Genomic Predictors of Response to Immune Checkpoint Inhibition

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Objectives

- Describe the essential rational of immune checkpoint inhibitory (ICI) therapy
- Define Comprehensive Genomic Profiling (CGP)
- Describe the advantages and limitations of Microsatellite Instability (MSI) as a predictor of ICI therapy
- Describe the advantages and limitations of Tumor Mutation Burden (TMB) as a predictor of ICI therapy

Comprehensive Genomic Profiling

- Small mutations
- Amplification/overexpression
- Deletion/loss of expression
- Splicing alterations
- Gene rearrangements
- Tumor mutation burden
- Microsatellite instability
- Mutational signatures

Palmetto concept of CGP:

- Base pair substitutions (SNVs)
- Insertions and/or deletions (MNVs)
- Copy number variants
- Translocations

EGFR	Mutation		Gefitinib, erlotinib, afatinib, osimertinib, dacomitinib, amivantamab, mobicertinib, poziotinib
KRAS			Sotorasib, adagrasib
ARAF			Sorafenib
BRAF			Dabrafenib + trametinib
MAP2K1			Trametinib, cobimetinib
ERBB2 (HER2)		Amplification	Trastuzumab, neritinib
MET			Crizotinib, cabozantinib, capmatinib, tepotinib
ALK		Alt splice	Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib
ROS1			Crizotinib, entrectinib
RET		Rearrangement	Pralsetinib, selpercatinib, cabozantinib, vandetanib
NRG1			Zenocutuzumab
NTRK1/2/3			Larotrectinib, entrectinib

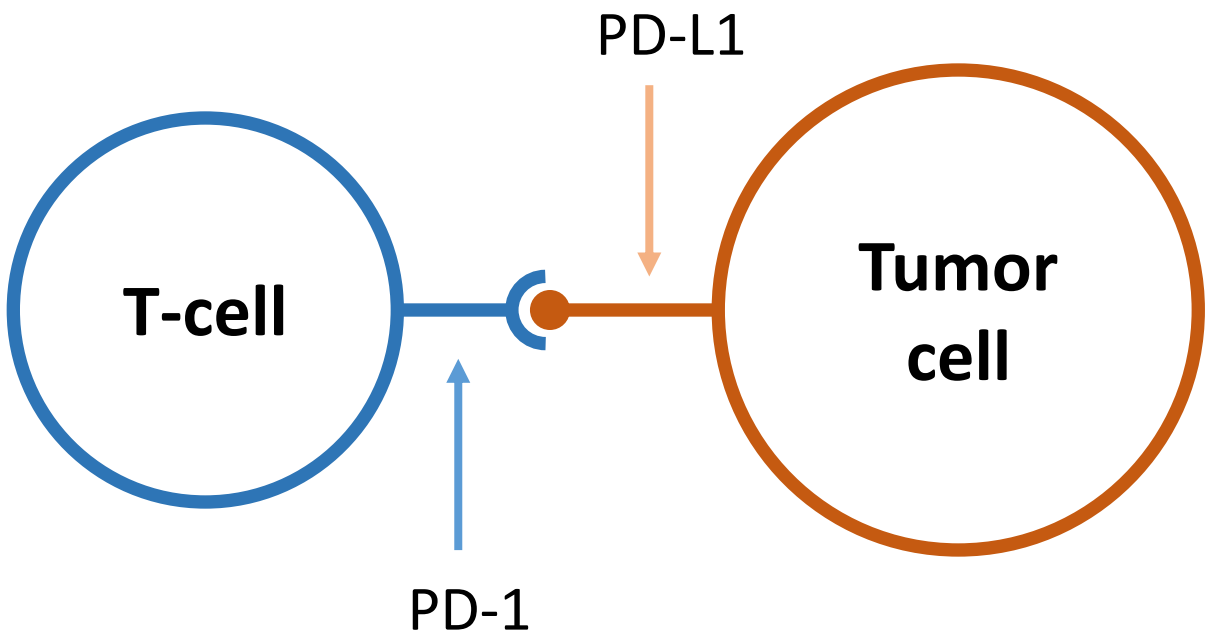
TCGA significantly mutated genes in lung

