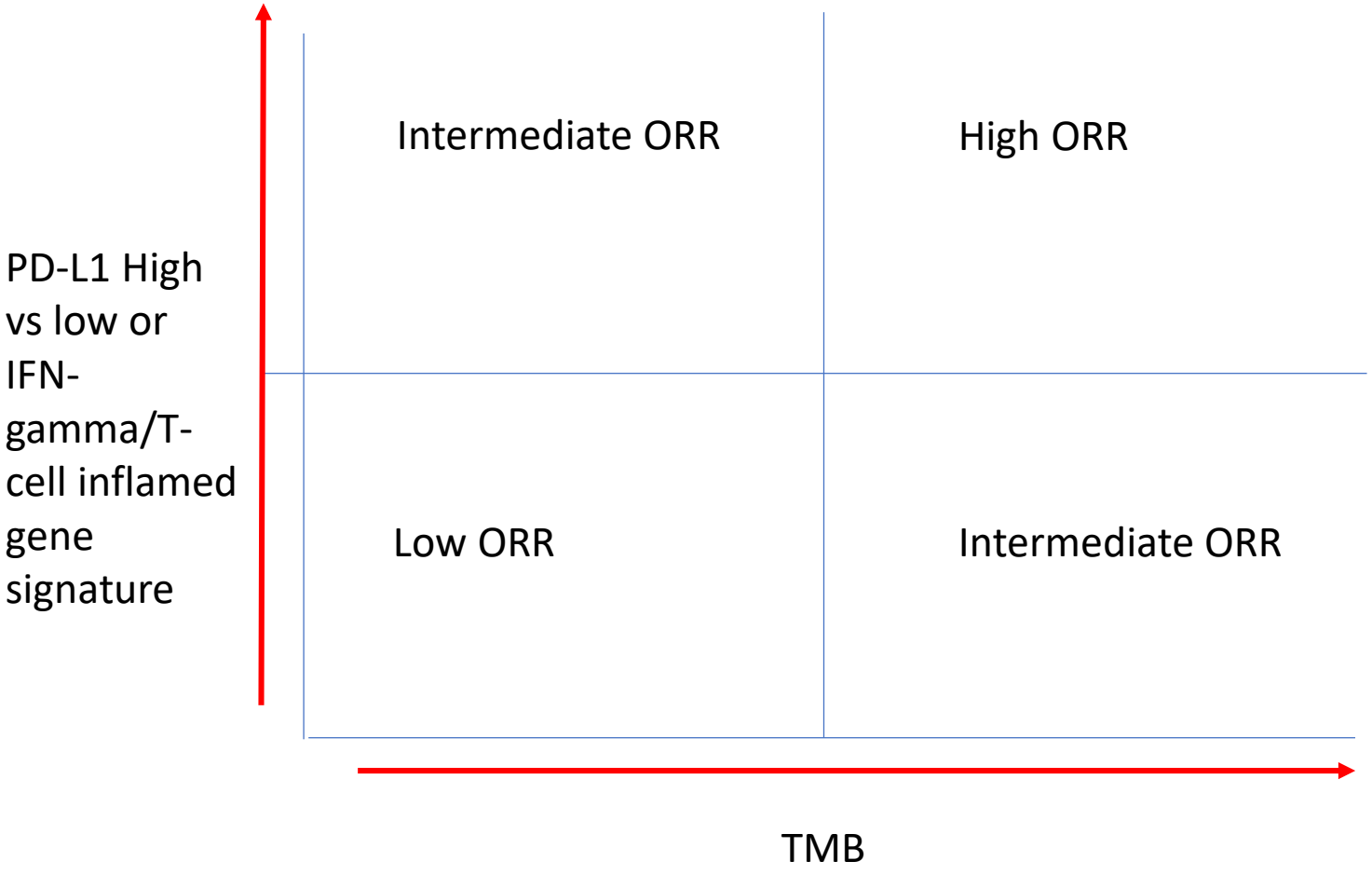
[a]Symbols (dots) in the scatterplot may represent multiple data points, especially for patients with <1% tumor PD-L1 expression. The black line shows the relationship between TMB and PD-L1 expression as described by a linear regression model.



Supplement to: Hellmann MD, Ciuleanu T-E, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018;378:2093-104. DOI: 10.1056/NEJMoa1801946

Ramalingan et al, AACR 2018

Combination Biomarkers May Provide Better Predictive Value



Inflamed T cell mRNA signature Identifies Most Objective Responders

The Journal of Clinical Investigation

RESEARCH ARTICLE

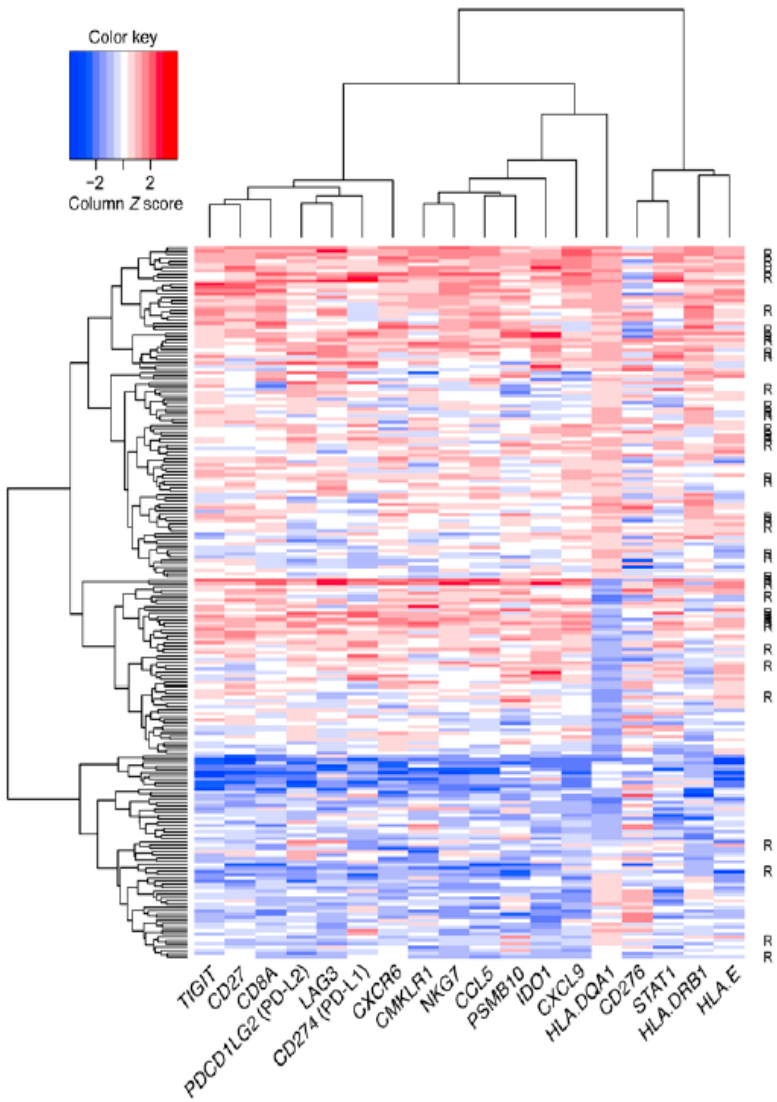


Figure 4. Heatmap for the final 18-gene T cell-inflamed GEP for 216 tumors from patients in KEYNOTE-012 and KEYNOTE-028 considered evaluable for objective response. Rows represent patients and columns genes. Expression levels have been standardized (centered and scaled) within columns for visualization. The "R" on the right side indicates whether the patient was a responder (by central imaging vendor in KEYNOTE-012 and by investigator assessment in KEYNOTE-028). The rows and columns have been grouped using unsupervised clustering.

