Cancer Immunotherapy: Current and Next Generation Biomarkers

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Definition of Cancer Immunotherapy

- Treatments that harness patients' immune system for cancer therapy
- Monoclonal antibodies
  - Target cancer cells
  - Modulate immune function: Immune Checkpoint Blockade (ICB)
- Cytokines (also blocking antibodies)
- Cancer vaccines (+/- dendritic cells)
- Adoptive cellular therapy (+/- engineered receptors: TCR or chimeric antigen receptor, CAR)

Approved Immune Checkpoint Inhibitors (ICIs) for Cancer

2011: Ipilimumab (anti-CTLA4 Ab) for advanced melanoma

2014: Pembrolizumab and Nivolumab (anti-PD1 Ab) for advanced melanoma

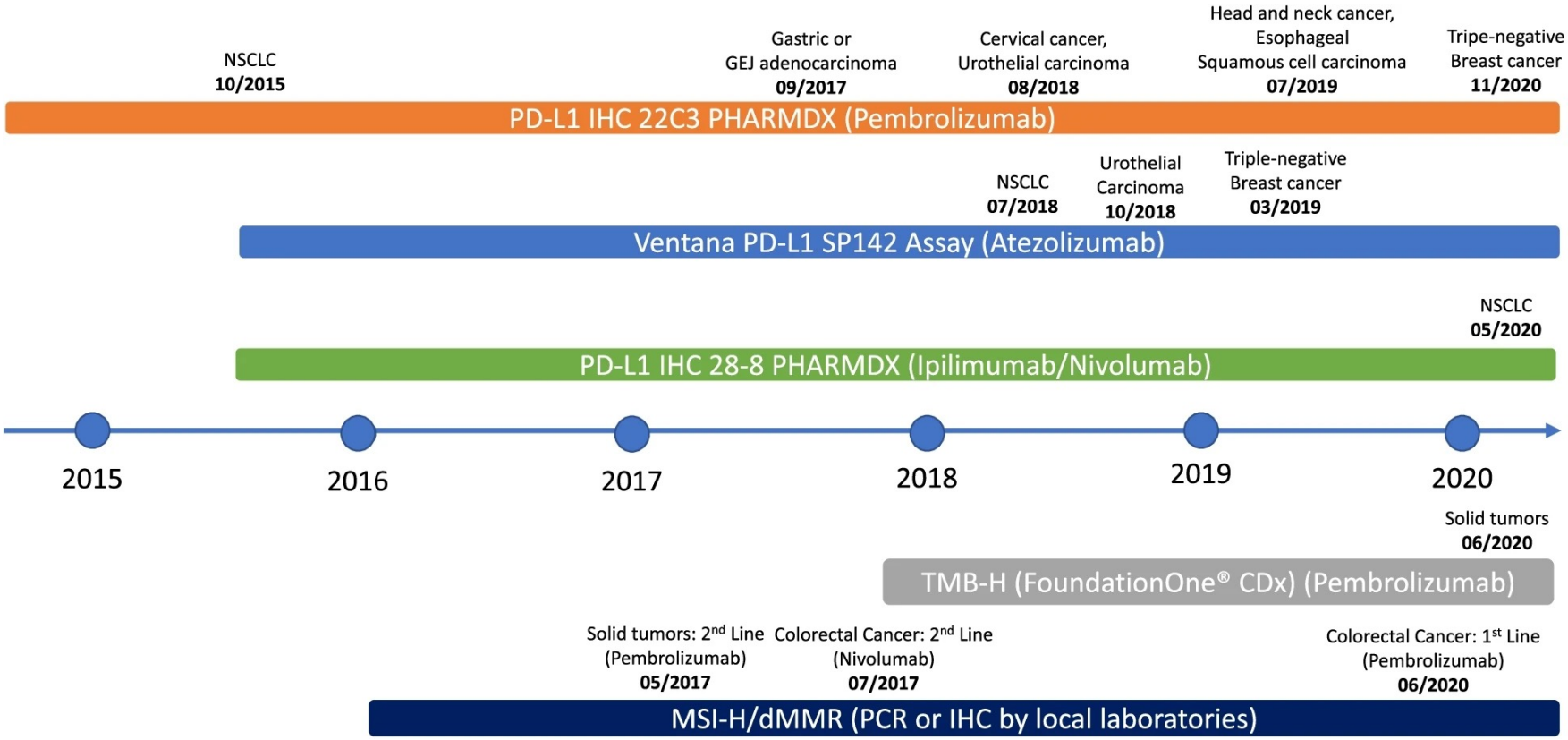
2015: Nivolumab for lung cancer and kidney cancer; Nivo/Ipi combination for melanoma

2016 on: multiple other cancer types; MSI tumors

These treatments still only work for subsets of patients and some cancer types (20-30% of hot tumors)

Need for biomarkers!

Current FDA Approved Biomarkers for ICIs



Sankar et al. Biomarker Research 2022

Current FDA Approved Biomarkers for ICIs

Biomarker	Cancer types	ICI agents	Accuracy
Programmed death-ligand 1 (PD-L1)	NSCLC, gastric, cervical, urothelial, HNSCC, esophageal, TNBC	Pembrolizmab Ipilimumab/Nivolumab Atezolizmab	low diagnostic accuracy: predictive of only 28.9% of ICI approvals 2011-2019.
Microsatellite instability/defective mismatch repair (MSI/dMMR)	Any (<5% of metastatic tumors): mainly CRC, endometrial, ovarian, gastric, other GI	Pembrolizmab Nivolumab	Good, but also depends on method: IHC, PCR, NGS
Tumor mutational burden (TMB)	Any: include CRC, esophageal, NSCLC, SCLC	Pembrolizmab	Wide variation in results, also depends on method: WES, targeted NGS panels