Adenocarcinoma	Squamous cell carcinoma	Both
KRAS	HLA-A	TP53
EGFR	PTEN	PIK3CA
BRAF	MLL2	KEAP1
MET	NFE2L2	CDKN2A
STK11	NOTCH1	RB1
ARID1A		
SETD2		
RBM10		
MGA		
SMARCA4		
NF1		

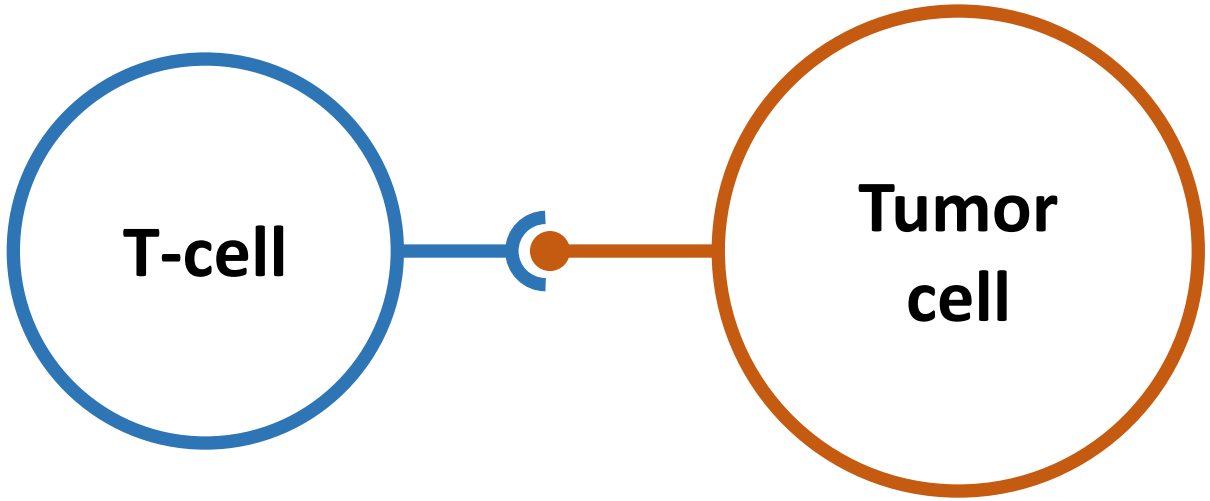
Targetable

May have significance for tumor-immune interactions

Immune checkpoint inhibition (ICI)



Other checkpoints/inhibitory interactions



TIM3 – GAL9
BTLA – HVEM
TIGIT – CD155/CD122
LAG3 – MHC I/II

Immune checkpoint inhibitors

Agent	Target	FDA IVD
Pembrolizumab (KEYTRUDA)	Anti-PD-1	22C3 IHC, MSI/dMMR, TMB
Nivolumab (OPDIVO)	Anti-PD-1	28-8 IHC, MSI/dMMR
Cemiplimab	PD1	22C3 IHC
Atezolizumab (TECENTRIQ)	PD-L1	SP142, SP263 IHC
Durvalumab (IMFINZI)	PDL1	
Avelumab (BAVENCIO)	PDL1*	
Ipilimumab (YERVOY)	Anti-CTLA4	

*Fc portion also engages NK cells and induces antibody-dependent cell-mediated cytotoxicity

MSI and ICI therapy

- First tumor-agnostic biomarker approved by FDA: pembrolizumab

  - treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options
  - treatment of patients with unresectable or metastatic MSI-H or dMMR colorectal cancer

MSI and ICI therapy

- Accelerated approval for nivolumab +/- ipilimumab

  - treatment of adults and pediatric patients 12 years and older with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer (CRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan

Microsatellite instability

- Microsatellites are short tandem repeats of 1-6 nucleotides
- Instability entails hypermutability at these sites due to loss of mismatch repair function, e.g.
  - Germline mutations of MLH1, MSH2, MSH6, PMS2 or
  - Methylation of the MLH1 promoter (somatic)

MSI assay considerations

- IHC versus molecular methods (PCR, NGS)
- For molecular methods, the type of repeat matters (mononucleotide versus di-, tri-, tetra-, pentanucleotide repeats)
- Number of repeats assessed
- Validation against other assay and particular tumor types (colorectal, endometrial, others)

MSI recommendations for NGS

ESMO

- IHC first
- PCR for indeterminate IHC
  - 5 poly(A) STR panel is preferred
- NGS considered an alternative, only in experienced centers. Possible fringe benefit of TMB noted.