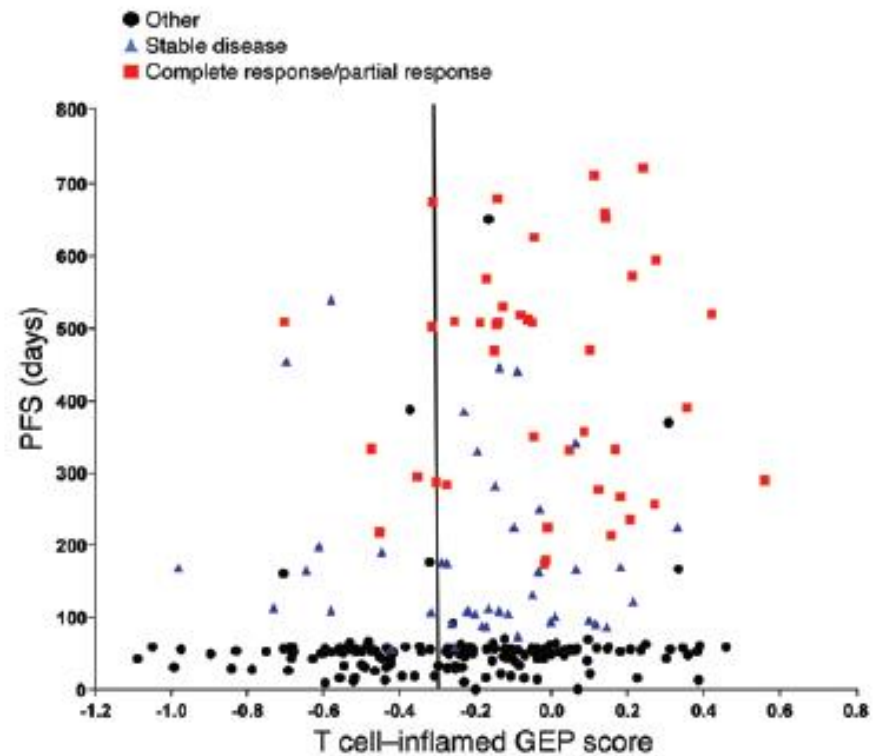


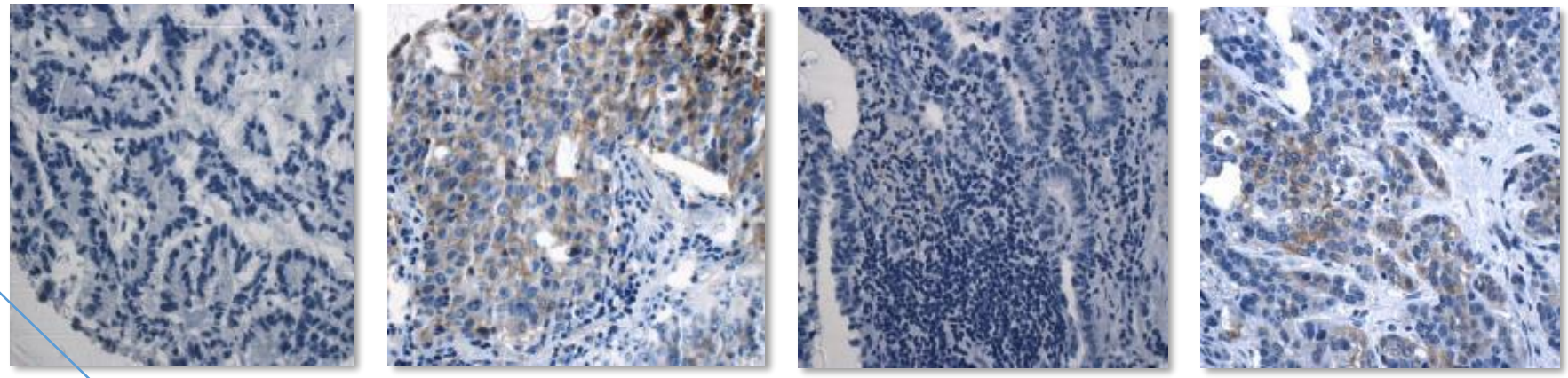
Figure 7. PFS time versus T cell-inflamed GEP score in 244 patients from KEYNOTE-012 and KEYNOTE-028 for the 9 cancer cohorts used to determine the T cell-inflamed GEP.

Mark Ayers, … , Antoni Ribas, Terrill K. McClanahan

J Clin Invest. 2017;127(8):2930-2940. https://doi.org/10.1172/JCI91190.

Presence of PD-L1 or TILs[1]

PD-L1⁻/TIL⁻	PD-L1⁺/TIL⁺	PD-L1⁻/TIL⁺	PD-L1⁺/TIL⁻



Schalper and Rimm, Yale University

NSCLC

45%	17%	26%	12%
Type 1	**Type 2**	**Type 3**	**Type 4**
45%	41%	13%	1%

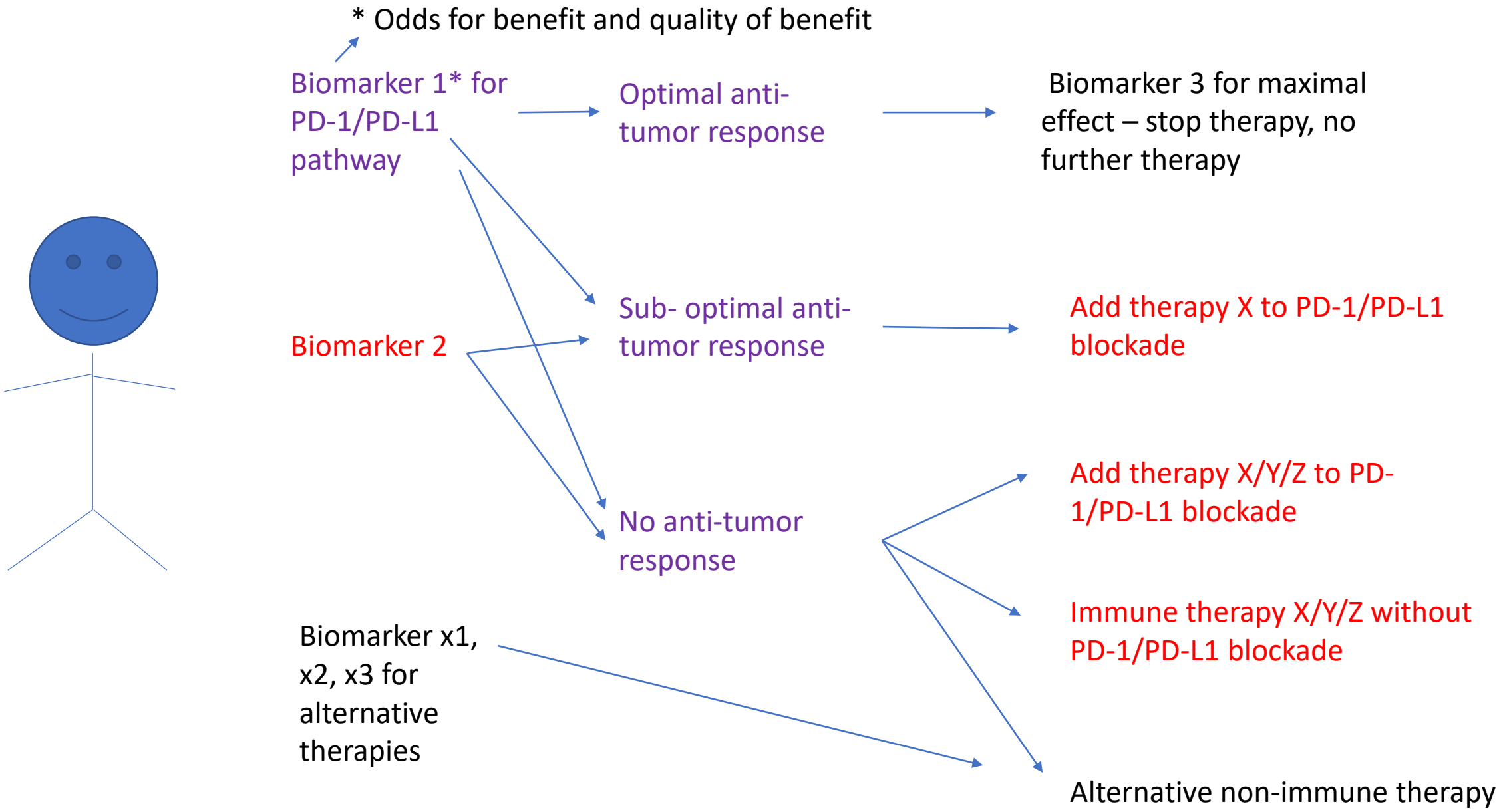
Melanoma

Table 2. Correlation of B7-H1 expression by melanocytes with the presence of immune cell infiltration.