FDA Approved PD-L1 IHC Assays

Test Name	PMA#	Tumor Type	ICI	Approval Year	Scoring System	PD-L1-Threshold	PD-L1 Staining
PD-L1 IHC 22C3 pharmDx	P150013	NSCLC	Pembrolizumab	2015	TPS	>=50%	tumor cells
PD-L1 IHC 22C3 pharmDx	P150013/ S006	gastric or GEJ adenocarcinoma	Pembrolizumab	2017	CPS	>=1	tumor cells, lymphocytes, macrophages
PD-L1 IHC 22C3 pharmDx	P150013/ S009	Cervical Cancer	Pembrolizumab	2018	CPS	>=1	tumor cells, lymphocytes, macrophages
PD-L1 IHC 22C3 pharmDx	P150013/ S011	urothelial carcinoma	Pembrolizumab	2018	CPS	>=10	tumor cells, lymphocytes, macrophages
PD-L1 IHC 22C3 pharmDx	P150013/ S014	head and neck squamous cell carcinoma	Pembrolizumab	2019	CPS	>=1	tumor cells, lymphocytes, macrophages
PD-L1 IHC 22C3 pharmDx	P150013/ S016	esophageal squamous cell carcinoma	Pembrolizumab	2019	CPS	>=10	tumor cells, lymphocytes, macrophages
VENTANA PD-L1 (SP142) Assay	P160002/ S006	urothelial carcinoma/ NSCLC	atezolizumab	2018	IC%/IC% or TPS	>=5%/>=10% or >=50%	tumor area/tumor area, tumor ells
VENTANA PD-L1 (SP142) Assay	P160002/ S009	Triple-Negative Breast Carcinoma	atezolizumab	2019	IC%	>=1%	tumor area
VENTANA PD-L1 (SP142) Assay	P160002/ S012	NSCLC	atezolizumab	2020	IC%/TPS	>=10%/>=50%	tumor area Tumor cells
PD-L1 IHC 28-8 pharmDx	P150025/ S013	NSCLC/SCCHN/UC	Nivolumab in combination with ipilimumab	2020	TPS	>=1%	tumor cells
PD-L1 IHC SP263	P160046	urothelial carcinoma	Durvalumab	2017	TPS/ICP/ IC+	>=25%/ICP > 1% and IC+ >=25%/ICP = 1% and IC+ = 100%.	tumor cells Immune cells

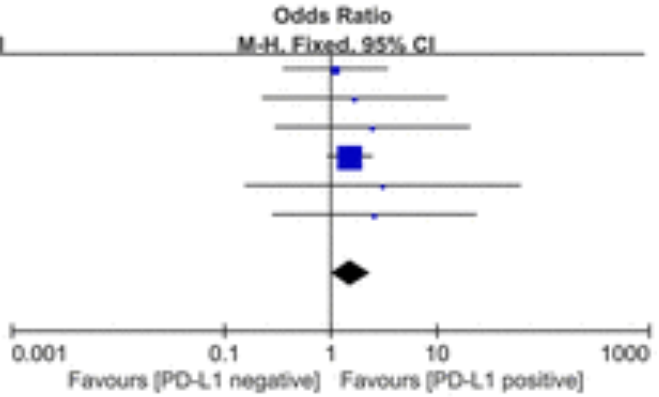
Wang *et al. Frontiers in Oncology* 2021

Tumor PD-L1 is a borderline predictor

(A) PFS

Study or Subgroup	PD-L1 positive Events	Total	PD-L1 negative Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Adams1 2018 [31]	9	105	5	64	15.8%	1.11 [0.35, 3.46]
Adams3 2018 [9]	3	12	2	12	4.2%	1.67 [0.22, 12.35]
Emens 2018 [10]	10	91	1	21	4.0%	2.47 [0.30, 20.43]
Schmid 2018 [36]	38	185	39	266	70.8%	1.50 [0.92, 2.46]
Sherene 2019 [29]	4	40	0	12	1.9%	3.08 [0.15, 61.38]
Voorwerk 2019 [12]	5	44	1	21	3.3%	2.56 [0.28, 23.46]
Total (95% CI)		**477**		**396**	**100.0%**	**1.55 [1.02, 2.36]**
Total events	69		48			

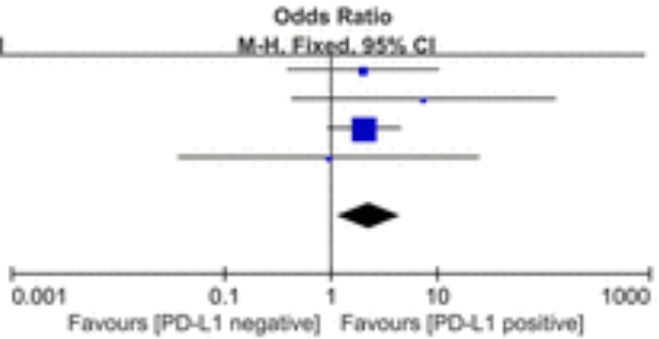
Heterogeneity: Chi² = 0.94, df = 5 (P = 0.97); I² = 0%
Test for overall effect: Z = 2.07 (P = 0.04)



(B) OS

Study or Subgroup	PD-L1 positive Events	Total	PD-L1 negative Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Adams3 2018 [9]	6	12	4	12	17.1%	2.00 [0.38, 10.41]
Emens 2018 [10]	13	91	0	21	5.9%	7.39 [0.42, 129.48]
Schmid 2018 [36]	15	185	11	266	70.8%	2.05 [0.92, 4.56]
Sherene 2019 [29]	1	40	0	12	6.2%	0.95 [0.04, 24.81]
Total (95% CI)		**328**		**311**	**100.0%**	**2.28 [1.16, 4.48]**
Total events	35		15			

Heterogeneity: Chi² = 1.02, df = 3 (P = 0.80); I² = 0%
Test for overall effect: Z = 2.40 (P = 0.02)



Zou et al. 2020

PD-L1 expressed on cancer cells or immune cells?