CAP [DRAFT]

- For CRC: IHC and/or PCR. NGS secondary.
- For GEJ/small bowel: IHC and/or PCR preferred over NGS
- For endometrial carcinoma: IHC is preferred over PCR and NGS
- Do not use TMB as a surrogate for MSI

MSI: bioinformatics approaches

- Count mutant microsatellites
  - Pang J, et al. J Clin Pathol 2020;73:83–89
  - Vanderwalde A, et al. Cancer Med 2018;7:746–756
- Use a dedicated scoring/calling algorithm
  - **MANTIS** (Kautto EA, et al. Oncotarget. 2017;8:7452–7463)
  - **MSIsensor** (Niu B, et al. Bioinformatics 2014;30:1015–1016)
  - **MSI-ColonCore** (Zhu L, et al. J Mol Diagn 2018;20:225-231)
  - **mSINGS** (Salipante SJ. et al. Clin Chem 2014;60:1192-1199)

MSI calling softwares

Software	Normal reference	Classes	Threshold
MSI-ColonCore	Baseline normal	MSI-H vs MSI-L vs MSS	>40% unstable loci (MSI-H)
mSINGS	Baseline normal	MSI vs MSS	>20% unstable loci
MSIsensor	**Paired normal***	MSI vs MSS	>3.5% unstable loci
MANTIS	Paired normal	MSI vs MSS	MSI score >0.4

Software	Availability
MSI-ColonCore	?
mSINGS	https://bitbucket.org/uwlabmed/msings
MSIsensor	https://github.com/ding-lab/msisensor
MANTIS	https://github.com/OSU-SRLab/MANTIS

*** Can also be used on tumor data alone**

Counting mutations: 2 exemplars

Pang J, et al. J Clin Pathol 2020;73:83-89

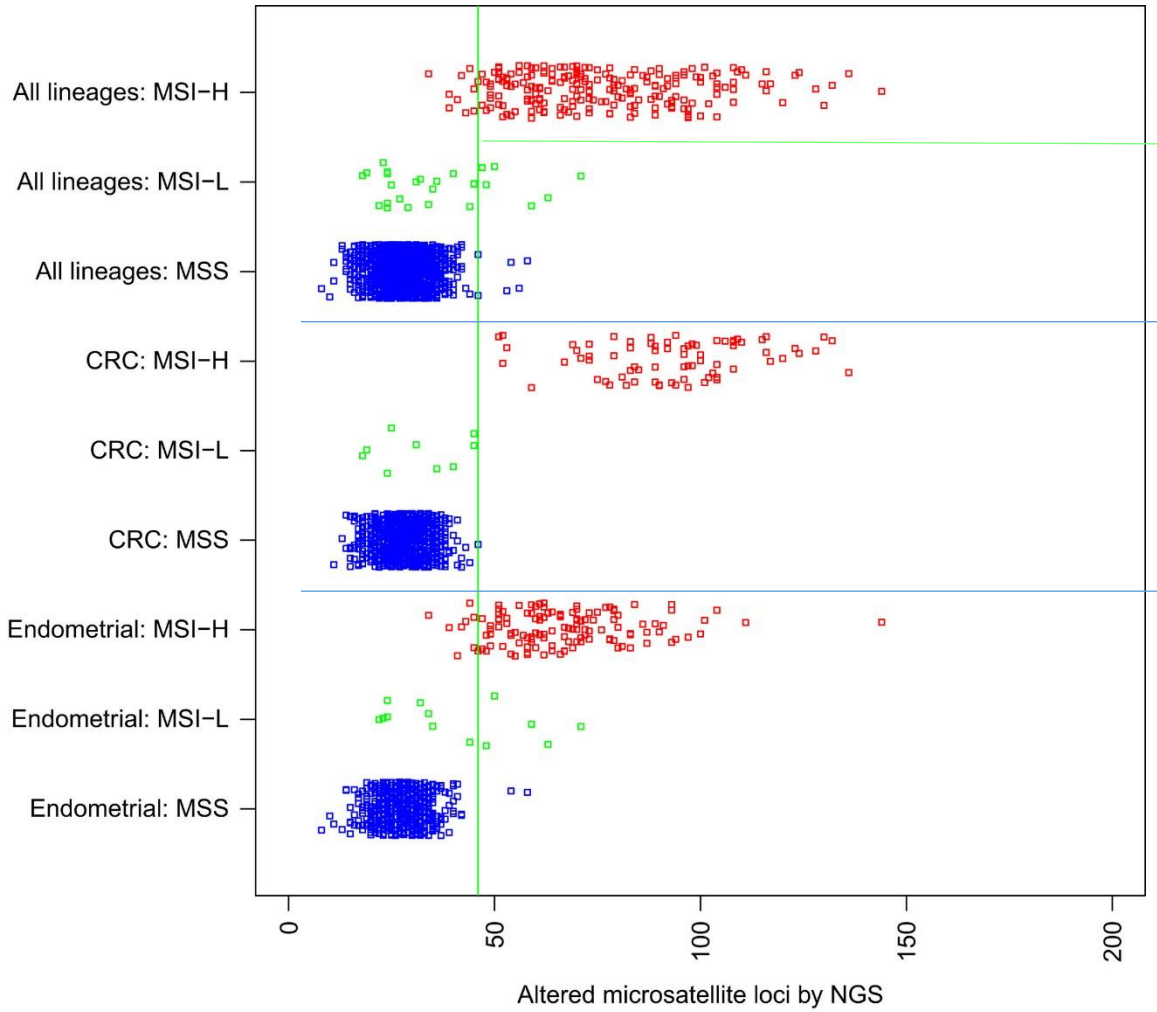
- Candidate repeats identified with Repeat Finder (github.com/OSU-SRLab/MANTIS)
- 8682 mononucleotide repeats after filtering
- **>=7 mutations considered MSI**