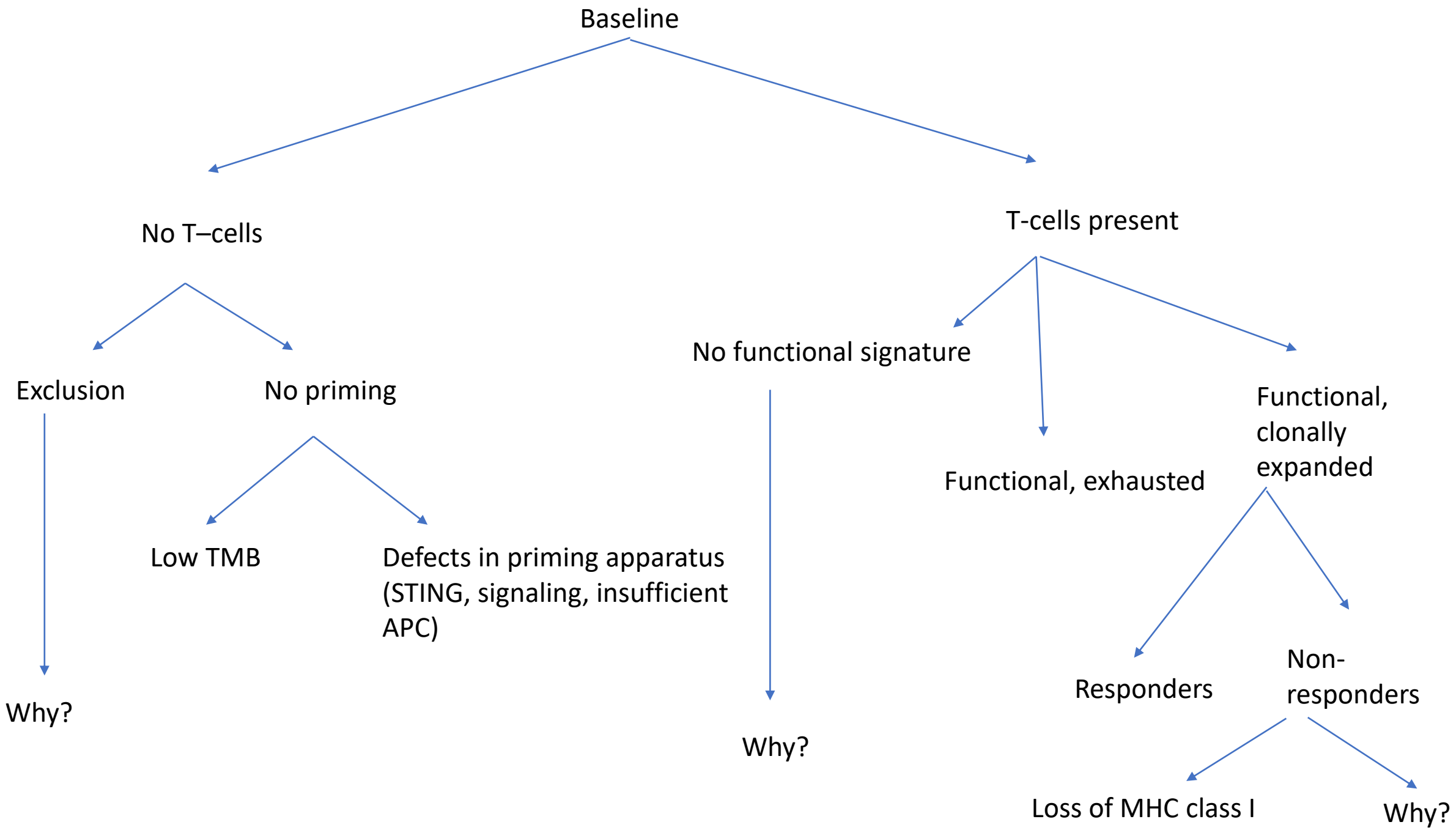
Histology	Total	Number of cases/total cases (%)				P*
		B7-H1⁺†		B7-H1⁻		
		TIL⁺‡	TIL⁻	TIL⁺	TIL⁻	
Benign nevi	40	14/14 (100)	0/14 (0)	4/26 (15)	22/26 (85)	<0.0001
Primary melanomas (in situ or invasive)	54	19/19 (100)	0/19 (0)	15/35 (43)	20/35 (57)	<0.0001
Metastases	56	23/24 (96)	1/24 (4)	7/32 (22)	25/32 (78)	<0.0001
All	150	56/57 (98)	1/57 (2)	26/93 (28)	67/93 (72)	<0.0001

*Fisher's exact test, two-sided, was conducted on the 2 × 2 matrix defined by B7-H1 (±) expression and TIL (±) for each lesion type. †More than 5% melanocytes with membranous expression on IHC. ‡Including mild, moderate, and severe lymphocyte infiltrates and their associated histiocytes/macrophages.

Taube et al



Biomarker 1 and Biomarker 2 could be assessed early post-treatment


Baseline
No T–cells
T-cells present
Exclusion
No priming
No functional signature
Functional, exhausted
Functional, clonally expanded
Low TMB
Defects in priming apparatus (STING, signaling, insufficient APC)
Why?
Why?
Responders
Non-responders
Loss of MHC class I
Why?

Summary of Immune Checkpoint Inhibitor Non-Response or Resistance

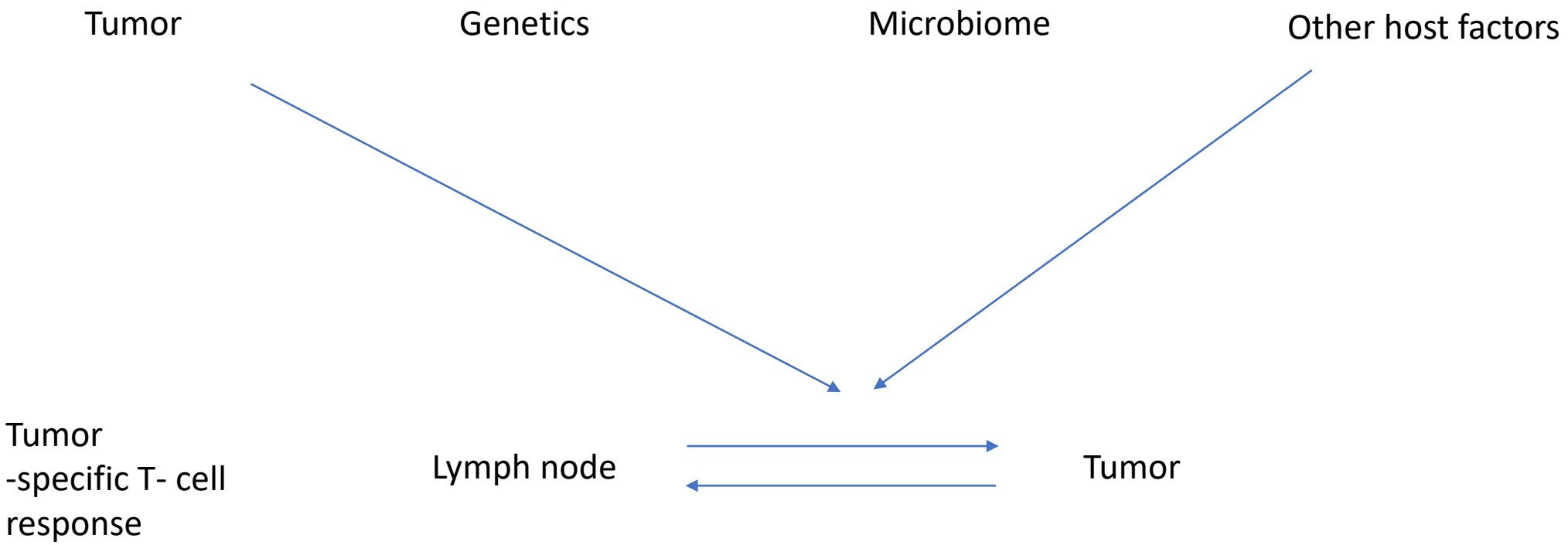
- Genetic component (HLA heterozygosity, other)?
- Low tumor mutation burden
- Lower microbiome diversity/presence or absence of bacterial species
- Increased/stabilized beta catenin
- STK11/LKB1 mutation
- Failure of Sting activation
- PTEN loss (dependent on VEGF)
- Increased VEGF
- Tumor Hypoxia
- IPRES signature/angiogenesis/ETM transition
- Increase in Myeloid cell signature
- Increased peripheral complement activation, wound healing, acute phase reactants
- Tumor/TME metabolism (glucose)
- *Induction of T-cell regulatory mechanisms (IDO, Tim-3, other immune checkpoints) or T-cell exhaustion*
- Increase in tumor DNA copy number loss (immune related genes) (Roh et al., Sci. Transl. Med. 9, March 2017)
- JAK mutations (IFN-γ pathway signaling) (Zaretsky et al, NEJM)
- Beta-2 microglobulin/HLA loss

→ Priming – Minimal to no T-cell response

→ Exclusion/Traffic signals?
Or lack of/inadequate activation of tumor APC

→ Feedback negative regulation +/- lack of additional agonist signals

→ Tumor cell or T-cell insensitivity



If response requires a component of new T cell influx into tumor, tumor microenvironment extrinsic factors may impact outcome of intervention

Do Host* Factors Play a Role in in Response and Toxicity of anti-PD-1? Combined Analyses of Nivolumab in Metastatic Melanoma

Table 2. Impact of Treatment-Related Select AEs and IM Use on Response to Nivolumab Therapy

		Any-Grade Treatment-Related Select AEs*				Grade 3 to 4 Treatment-Related Select AEs		Patients Receiving Systemic IM	
	All Patients (N = 576)	Any (n = 255)	None (n = 321)	1-2 (n = 242)	≥ 3 (n = 13)	Yes (n = 18)	No (n = 558)	Yes (n = 114)	No (n = 462)
ORR, No. of patients (%)	181 (31.4)	124 (48.6)	57 (17.8)	113 (46.7)	11 (84.6)	5 (27.8)	176 (31.5)	34 (29.8)	147 (31.8)
95% CI	27.6 to 35.4	42.3 to 54.9	13.7 to 22.4	40.3 to 53.2	54.6 to 98.1	9.7 to 53.5	27.7 to 35.6	21.6 to 39.1	27.6 to 36.3
P		< .001		< .0001†	< .001†	1.00		.736	

Abbreviations: AE, adverse event; IM, immune-modulating agent; ORR, objective response rate.
*Data in these columns are for patients with the indicated numbers of any-grade treatment-related select AEs: any AE, no AEs, 1-2 AEs, and ≥ 3 AEs.
†Versus no treatment-related select AEs.

J Clin Oncol 35:785-792.