Current FDA Approved Biomarkers for ICIs

Biomarker	Cancer types	ICI agents	Accuracy
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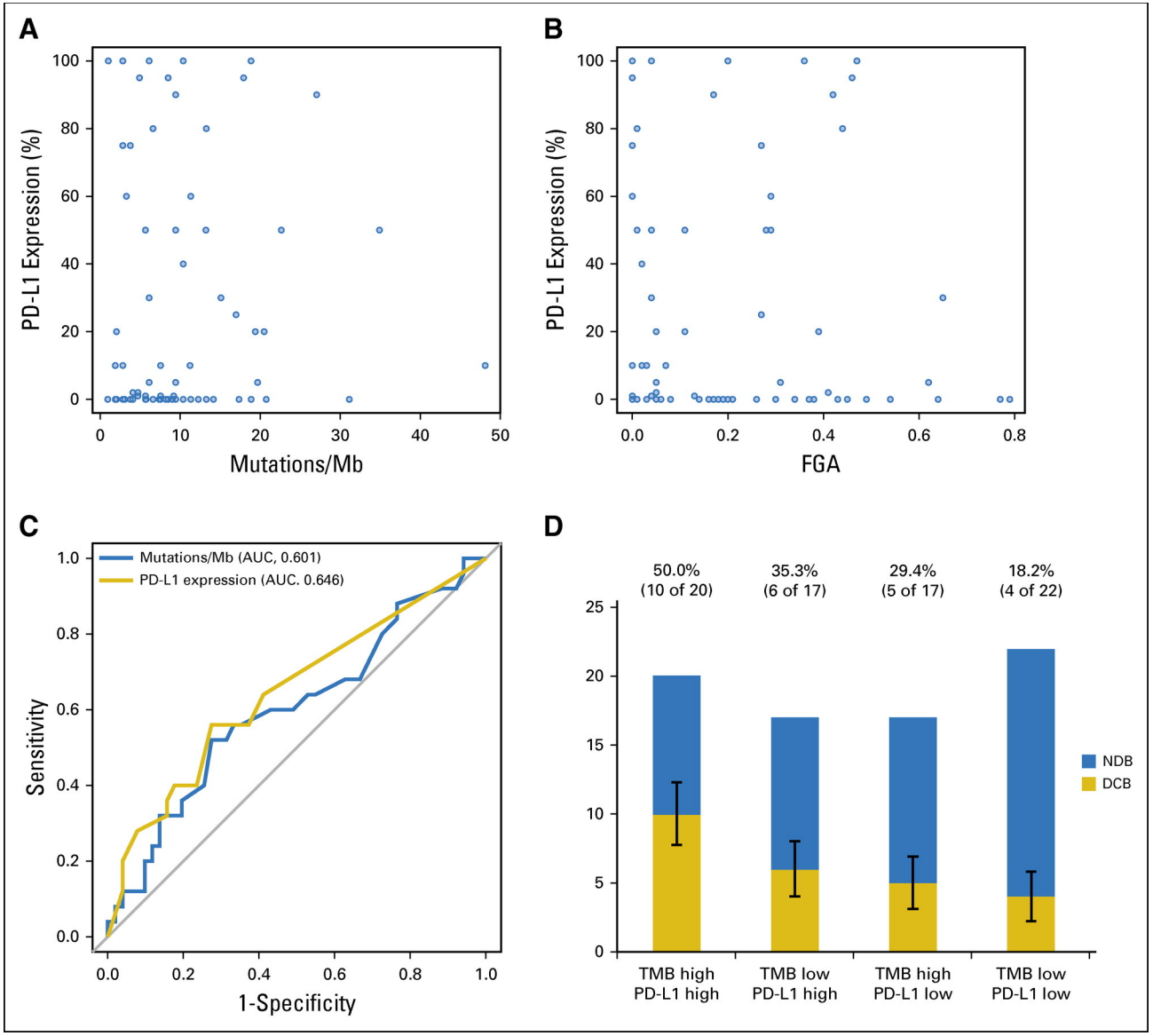
Tumor mutational burden (TMB)	Any: include CRC, esophageal, NSCLC, SCLC	Pembrolizmab	Wide variation in results, also depends on method: WES, targeted NGS panels

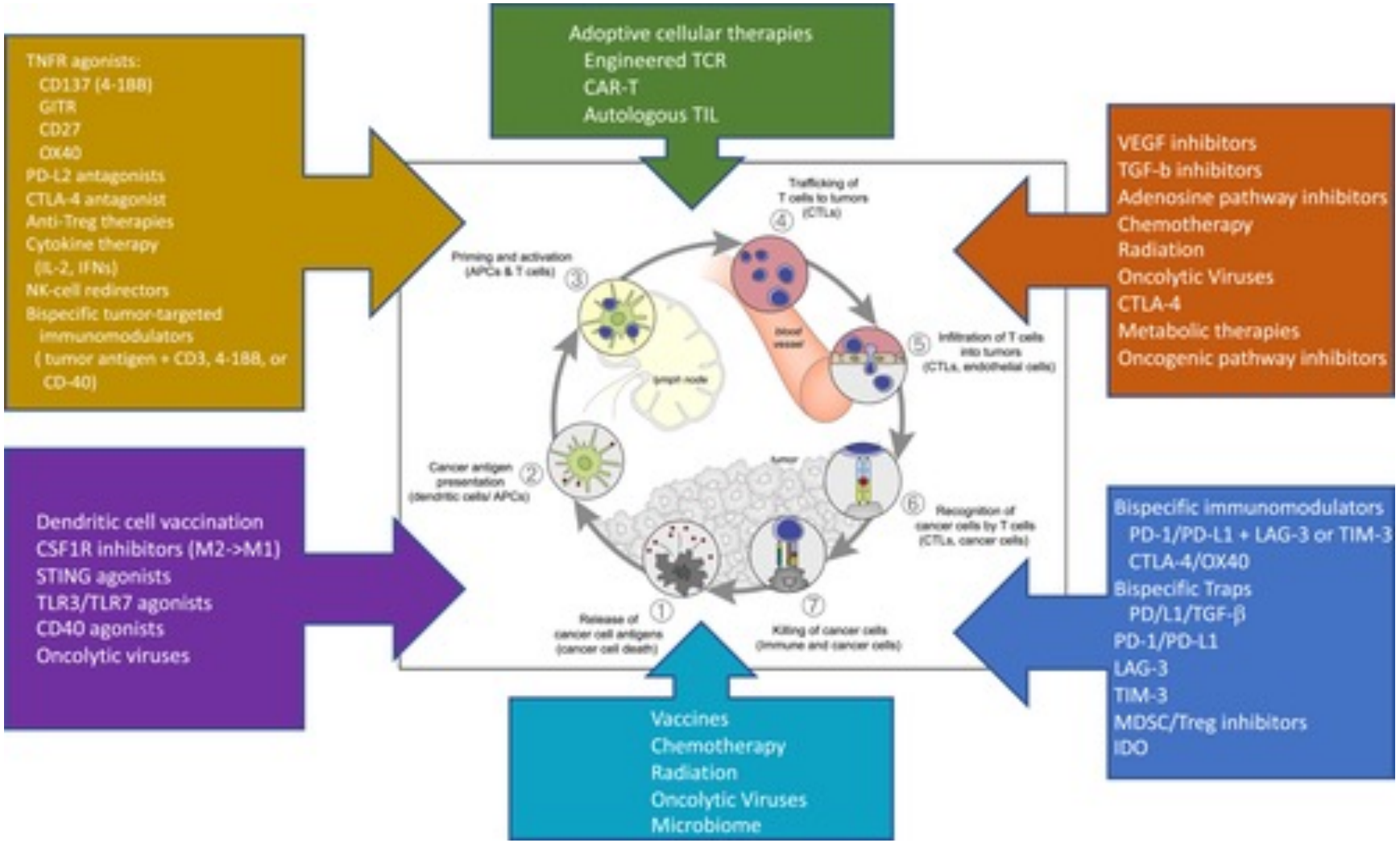
Tumor mutational burden (TMB)