Vanderwalde A, et al. Cancer Medicine 2018;7:746–756

- Candidate repeats identified with MISA algorithm (pgrc.ipk-gatersleben.di/misa/)
- 7317 mono-, di-, tri-, tetranucleotide repeats after filtering
- **>=46 mutations considered MSI**

See also: Yamamoto H and Imai K. Semin Oncol 2019;46:261-270. PMID 31537299

Determining a cut-off for MSI



Green threshold line at 46 mutations

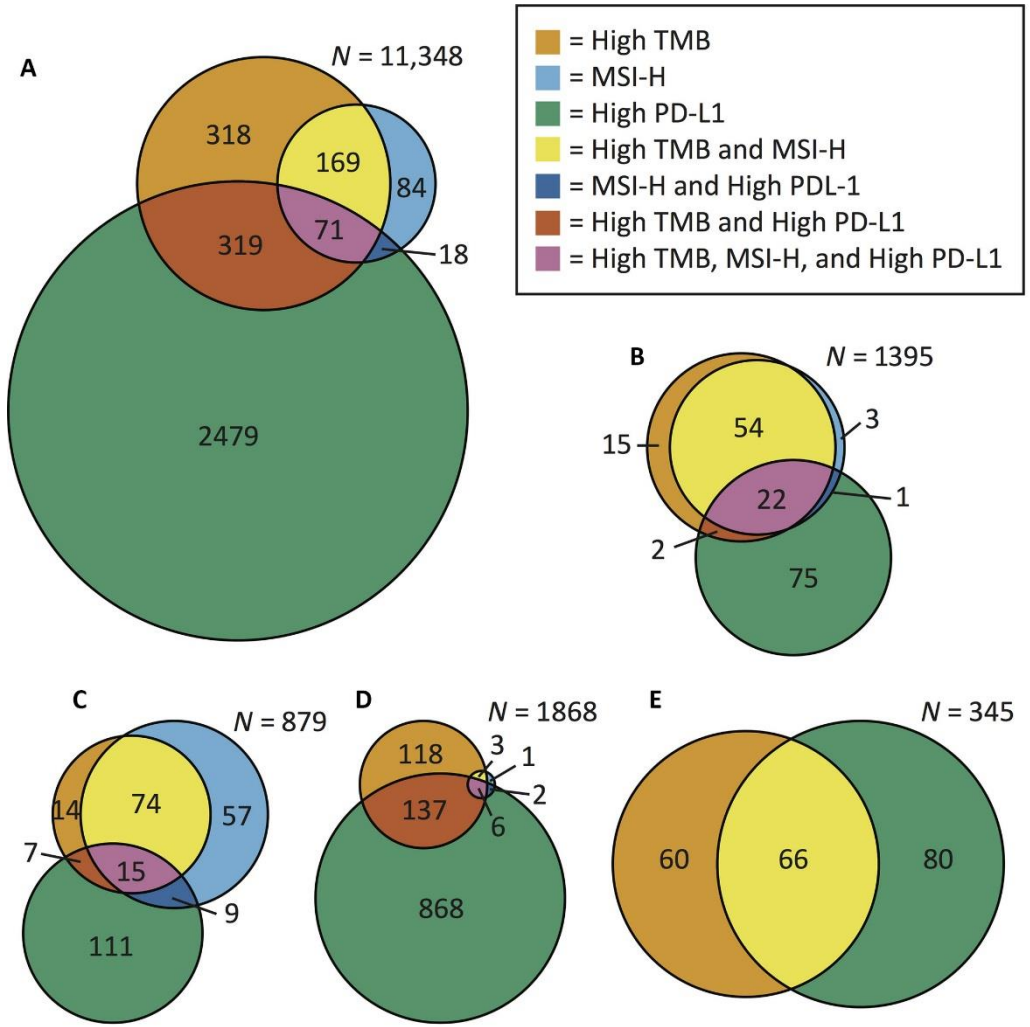
Colorectal carcinoma

Endometrial carcinoma

Tumor Mutation Burden

- Mutations that change amino acid sequences may give rise to neoantigens and increased tumor immunogenicity
- Generally, little correlation between PD-L1 IHC and TMB
  - Considered independent but potentially complementary
  - Tumor inflammation versus neoantigenicity
- “The mechanism(s) underlying the association between TMB and benefit with immunotherapy is not entirely clear”
  - Hellmann et al. Cancer Cell 2018;33:834-852

Correlation of TMB, MSI, PD-L1



A: All cancers

B: Colorectal carcinoma

C: Endometrial

D: NSCLC

E. Melanoma

Omitted from original figure: ovarian surface epithelial carcinomas, neuroendocrine tumors, cervical cancer

CheckMate: Nivolumab +/- ipilimumab

Trial	Therapy	N	Variants counted	TMB high cut-off	Result
CheckMate 012 (Hellmann 2018, PMID 29657128)	Nivolumab + ipilimumab vs chemotherapy	75	Nonsynonymous mutations (SNVs and indels)	158/exome	**Superior PFS** for high TMB: 17.1 vs 3.7 months (HR 0.41, CI 0.23-0.73)
CheckMate 026 (Carbone 2017, PMID: 28636851)	Nivolumab vs chemotherapy	312	Nonsynonymous SNVs	243/exome	*No statistically significant difference in PFS*
CheckMate 227 (Hellmann 2018, PMID 29658845)	Nivolumab + ipilimumab vs chemotherapy	299	SNVs and indels (FoundationOne CDx)	10/Mb	**Superior PFS** for high TMB: 7.2 vs 5.5 mo (HR 0.58, 97.5% CI 0.41-0.81, p<0.001)
	Nivolumab vs chemotherapy	150		13/Mb	*No statistically significant difference in PFS*

CheckMate-227 and TMB

Publication	Therapy	N	Variants counted	TMB high cut-off	Result
Hellmann, et al. NEJM **2018**; 378:2093-2094	Nivolumab + ipilimumab vs chemotherapy	299	SNVs and indels (FoundationOne CDx)	10/Mb	**Superior PFS** for high TMB: 7.2 vs 5.5 mo (HR 0.58, 97.5% CI 0.41-0.81, p<0.001)
	Nivolumab vs chemotherapy	150		13/Mb	*No statistically significant difference in PFS*
Hellmann, et al. NEJM **2019**; 381:2020-2031	Nivolumab + ipilimumab vs chemotherapy	1739	N/A	N/A	*TMB not predictive of benefit with regard to overall survival (and not mentioned in the paper)*