*Genetic, Tumor–Induced Systemic Immune Modulation, Other Systemic Immune Modulation

Create new tumor-specific T-cells or enhance in vivo Ag presentation

- Anti-CD40, FLT3, TLR agonists
- STING agonists, **T-VEC**, Other oncolytic viruses, Vaccines, **Chemotherapy**, Targeted agents, Epigenetic Modifiers, MEKi
- Adoptive Transfer: **CAR-T**

Expansion and Increase Function of Ag-specific T cells

- CTLA-4, others
- Enhancing TCR signaling
- Transcription factor agonists
- **Cytokines and Modified Cytokines**
- Co-stimulatory Agonists – 4-1BB, OX-40, GITR, ICOS, CD27
- Adoptive Transfer: TIL, **CAR-T**

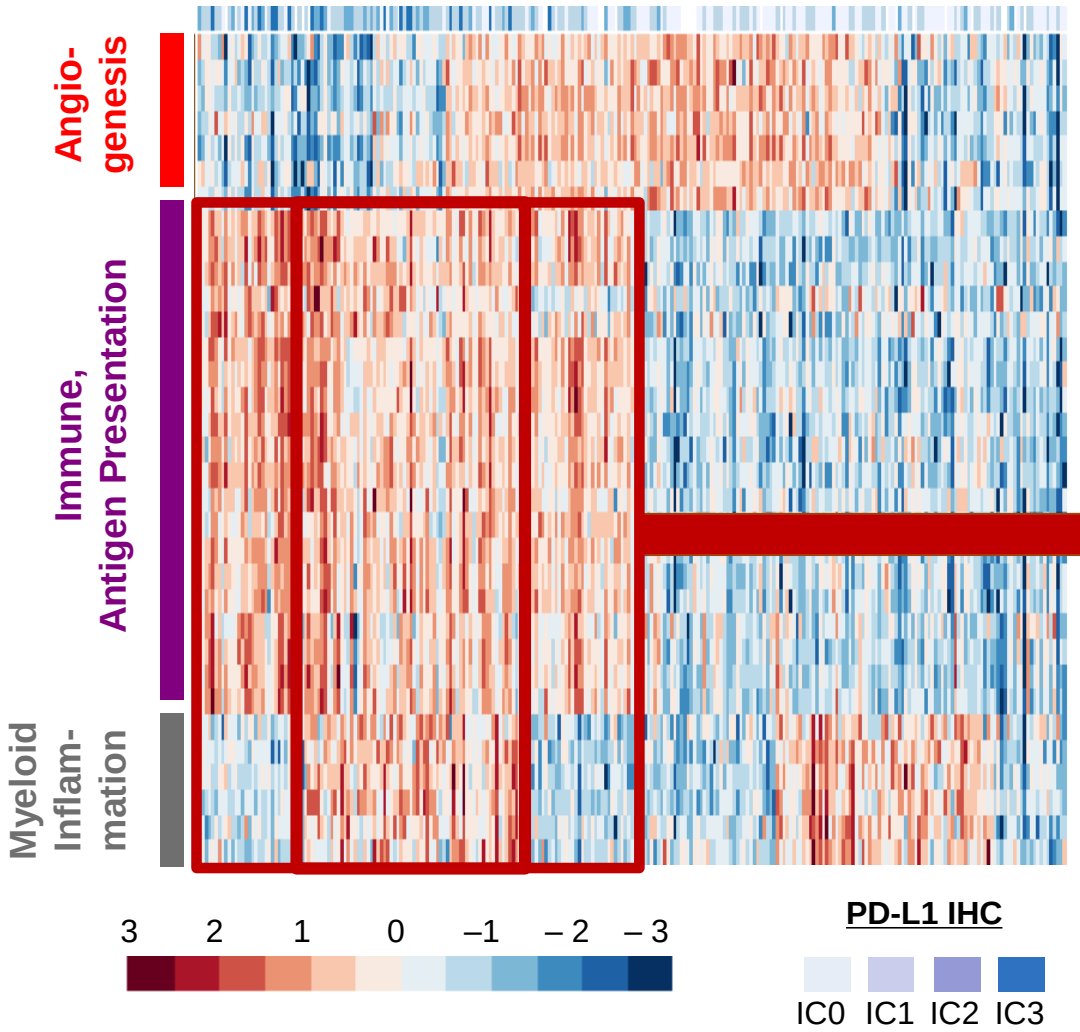
Co-opt non-specific TIL

- **Activate with TCR-CD3 Constructs (CEA, gp100)**

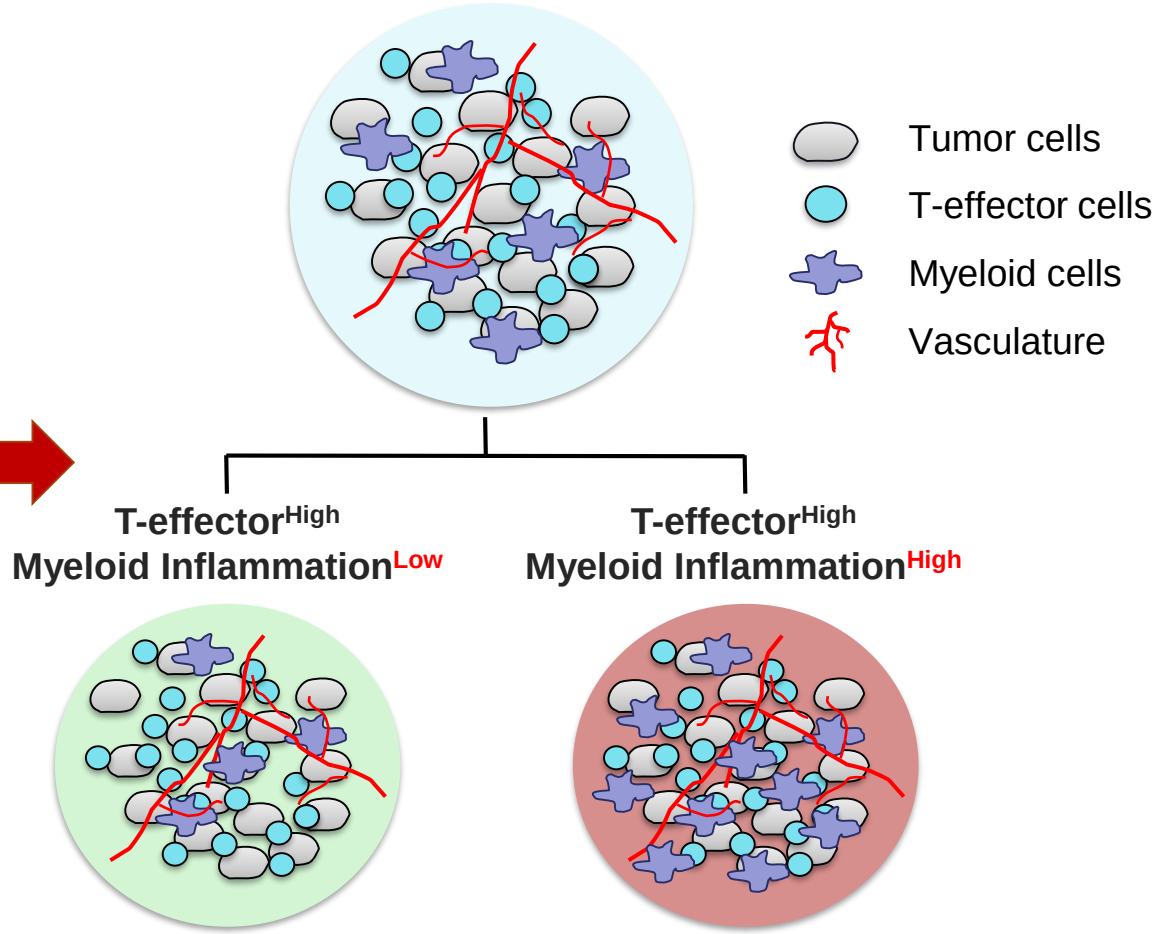
Checkpoints within tumor	**CTLA-4, LAG3**, TIM3, TIGIT, B7-H3, B7-H4, PD-1H (Vista), CD200, CEACAM1, **KIR**
MDSC/TAMS	**HDACi**, MER-TKi, CCR2i, CSF-1Ri, CD40, CKITi, ibrutinib, Anti-CD47 (‘Don’t Eat Me Signals’), SIGLECs
Treg	Anti-CCR4, **anti-CTLA-4, anti-CD25**
Inhibitory Cytokines	Antibodies and small molecule inhibitors of TGF-beta or its receptors
Hypoxia/Adenosine	Adenosine 2AR inhibitors Anti-CD39, anti-CD73
Metabolic Inhibitors and Prostaglandins	**IDO inhibitors**, Cox2 inhibitors, modulators of tumor/T-cell glucose consumption (PPAR-alpha inhibitors)
Barriers to T-cell infiltration	**Anti-VEGF**, anti-SEMA-4D, anti-CTLA-4