NBD = no durable benefit
DCB = durable clinical benefit

Rizvi et al. Thoracic Oncology 2021

Need for better biomarkers: Cancer immunity cycle, revisited



Immunological Reviews, Volume: 290, Issue: 1, Pages: 6-23, First published: 29 July 2019, DOI: (10.1111/imr.12766)

Immune Contexture of Tumors

Table. Comparison of Pooled Diagnostic Sensitivity and Specificity and Predictive Values for Responders vs Nonresponders After Anti–PD-1/PD-L1 Therapy Between Different Assay Modalities[a]

Modality	Pooled Sensitivity	Pooled Specificity	Pooled PPVs	Pooled NPVs
PD-L1 IHC (n = 24)	0.50 (0.48-0.53)	0.63 (0.62-0.65)	0.34 (0.32-0.36)	0.78 (0.76-0.79)
TMB (n = 10)	0.57 (0.51-0.62)	0.70 (0.66-0.73)	0.42 (0.38-0.47)	0.80 (0.77-0.83)
GEP (n = 9)	0.71 (0.67-0.75)	0.51 (0.48-0.54)	0.42 (0.39-0.46)	0.77 (0.74-0.81)
mIHC/IF (n = 7)	0.60 (0.53-0.66)	0.78 (0.73-0.82)	0.63 (0.56-0.70)	0.75 (0.70-0.80)
Multimodality (n = 6)	0.58 (0.50-0.65)	0.79 (0.75-0.82)	0.41 (0.33-0.48)	0.88 (0.85-0.91)

Abbreviations: GEP, gene expression profiling; mIHC/IF, multiplex immunohistochemistry/immunofluorescence; PD-L1 IHC, programmed cell death ligand 1 immunohistochemistry; TMB, tumor mutational burden.

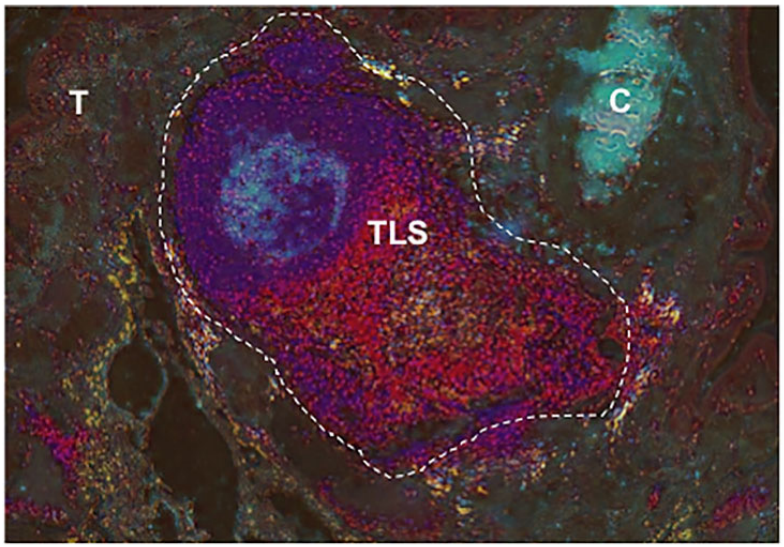
[a] All data are reported as a proportion (95% CI). Nonoverlapping 95% CIs suggest statistical significance.

From: **Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade: A Systematic Review and Meta-analysis**

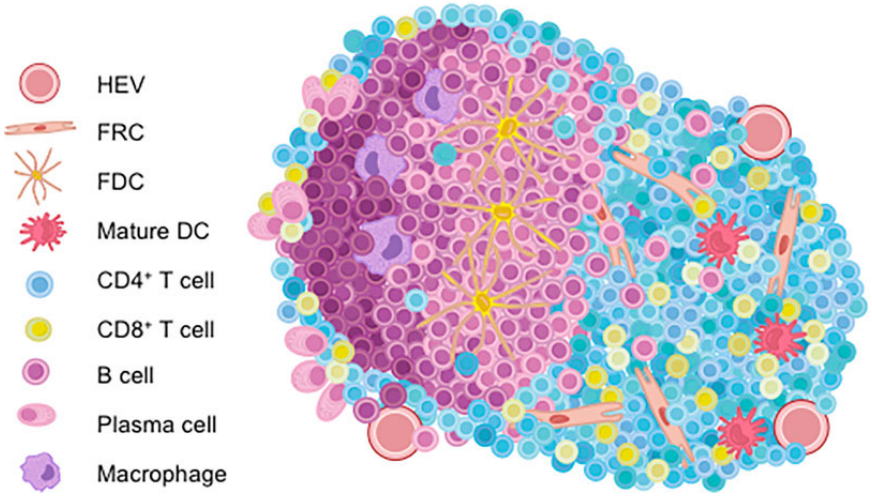
JAMA Oncol. Published online July 18, 2019. doi:10.1001/jamaoncol.2019.1549

Tertiary Lymphoid Structures (TLS)