Pembrolizumab and TMB

Trial	Therapy	N	Variants counted	TMB high cut-off	Result of TMB analysis
KEYNOTE-001	Pembrolizumab monotherapy (phase I)	34	Nonsynonymous mutations	200/exome	Superior PFS for high TMB: Median PFS not reached vs 3.4 months, HR 0.15 (p=0.006)
KEYNOTE-021 (abstract OA04.05 presented at IASCL 2019)	Pembrolizumab + chemotherapy vs placebo + chemotherapy	70	Cut-off of 175 mutations/exome for ORR, also assessed as a continuous variable (log10 transformed) for OS		Not predictive of overall survival as cont var (pembro vs chemo), ORR similar between high and low TMB
KEYNOTE-189 (abstract OA04.06 presented at IASLC 2019)	Pembrolizumab + chemotherapy vs placebo + chemotherapy	293	Cut-off of 175 mutations/exome, also assessed as a continuous variable (log10 transformed)		Not predictive of overall survival as cont var (pembro vs chemo), OS similar between high and low TMB

KEYNOTE-158

- 790 patients with advanced/incurable solid tumor malignancies with progression on or intolerance to one or more lines of standard therapy
- Participants given pembrolizumab 200 mg IV q3w up to 35 cycles
- Primary endpoint was overall response rate (ORR), incl complete and partial responses
- TMB-H defined as 10 or more mutations/Mb (Foundation Medicine), N=102 (13%)

Marabelle, et al. Lancet Oncol. 2020;21:1353-1365

KEYNOTE-158

- ORR superior in TMB-H
  - TMB-H: 29% ORR (95% CI 21-39)
  - TMB-L: 6% ORR (95% CI 5-8)
- Median PFS equivalent in both groups, but apparently more long-term survivors in TMB-H cohort
- OS Kaplan-Meier curves do not diverge much
- Take-home point: Good PFS seen in a subset of a subset

Marabelle, et al. Lancet Oncol. 2020;21:1353-1365

Optimal TMB threshold? Goodman 2017

- 151 ICI-treated patients with TMB-H defined as 20 mut/Mb
- Response rate: 58% in TMB-H versus 20% in low
- PFS: 12.8 m in TMB-H versus 3.3 in low ($p<0.0001$)
- Median OS: not reached in TMB-H vs 16.3 m in low ($p=0.0036$)

- Interestingly, dual checkpoint blockade (i.e., nivo+ipi) achieved a higher response rate (77%) and superior PFS independent of TMB

Goodman AM, et al. Mol Cancer Ther. 2017;16:2598-2608

Palmeri, et al. ESMO Open. Feb 2022

- 157 pts with MSI-H (8), TMB-H (122), or both (27)
- TMB-H defined at 20 muts/Mb
- ICI vs chemo: ORR
  - MSI-H: 50%, TMB-H: 64%, Both: 50%; vs.
  - Chemo: 34.4%
- Median PFS for MSI-H and/or TMB-H:
  - ICI: 24.2 m (9.6 m-NR) vs.
  - Chemo: 6.8 m (3.9-10.9), p=0.042

MSK-IMPACT experience

- 1662 patients treated with ICI across various regimens and histologies
- Higher TMB thresholds associated with better overall survival
- Multivariate analysis: HR 0.6 for ICI-treated patients in top 20^{th} percentile range per histology
- "…suggests that there is not likely to be a universal number defining high TMB that is predictive of clinical benefit to ICI across all cancer types, and that the optimal cutpoint is likely to vary for different cancers"

Samstein RM, et al. Nat Genet. 2019;51:202-206

Tumor types with the highest TMB

Zehir A, et al. Nature Medicine 2017;23:703 (N=10000)

- Bladder cancer
- Melanoma
- Colorectal carcinoma
- NSCLC
- Endometrial carcinoma
- Esophagogastric carcinoma
- Glioma

Chalmers Z, et al. Genome Med 2017;9:34 (N=100000)

- Cutaneous SCC
- Melanoma (cutaneous or unknown primary)
- DLBCL
- Pulmonary large cell NEC
- SCLC
- Pulmonary SCC

High TMB predicts response rate

- Endometrial carcinoma (KEYNOTE 158)
- Cervical carcinoma (KEYNOTE 158)
- Colorectal carcinoma (Chalabi + Goodman)
- Melanoma (Goodman, Hugo, Miao)
- Bladder carcinoma (Mariathasan, Synder, Miao)
- Pulmonary adenocarcinoma (Goodman, Rizvi, Rizvi, Hellmann)