McDermott et al, Nat Med. 2018 Jun;24(6):749-757

Transcriptome Map of Angiogenesis and Immune-Associated Genes in RCC Tumors



T-effector^{High} Subpopulation

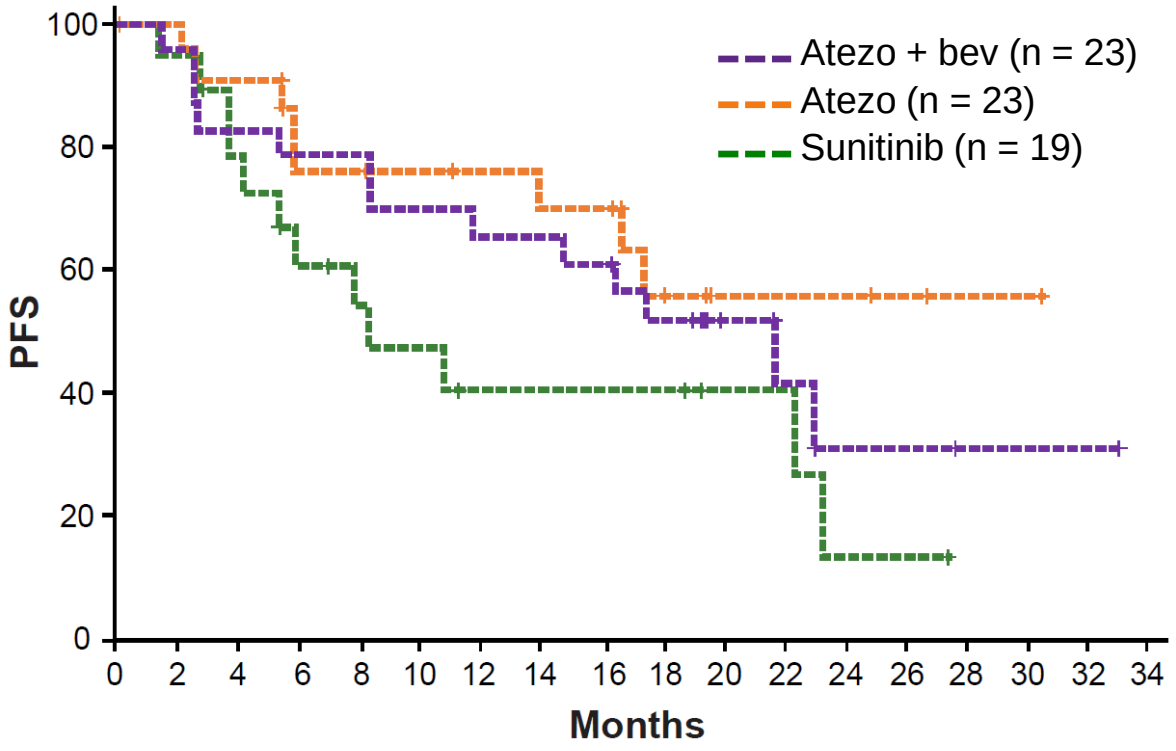


1. Brauer, *Clin Cancer Res*. 2012; 2. Herbst, *Nature* 2014; 3. Powles, SITC 2015; 4. Fehrenbacher, *Lancet* 2016. McDermott, AACR 2017.

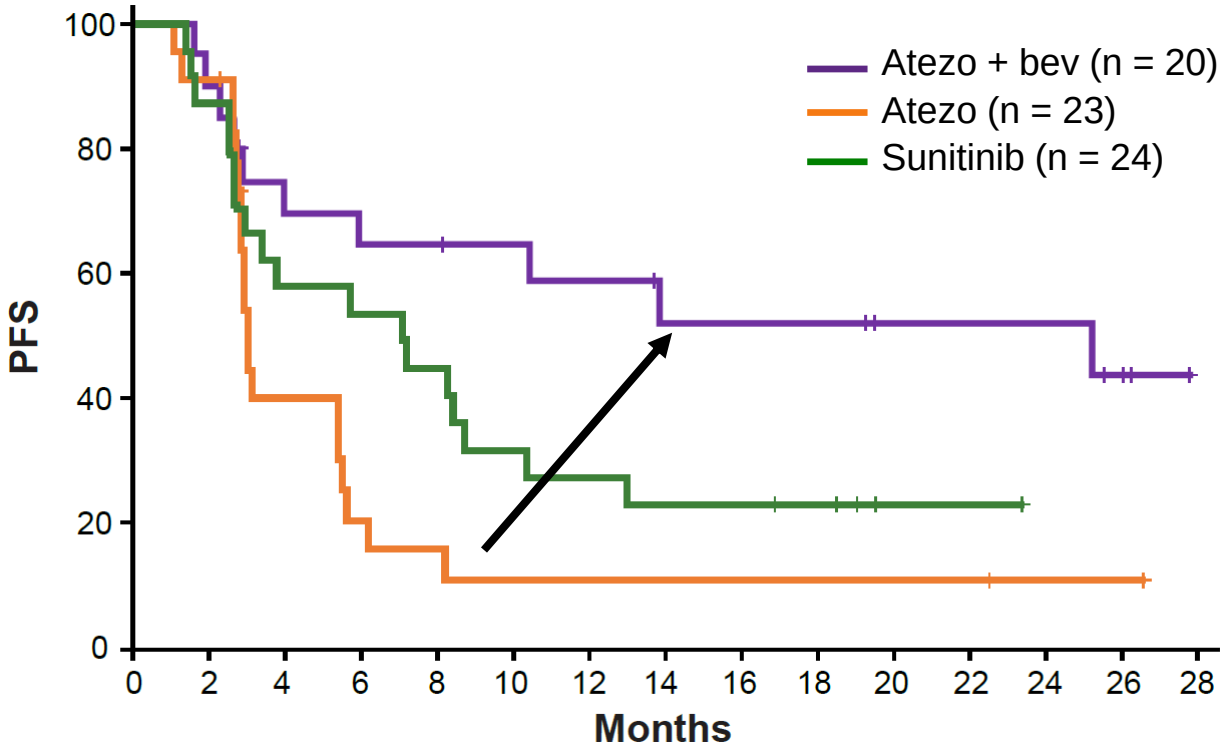
McDermott et al, Nat Med. 2018 Jun;24(6):749-757

Addition of Bevacizumab to Atezolizumab in 1L Was Associated With Improved Benefit in T-Effector^{High} Myeloid Inflammation^{High} Subgroup

T-effector^{High} Myeloid Inflammation^{Low}



T-effector^{High} Myeloid Inflammation^{High}



PFS measured by independent review facility.
T-effector gene signature: *CD8A, EOMES, PRF1, IFNG, CD274*.
High, ≥ median expression; low, < median expression. McDermott, AACR 2017.

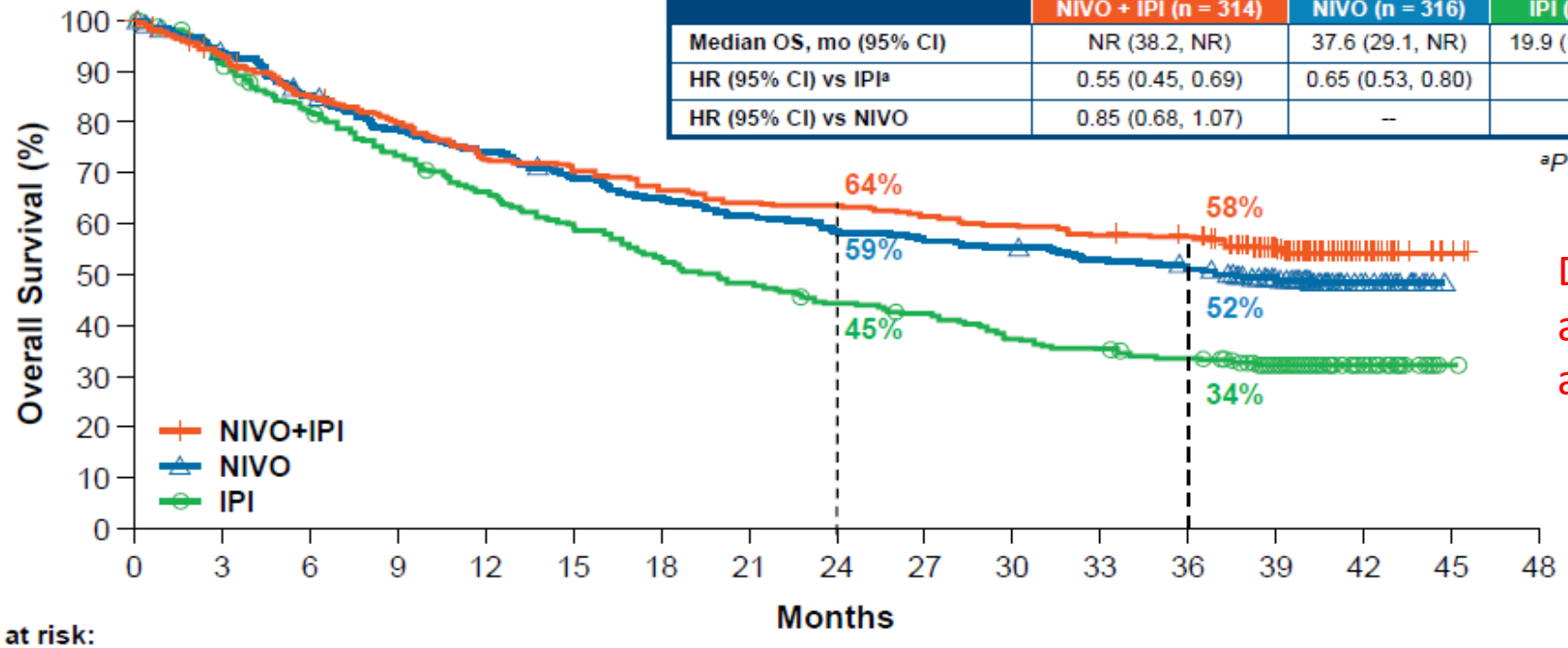
No effect of Atezo + bev over Atezo alone in Teff-low groups