A

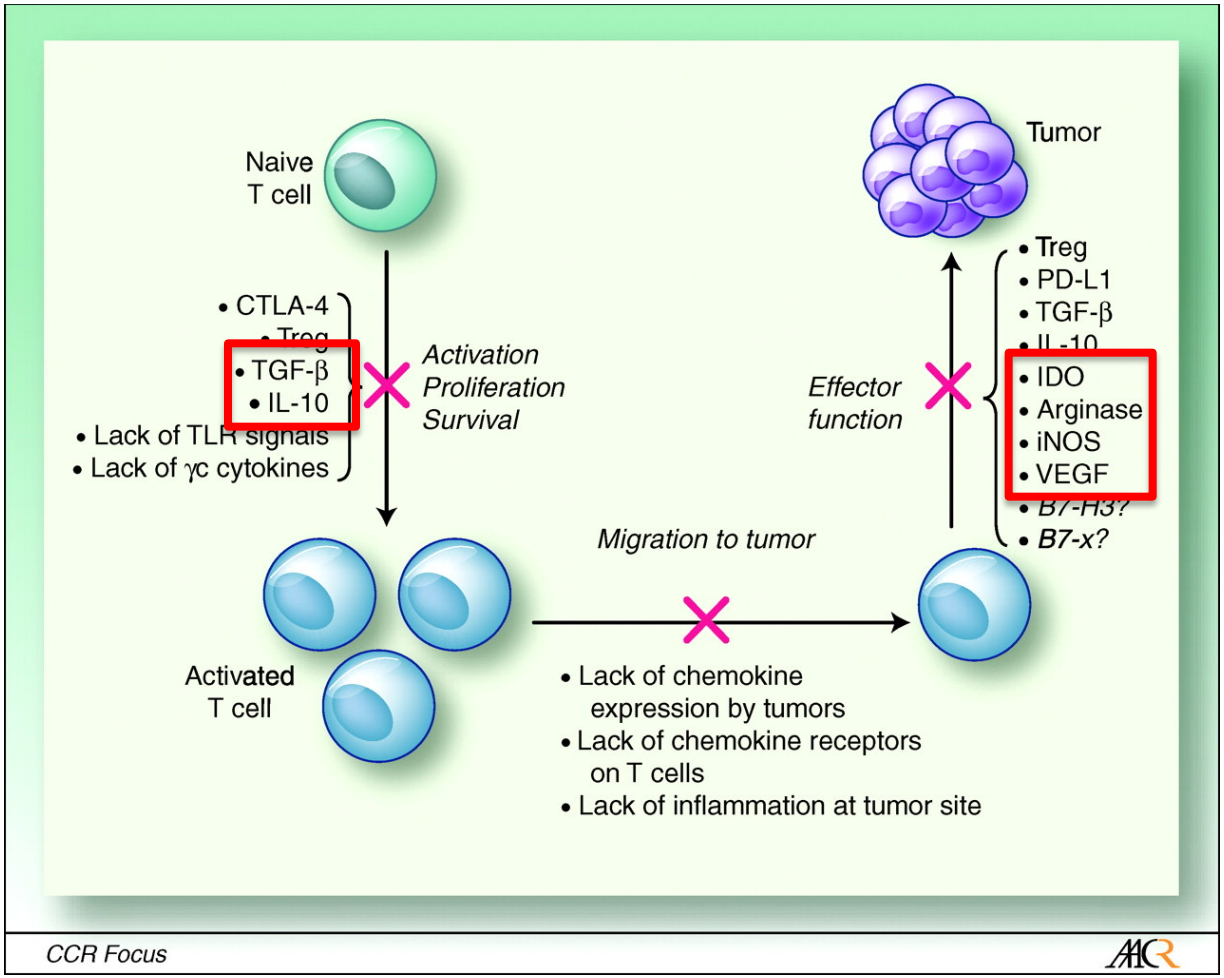


B



Domblides *et al. Frontiers in Immunology* 2021

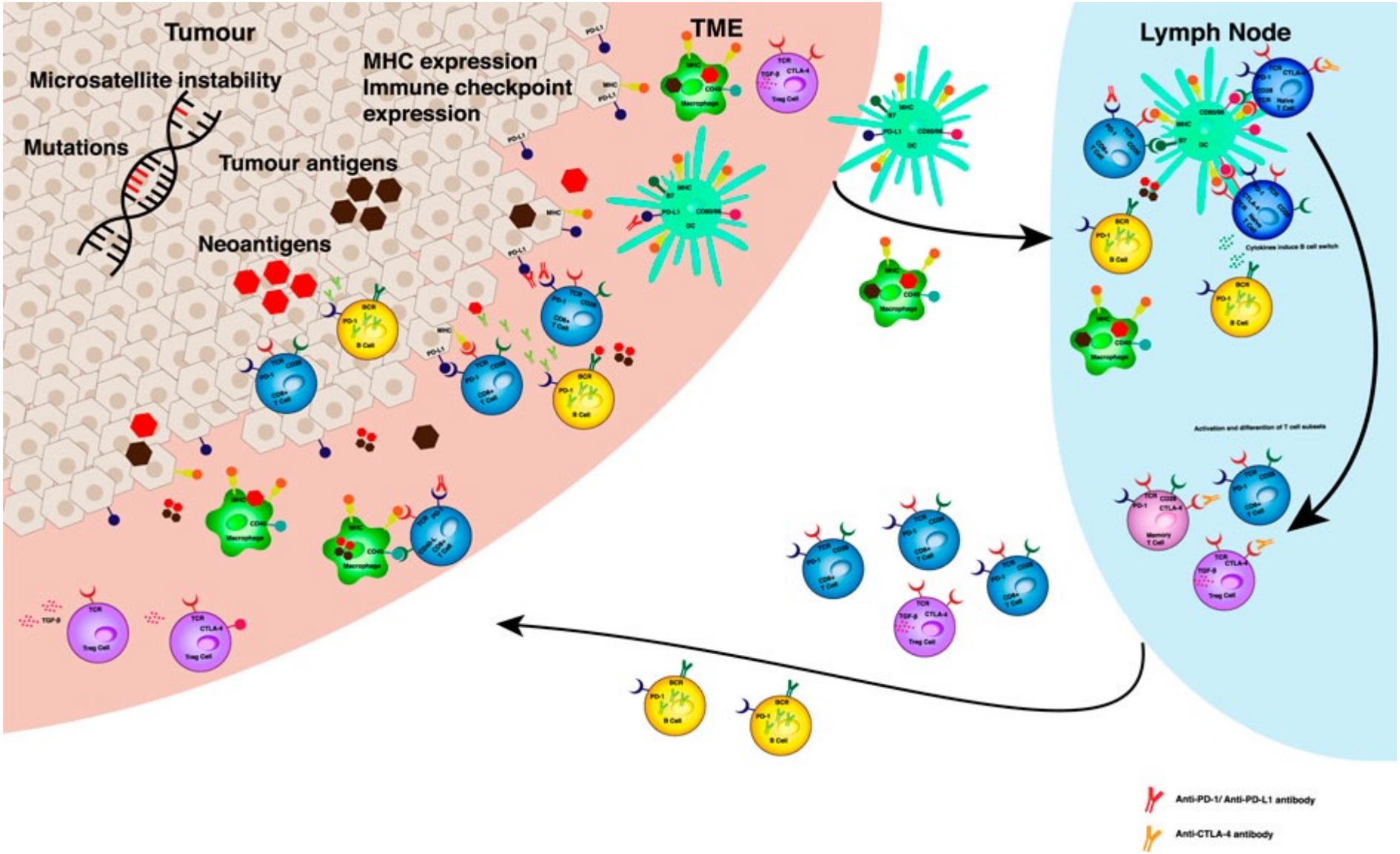
Many barriers in vivo to effective anti-tumor T cell immunity