Neoantigenicity of mutations correlate well with CD8+ T-cell score in above tumor types. For cohort and analysis details see McGrail DJ, et al. Ann Oncol. 2021;32:661-672

TMB does not predict response rate

- Anal carcinoma (KEYNOTE-158)
- Gastric carcinoma (Kim)
- HNSCC (Goodman + Miao, MDACC)
- Pulmonary SCC (Hellmann, Goodman + Rizvi + Miao)
- Mixed metastatic SCCs (Goodman)
- TNBC (Voorwerk, MDACC)
- Prostate adenocarcinoma (Subudhi)

Neoantigenicity of mutations did not correlate with CD8+ T-cell score in above tumor types. See McGrail DJ, et al. Ann Oncol. 2021;32:661-672

Dissenting opinions

“Is TMB ready for clinical application in NSCLC? The answer is clearly no based on the current evidence... Despite all this, there appears to be groupthink to rush TMB for approval by the US Food and Drug Administration and wide-spread use in practice.”

- Addeo A, Banna GL, and Weiss GJ. Tumor Mutation Burden—From Hopes to Doubts. JAMA Oncology 2019;5:934-935

Further critique of TMB as an IVD

- Editorial by Addeo and Prasad, 2020: *The FDA approval of pembrolizumab for patients with TMB >10 mut/ Mb: was it a wise decision? No*
  - “There is nothing logical about the cut off of 10 mut/Mb… arbitrary and capricious”
  - “We do not know if patients live longer or better… [response rate] is a poor surrogate for survival”
  - “Overall survival was longer in the TMB-low cohort, i.e. where the drug was not approved”
  - “The cut off has fallen from prior publications, which means more prescriptions and more profits”