CA209-067 – Ipi/Nivo or Nivolumab vs. Ipilimumab

Figure 1. OS (Intent-to-Treat)[1]

	NIVO + IPI (n = 314)	NIVO (n = 316)	IPI (n = 315)
Median OS, mo (95% CI)	NR (38.2, NR)	37.6 (29.1, NR)	19.9 (16.9, 24.6)
HR (95% CI) vs IPI[a]	0.55 (0.45, 0.69)	0.65 (0.53, 0.80)	--
HR (95% CI) vs NIVO	0.85 (0.68, 1.07)	--	--

[a]$P < 0.0001$



Patients at risk:

NIVO+IPI	314	292	265	247	226	221	209	200	198	192	186	180	177	131	27	3	0
NIVO	316	292	265	244	230	213	201	191	181	175	171	163	156	120	28	0	0
IPI	315	285	253	227	203	181	163	148	135	128	117	107	100	68	20	2	0

Despite higher ORR and PFS in ipi/nivo arm

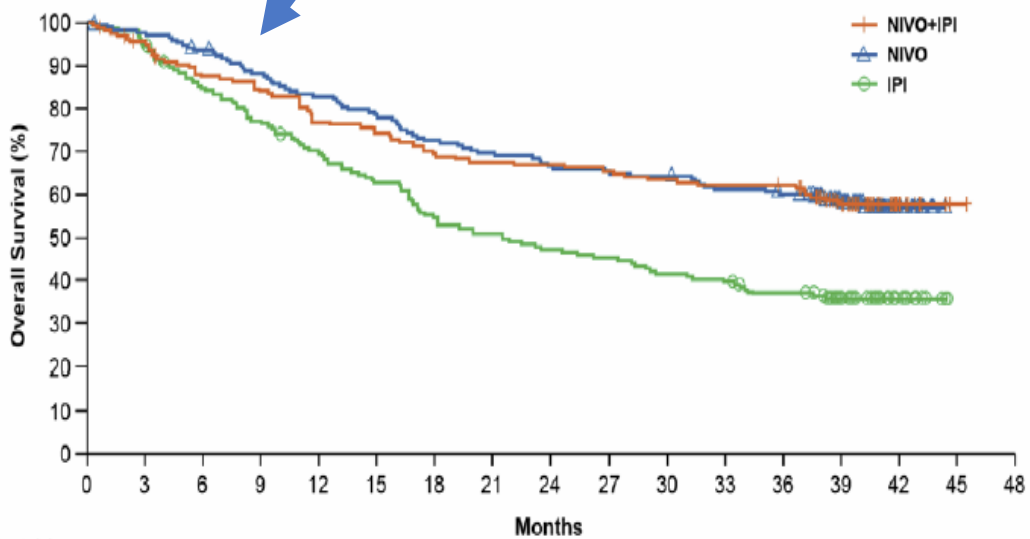
1. Wolchok JD et al. *N Engl J Med*. In press.

Wolchok et al, NEJM 2017

Post-hoc analyses of survival by PD-L1 status

Supplement to: Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2017;377:1345-56. DOI: 10.1056/NEJMoa1709684

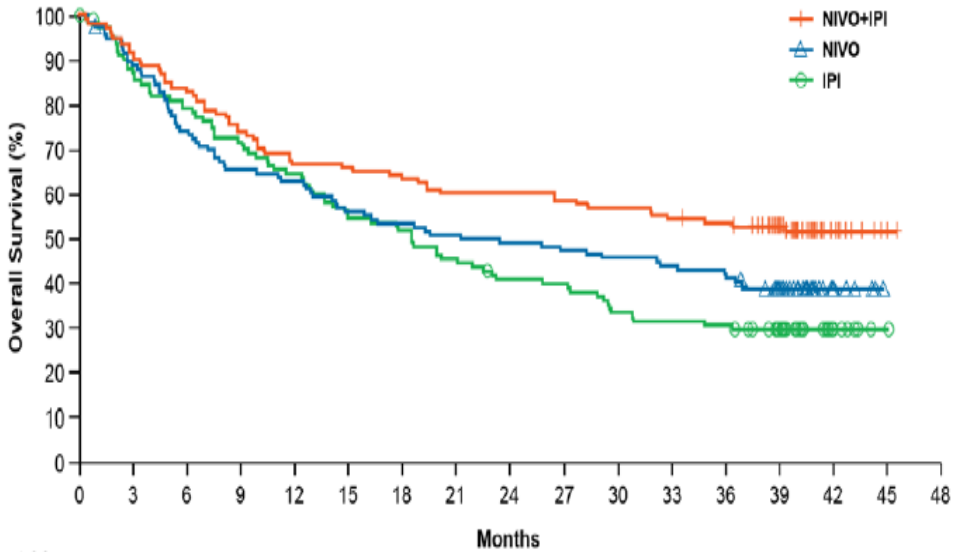
(C) PD-L1 expression level ≥1%



Patients at risk:

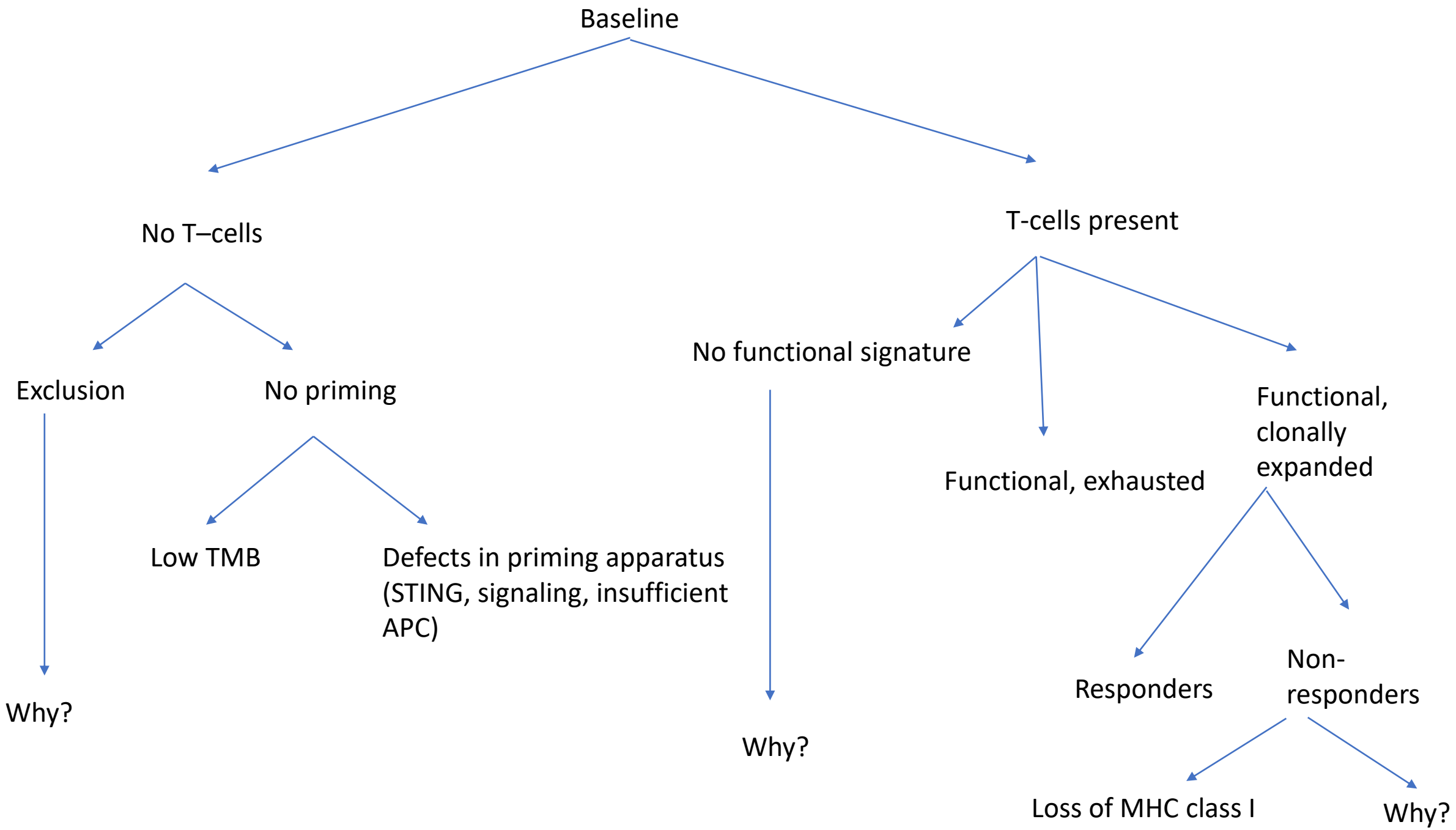
NIVO+IPI	155	144	132	127	116	112	105	102	101	99	96	94	93	66	14	1	0
NIVO	171	165	158	148	139	131	122	117	112	109	108	102	99	76	18	0	0
IPI	164	155	137	125	113	101	88	82	76	73	67	64	58	38	10	0	0

(D) PD-L1 expression level <1%



Patients at risk:

NIVO+IPI	123	113	102	91	82	82	79	74	74	72	70	67	65	50	11	2	0
NIVO	117	103	86	76	73	65	62	59	57	55	53	51	49	37	7	0	0
IPI	113	96	87	79	71	61	57	50	44	43	36	34	33	24	8	1	0


Baseline
No T–cells
T-cells present
Exclusion
No priming
No functional signature
Functional, exhausted
Functional, clonally expanded
Low TMB
Defects in priming apparatus (STING, signaling, insufficient APC)
Why?
Why?
Responders
Non-responders
Loss of MHC class I
Why?