Lizée G et al. *Clin Cancer Res* 2007;13:5250-5255

Moving beyond the tumor

Tumor-Draining Lymph Nodes



Goode *et al. Frontiers in Molecular Biosciences* 2021

Blood Biomarkers

- Immune cell subsets: PD1+ CD8 T cells, Tregs
- Serum cytokines: IL-6
- Immune cells signaling responses
- T cell receptor (TCR) clonality
- Peripheral blood shed PD-L1
- Cell-free (cf)DNA

Multi-parameter blood biomarkers

Biomarker	Cancer type	No. of patients	Main results	Reference
(%Ki-67^{+} cells/PD-1^{+}CD8^{+} T cells 3-wk post-treatment)/baseline tumor burden (Ki67/TB)	Melanoma	Discovery cohort: 23	Higher Ki67/TB significantly associated with superior ORR (p=0.03) and PFS (p=0.004).	Huang et al. (34)
		Validation cohort: 18	Higher Ki67/TB associated with superior ORR (p=0.14) and PFS (p=0.06).	
(%Ki-67^{+} cells/PD-1^{+}CD8^{+} T cells 1-wk post-treatment)/(%Ki-67^{+} cells/PD-1^{+}CD8^{+} T cells at baseline) (Ki-67$^{D7/D0}$)	TET	Discovery cohort: 31	Higher Ki-67$^{D7/D0}$ significantly associated with durable clinical benefit (PR, or SD for 6 months or longer; p<0.001) and PFS (p=0.027)	Kim et al. (32)
	NSCLC	Discovery cohort: 33	Higher Ki-67$^{D7/D0}$ significantly associated with durable clinical benefit (PR, or SD for 6 months or longer; p<0.01), PFS (p=0.004), and OS (p=0.001)	
		Validation cohort: 46	Higher Ki-67$^{D7/D0}$ significantly associated with durable clinical benefit (PR, or SD for 6 months or longer; p<0.01), PFS (p=0.002), and OS (p=0.037)	
%FoxP3^{-}PD-1^{hi}CD4^{+} T cells/CD4^{+} T cells (4PD1^{hi}) 3-wk post-treatment Fold change of 4PD1^{hi}	Melanoma	52	Higher frequency of 4PD1^{hi} 3-wks post treatment (p=0.0005) and fold change of 4PD1^{hi} (p=0.046) associated with poorer OS.	Zappasodi et al. (36)
TCR diversity of PD-1^{+}CD8^{+} T cells at baseline and post-treatment	NSCLC	Discovery cohort: 25 Validation cohort: 15	Higher baseline diversity in PD-1^{+}CD8^{+} T cells (p=0.021) and increased clonality after treatment (p=0.002) associated with superior PFS.	Han et al. (39)
%CD27^{-}CD28^{-} cells/CD4^{+} T cells at baseline	NSCLC	51	Higher frequency of CD27^{-}CD28^{-}CD4^{+} T cells associated superior PFS (p=0.001).	Zuazo et al. (42)
Ratio of the frequency of Treg cells and PMN-MDSCs at baseline	NSCLC	Discovery cohort: 34	Higher ratio of the frequency of Treg cells and PMN-MDSCs associated with superior PFS (p=0.0079).	Kim et al. (44)
		Validation cohort: 29	Higher ratio of the frequency of Treg cells and PMN-MDSCs associated with superior PFS (p=0.0017).	
%Effector/memory (CCR7^{-}CD45RA^{-}) cells/CD8^{+} T cells at baseline	NSCLC	263 (flow cytometry analysis in 144)	Lower frequency of effector/memory CD8^{+} T cells with development of hyperprogressive disease (p<0.001) and poor PFS (p<0.001) and OS (p<0.001).	Kim et al. (53)
%TIGIT^{+} cells/PD-1^{+}CD8^{+} T cells at baseline			Higher frequency of TIGIT^{+} cells among PD-1^{+}CD8^{+} T cells in peripheral blood at baseline significantly associated with development of hyperprogressive disease (p<0.001) and poor PFS (p<0.001) and OS (p=0.01).	

NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease; TET, thymic epithelial tumor; CCR7, C-C chemokine receptor type 7.

Kim et al. 2020

Functional (dynamic) immune assays

- Most assays measure quantities (static)
- Immune system is dynamic -> need to assess functional status of patients' immune system
- Measure functional responses after specific interrogation:
  - Cytokine signaling responses
  - Cytotoxicity responses
  - Cytokine production