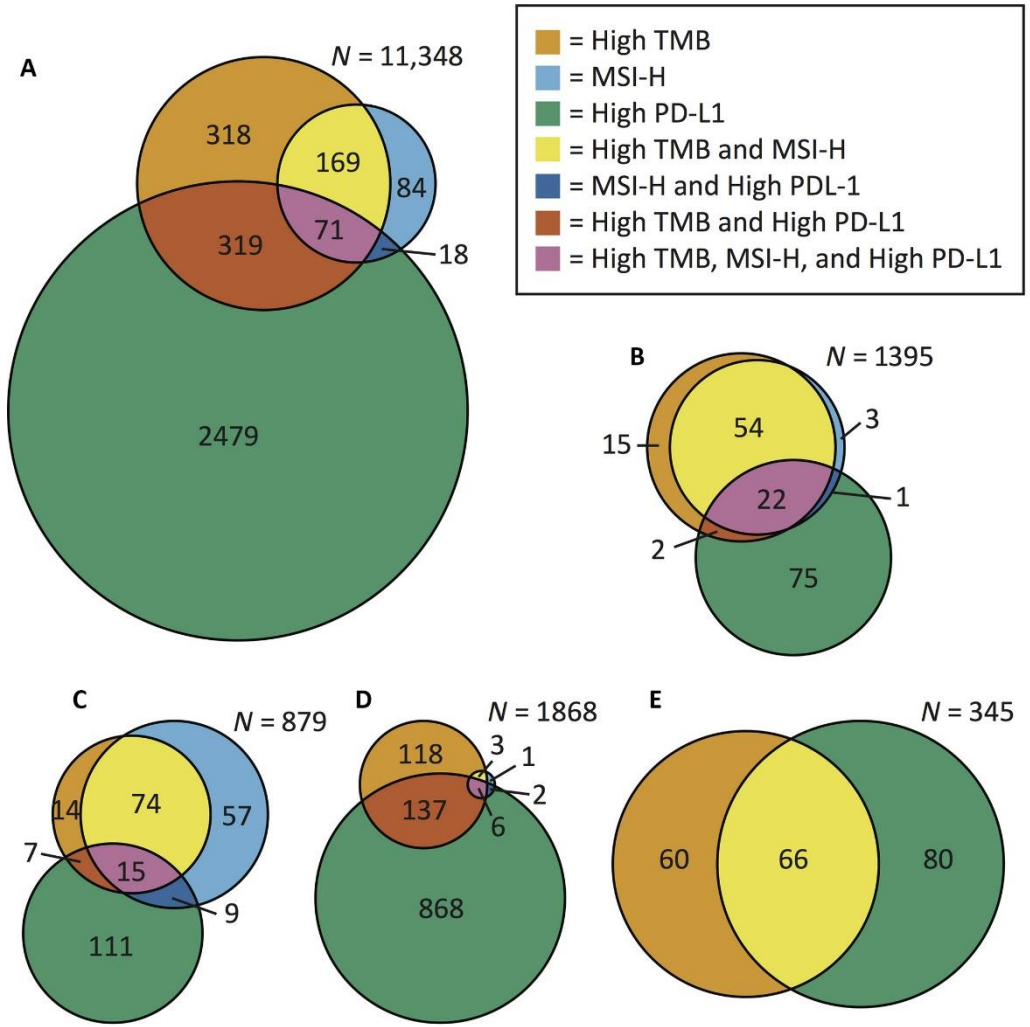
Ann Oncol 2020;31:1112-1114

Defense of TMB as an IVD

- Counterpoint by Subbiah, et al: *…a decision centered on empowering patients and their physicians*
  - Durable responses in a subset of patients
  - Drug access enhanced for patients with pediatric and rare adult solid tumors, minorities, economically disadvantaged patients
  - TMB ≥10 threshold was consensus recommendation, admittedly never intended to be optimal for all clinical scenarios
  - Strong biologic rationale: MSI-H patients “because it results in high TMB” respond well to ICI, as do MSS with high TMB (citing Goodman 2019)

Ann Oncol 2020;31:1115-1118

Correlation of TMB, MSI, PD-L1



A: All cancers

B: Colorectal carcinoma

C: Endometrial

D: NSCLC

E. Melanoma

Omitted from original figure: ovarian surface epithelial carcinomas, neuroendocrine tumors, cervical cancer

TMB in MSS tumors: Goodman 2019

- 60 patients from 14 histologic tumor types tx with ICI
- TMB was dichotomized into two groups: low-to-intermediate (0-19 mutations/mb) versus high (≥20 mutations/mb)
- 82% of MSI-H were TMB-H
- Median PFS for MS-stable/TMB-high versus MS-stable/ TMB-low/TMB-intermediate tumors was 26.8 versus 4.3 months (P = 0.0173)

Goodman A, et al. Cancer Immunol Res. 2019;7:1570-1573

Meta-analyses of high TMB

Paper	Patients (studies)	Result (overall survival)
Cao D, et al. OncoImmunology 2019;8:9, e1629258	103078 (45)	HR=0.40 (95% CI 0.30-0.53, p<.00001)