Endpoint Dilemma – Hypothesis

- ORR and PFS possibly more predictive comparing immune therapy to non-immune therapy
  - But OS benefit may be seen absent improvement in ORR and PFS (durable response)
- ORR and PFS may be less useful comparing immune therapy to immune therapy, particularly when adding to anti-PD-1/PD-L1
  - Increase in PFS and ORR (in patients already receiving 'some' anti-tumor benefit) may not contribute to OS, at least in short-term
  - Short term OS increment may be seen only from 'converting' subset with no anti-tumor effect (primary resistance?)

Clinical Challenges - 1

- Optimal predictive biomarkers for anti-PD-1
- Mechanisms of non-response and resistance in T-cell inflamed tumors
    - Precise characterization of tumor Ag specific response
    - Rapid characterization of mechanisms in individuals
    - Defining true clinical resistance
- Mechanisms of non-response and resistance in T-cell infiltrated non-inflamed tumors
    - Co-opting activity of non-specific T cells
    - Other (non-PDx pathway) mechanisms of T-cell inhibition
- Addressing the cold (no T-cells) tumor
    - Mechanism
    - Inducing Ag-specific T cell response
    - Promoting T-cell infiltration
    - *Harnessing innate immunity*
- Biomarker ‘2’ for combination strategy
    - Detecting clinical signal of activity

Anti-PDx Resistance and Subsequent Signal Detection

- Progression while on anti-PD-1 in single or oligoclonal sites amenable to surgery/RT does not equal loss of benefit/resistance
- Mixed response early in treatment should not equate to PD/resistance unless progression is confirmed on subsequent scan 4-8 weeks later
- Progression in multiple sites while on drug and >= 12 weeks from starting Rx likely signifies true PD/drug resistance
- In patients with prior PR/CR/SD (with tumor regression), progression after drug holiday of > 4-6 months may not signify true resistance
- If prior response to ipi/nivo, progression on anti-PD-1 alone does not signify resistance to ipi/nivo
- Use progressing lesions only when assessing response to new agent or new agent + anti-PDx after declaring anti-PDx resistance

SITC Meeting and Consensus Conference April 1 – White paper to follow

Clinical Challenges -2

- Managing tumor microenvironment heterogeneity
- Optimal surrogate clinical endpoint for long-term survival/survival benefit
- Prognostic and predictive biomarkers in the adjuvant setting
- Specificity of drug delivery (targeting immune modulator to tumor)
- Library of tumor-specific cell surface targets
- Cost–effective T-cell engineering and cell therapies
  - universal T-cell, targeting, and function, and control
- Prediction and management of toxicity
- Cost and access

Scientific Challenges

- In depth individualized characterization of host immune – tumor relationship
  - Assessing the tumor Ag-specific immune response
- Critical thresholds for effective anti-tumor immune response
- <u>T-cell subset biology and contribution to anti-tumor effect</u>
- Critical immune suppressive pathways within tumor and extrinsic to microenvironment
- Biology of immune cell (T-cell, NK, other) traffic and tumor exclusion
- Features, evolution and dynamics of immune modulator perturbed state (early on-treatment changes)
- Managing tumor microenvironment heterogeneity

Host genetics → Lifetime environmental exposures → TCR repertoire →

Carcinogenesis:
Mutations
Altered gene expression
Chronic inflammation

→ Tumor evolution
Metastases
Evolution of Tumor-Host immune relationship
Microbiome

→ Patient Presenting for Treatment

→ Tumor microenvironment and Host Anti-tumor immune response

T-cells
- How many?
- What type?
- Recognize tumor antigens?
- Breadth of antigen recognition (one, a few, many)
- Affinity of TCR for peptide-MHC complex
- Functional state
- Differentiated state
- Expression of inhibitory receptors
- Metabolic state and access to glucose and Oxygen
- Where located?

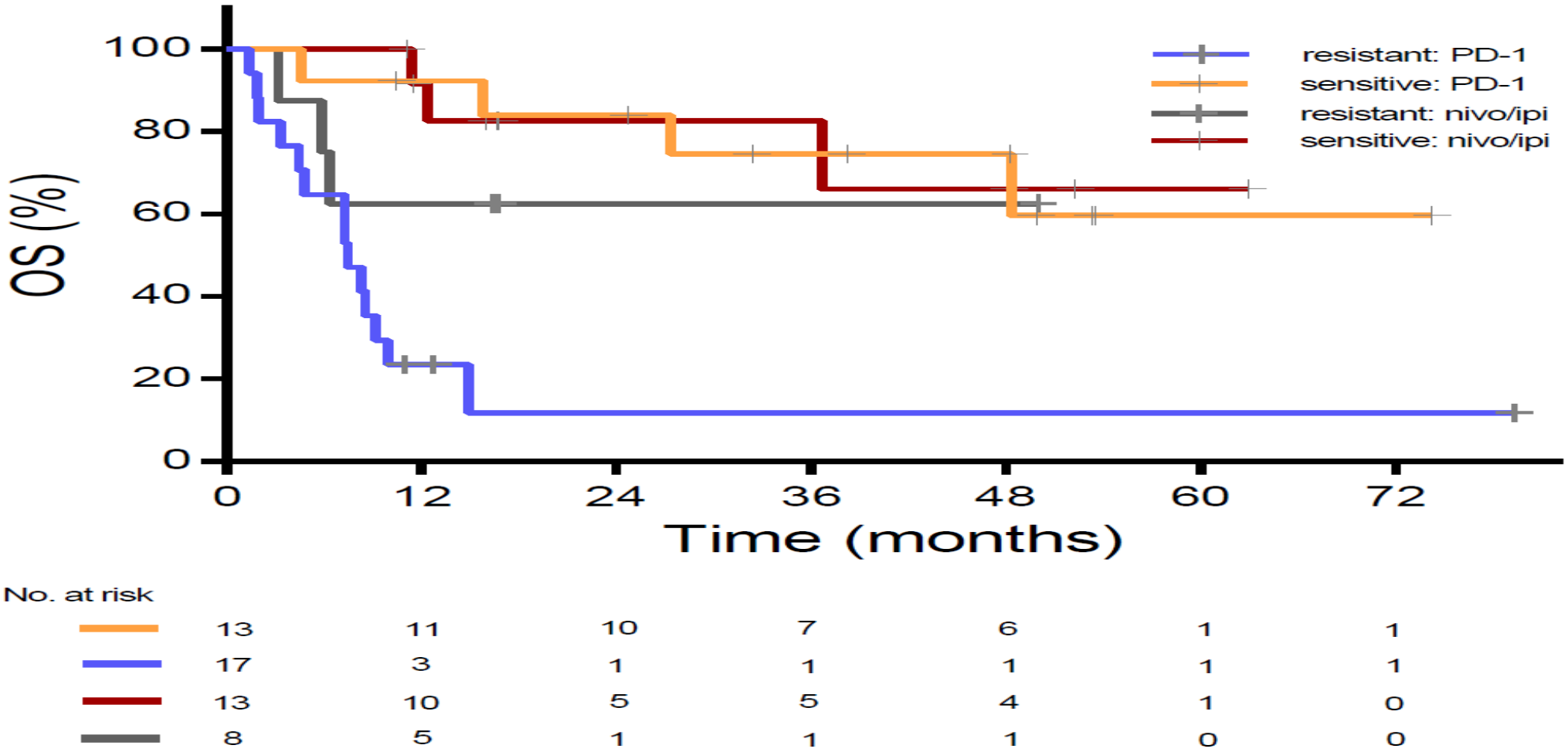
Tumor
- Antigens/neo-antigens
- Density of peptide/MHC complexes
- Expression of inhibitory ligands
- Expression of stimulatory ligands
- Production of inhibitory cytokines
- Production of other inhibitory substances
- Expression of chemokines
- Innate resistance to lytic mechanisms

Stroma/Other Immune Cells
- Treg
- MDSC
- Monocytes/macrophages/ APC
- B-cells
- NK and NKT cells
- Tumor Vasculature
- Fibroblasts
- Secreted Inhibitory Factors
- Complement