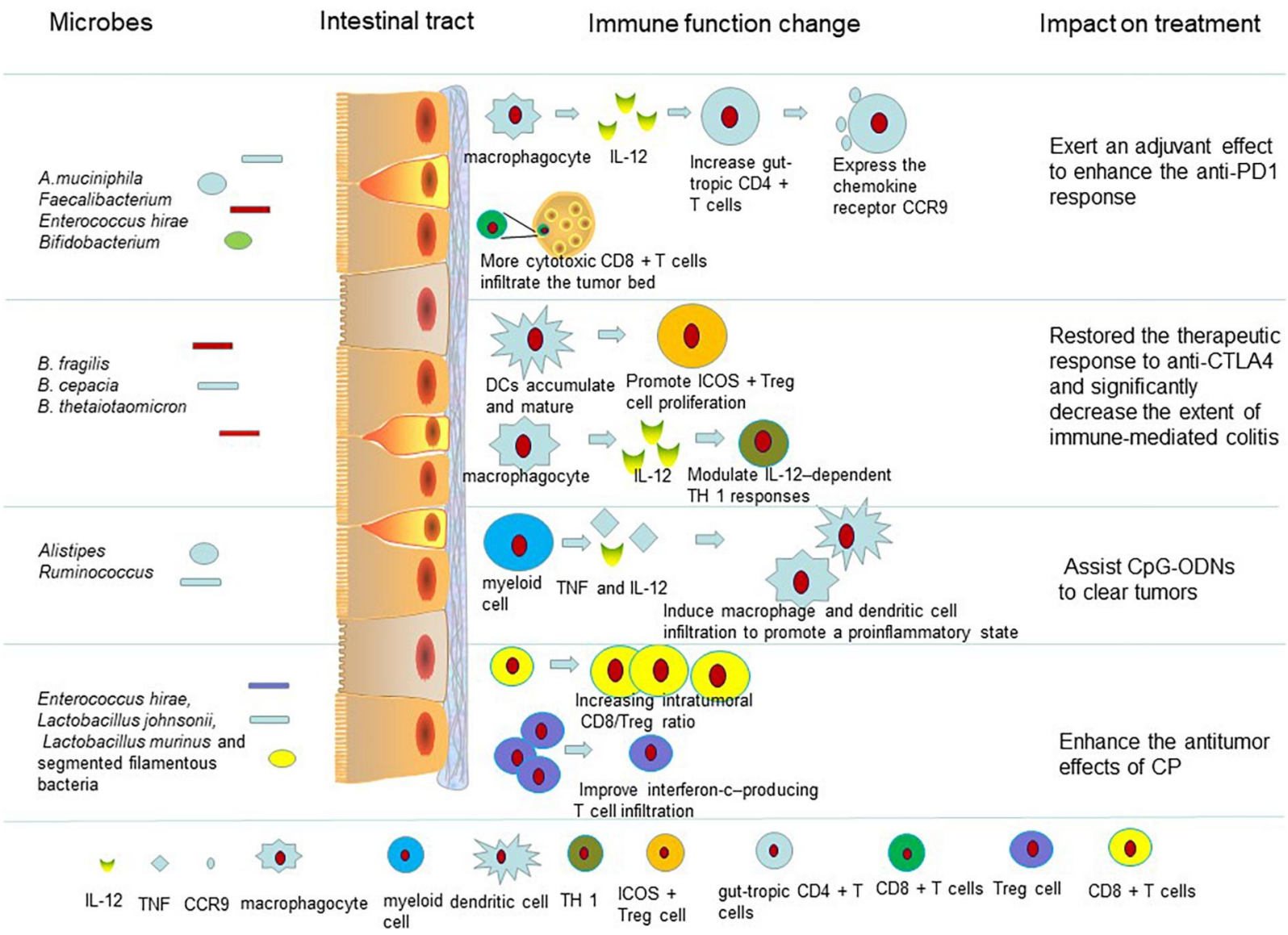
Biomarkers for irAEs

Biomarker	Cancer type	# pts	Main results	Reference
Fold change of effector Treg cells 1-wk post- Th17 to Th1 ratio at baseline %Ki-67^{+}/PD-1^{+}CD8^{+} T cells 1-wk post-treatment %TNF-α^{+}/CD4^{+} or CD8^{+} T cells 1-wk post-treatment	TET NSCLC	31 60	Patients with irAEs can be distinguished into 4 distinct subtypes according to the T-cell parameters and each T-cell parameter predicts the corresponding subtype of irAEs	Kim et al. (63)
Memory cytotoxic (CD45RO^{+}GzmB^{+}Ki-67^{+}) CD4^{+} T cells	Melanoma	3	Activated memory CD4^{+} T cells were highly enriched in inflammed, affected region of cases with	Johnson et al. (68)
Early B cell changes (decline in B cells, increase in CD21^{lo} B cells)	Melanoma	23	Decline in B cells but an increase in CD21^{lo} B cells more prominent in patients with severe irAEs that received combined anti-PD-1 and anti-CTLA-4	Das et al. (72)
Cytokine expression-based score	Melanoma	98	Eleven cytokines were integrated into a single score (CYTOX) and it significantly predicted development of severe irAEs in patients treated with combined anti-PD-	Lim et al. (77)
		49	CYTOX score significantly predicted development of	
Auto-Abs (rheumatoid factor, antinuclear Ab, antithyroglobulin, and antithyroid peroxidase)	NSCLC	137	Preexisting rheumatoid factor or auto-Abs significantly correlates with development of any grade irAEs	Toi et al. (75)
Anti-thyroid Abs (anti-microsomal and anti-thyroglobulin)	NSCLC	51	Presence of anti-thyroid Abs either at baseline or during anti-PD-1 treatment was significantly associated	Osorio et al. (76)

NSCLC, non-small-cell lung cancer; TET, thymic epithelial tumor.

Kim et al. 2020

GI Microbiome



Current state of biomarkers: Work in progress

	Biomarker			Method of detection	Tissue type	Association with positive predictive value	Level of evidence* and results
Tumor-related	PD-L1 pathway	Tissue PD-L1		Immunohistochemistry	Tumor	Positive PD-L1 tumor expression	Concordant results
		Soluble PD-L1		ELISA chemiluminescence	Blood	Conflicting results	Conflicting results
	Antigen recognition	MSI-MDRD		Immunohistochemistry	Tumor	Mismatch repair deficiency: hypermutator phenotypes	Concordant results
		TMB	Tumoral TMB	WES; NGS (Foundation One CDx™ and MSK-IMPACT)	Tumor	High mutational rate (TMB≥175 mut/exome)	Conflicting results
			Blood TMB	WES; NGS	Blood	High mutational rate	Conflicting results
		Tumor-specific genotype		Direct sequencing; NGS	Tumor	Absence of EGFR, ALK or ROS1 mutations	Conflicting results
	Others	CTC		Enrichment (CellSearch®) and detection (IF staining)	Blood	Low baseline CTC count	Results to be validated
		ctDNA		Multiplex targeted NGS; digital droplet PCR; RNASeq; SNP array	Blood	Undetectable ctDNA levels at week 8 of treatment; early decrease in ctDNA burden at 1 month of treatment	Concordant results
		Epigenetic		Bisulfite conversion of genomic DNA, whole-genome amplification and array-based capture and scoring of CpG loci	Tumor	EPIMMUNE signature	Results to be validated
Tumor/host interaction: microenvironment	TILs			Immunohistochemistry; flow cytometry	Tumor	$CD3^+CD8^+$ infiltration; TILs density>5%	Concordant results
	B cells and TILs			Immunohistochemistry; flow cytometry	Tumor	TLS presence	Results to be validated
Host-related	Gene expression signature			Microarray analysis	Tumor	IFN-γ gene signature	Concordant results
	Circulating immune cells	$CD3^+$ T cells		Complete blood count; flow cytometry	Blood	Expansion of $PD\text{-}1^+$ CD8 T cells during treatment; low baseline proportion of $CD28^-CD57^+KLRG1^+$ CD8 T cells	Results to be validated
		Neutrophils and MDSC		Complete blood count; flow cytometry	Blood	Low baseline proportion of circulating M-MDSCs; low baseline dNLR	Results to be validated
	Soluble systemic immune or inflammatory markers	LDH		Spectrophotometry	Blood	Low baseline LDH levels	Conflicting results
		CRP		Immunoturbidimetry	Blood	Low baseline CRP levels	Results to be validated
		Albumin		Immunoturbidimetry	Blood	High baseline albumin levels	Results to be validated
		Cytokines		ELISA chemiluminescence	Blood	Early decrease of IL-8 during treatment; increase of TNF-α and IFN-γ during treatment	Results to be validated
	Microbiota			Bacterial 16S ribosomal RNA gene sequencing	Gut microbiota	High gut diversity at baseline Akkermansia enrichment	Results to be validated

Prospective study 1	Concordant results A
Retrospective study with independent cohorts 2	Conflicting results B
Retrospective study without a control group 3	Results to be validated in further studies C

Duchemann *et al. Translational Lung Cancer Research* 2021

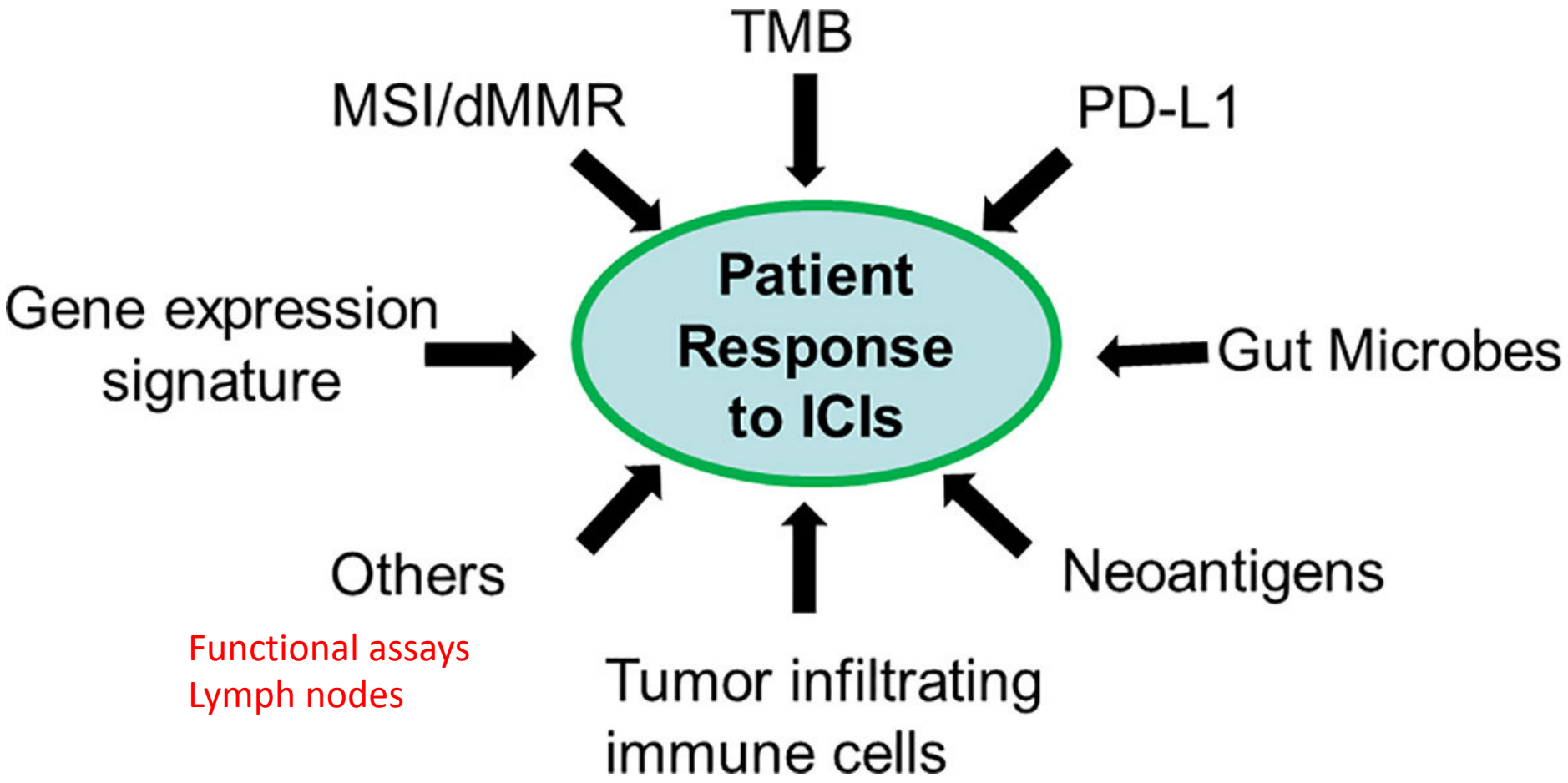
Biomarkers for CAR T therapy?

IFNγ production by CAR T cells and IFNγ responsiveness of host immune cells are critical for tumor immune landscape remodeling to promote a more activated and less suppressive tumor microenvironment.

Alizadeh *et al. Cancer Discovery* 2021

Precision Immunotherapy

Integrative computational algorithms:



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Thanks!

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