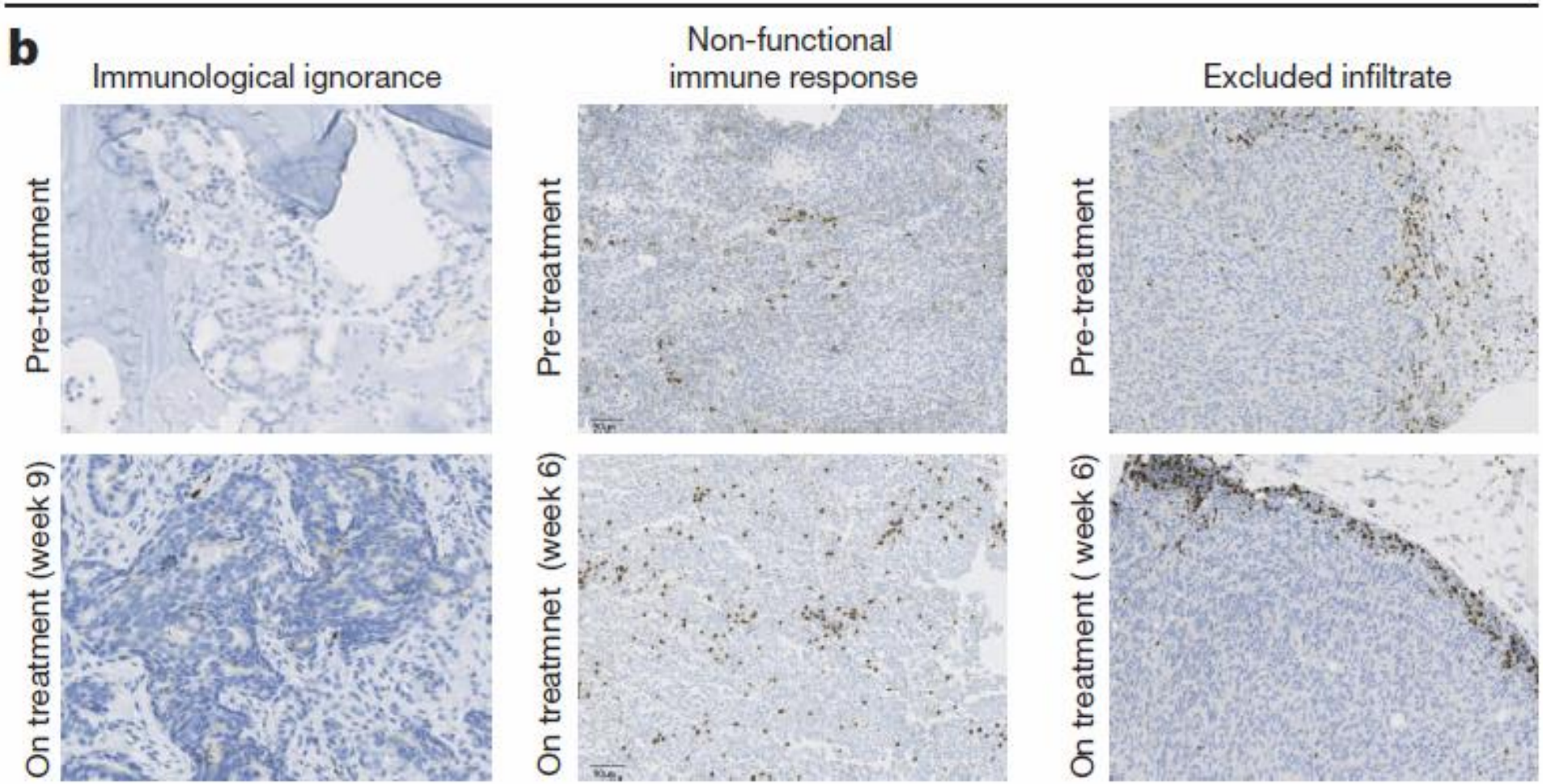
Other
- Microbiome
- Lymph nodes
- Blood

$\sum$ → Immune Intervention → Outcome

Serum Protein Signature Based on MALDI-TOF Spectometry Associated with Outcome After anti-PD1 Therapy in Metastatic Melanoma – Presented by Weber et al, ITOC-4, March 21, 2017



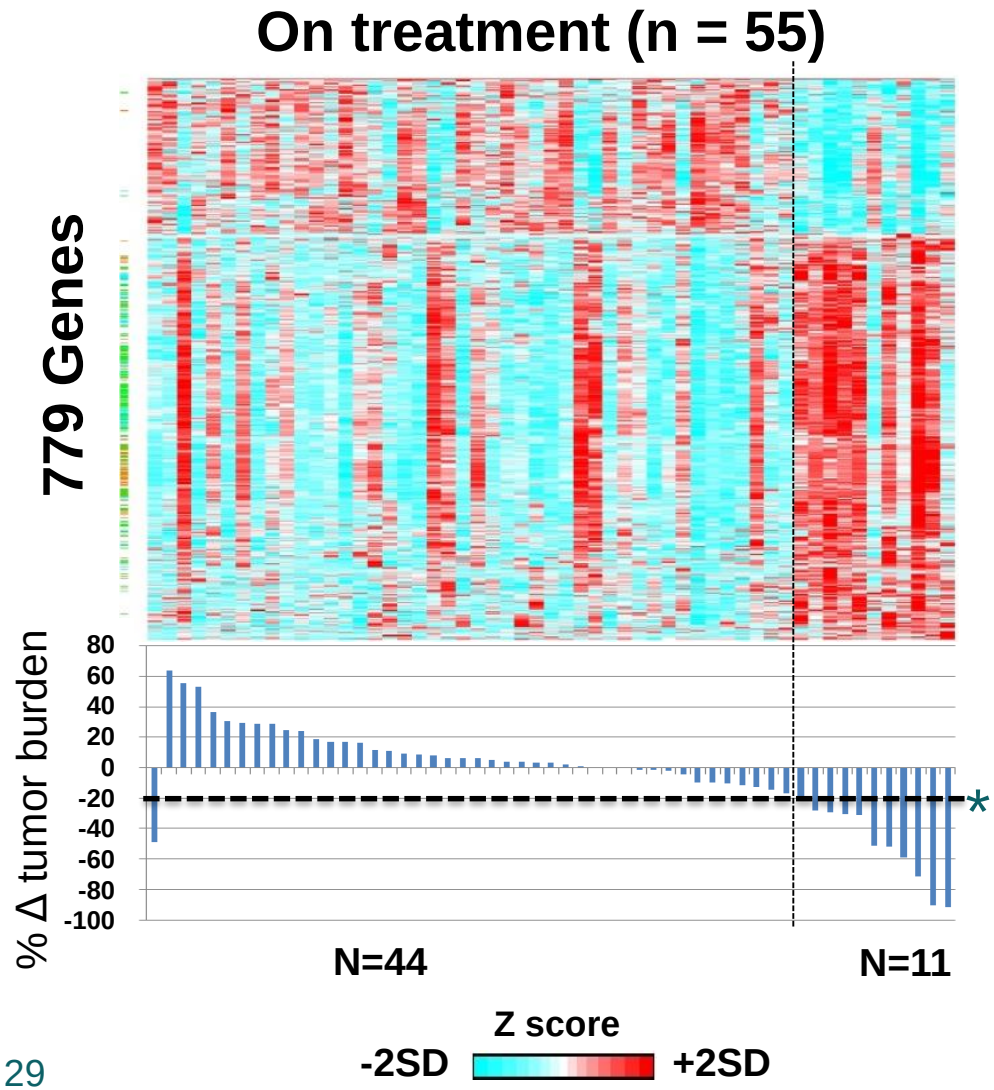
Cancer Immunol Res. 2018 Jan;6(1):79-86



Predictive correlates of response to the anti–PD–L1 antibody MPDL3280A in cancer patients

Roy S. Herbst[1], Jean-Charles Soria[2], Marcin Kowanetz[3], Gregg D. Fine[3], Omid Hamid[4], Michael S. Gordon[5], Jeffery A. Sosman[6], David F. McDermott[7], John D. Powderly[8], Scott N. Gettinger[1], Holbrook E. K. Kohrt[9], Leora Horn[10], Donald P. Lawrence[11], Sandra Rost[3], Maya Leabman[3], Yuanyuan Xiao[3], Ahmad Mokatrin[3], Hartmut Koeppen[3], Priti S. Hegde[3], Ira Mellman[3], Daniel S. Chen[3] & F. Stephen Hodi[12]

CA209-009 – Nivo/RCC- Genes association with tumor burden reduction: On treatment



Lower expression

- **Cellular component organization** ($P < 10^{-18}$)
- **Signaling** ($P < 10^{-16}$)
- Downregulated by ipilimumab in melanoma[1] ($P < 10^{-5}$)

Higher expression

- Upregulated by ipilimumab in melanoma [1]($P < 10^{-135}$)
- **Immune system** (188; $P < 10^{-82}$)
- **Known Myeloid lineage (51):** e.g. CD68, CD86, CASP1, CSF3R
- **Known Lymphoid lineage (>65):** TCRα/β,CD3D, CD8A, CD28
- **Cytolytic function:** KLRG1, granzymes, PRF1
- **Immune checkpoint molecules:** TIGIT, CTLA-4, PD-L2, IL10RA

* 20% reduction in tumor burden

Bold: denotes Gene Ontology Process

1. Ji RR, et al. *Cancer Immunol Immunother* 2012;61:1019–31.