Wu Y, et al. Front Oncol. 2019;9:1161	4431 (29)	HR=0.68 (95% CI 0.53-0.89, P=0.004)
Kim JY, et al. Cancers (Basel). 2019;11:E1798	5712 (26)	HR=0.53 (95% CI 0.42-0.67)
Zhu J, et al. Front Pharmacol. 2019;10:673	2661 (8)	HR=0.47 (95% CI 0.35-0.63)
Osipov, et al. Clin Cancer Res 2020;26:4842	12450 (117)	RR improved, OS (subset of studies) not significant
Galvano, et al. ESMO Open. 2021;6:100124	3848 (5)	HR=0.67 (95% CI 0.59-0.77)

Therapies, TMB definitions vary between studies

TMB assay design considerations

- WES or panel-based approach
- Coverage and breadth required
- Germline filtering strategy
  - Paired normal sequencing
  - Population database filtering
- Types of variants counted
  - Missense +/- insertion/deletion
  - Nonsynonymous +/- synonymous
  - Exonic +/- intronic
- Removal/filtering of deamination artifacts
- Lower limit of variant calling
  - 5%, 10%, etc
- Threshold for interpretation
  - Varies by study
  - Reported metric varies (number of mutations, percentile by tumor type)

Call for standardization

"Different tests may report different measurements… it is imperative to create some sort of standardization to arrive at clinically-meaningful results"

- Friends of Cancer Research TMB Harmonization Working Group

https://www.focr.org/tmb, last accessed 2/10/19

"…the clinical merit of [TMB] in terms of reliability and reproducibility has yet to be demonstrated."

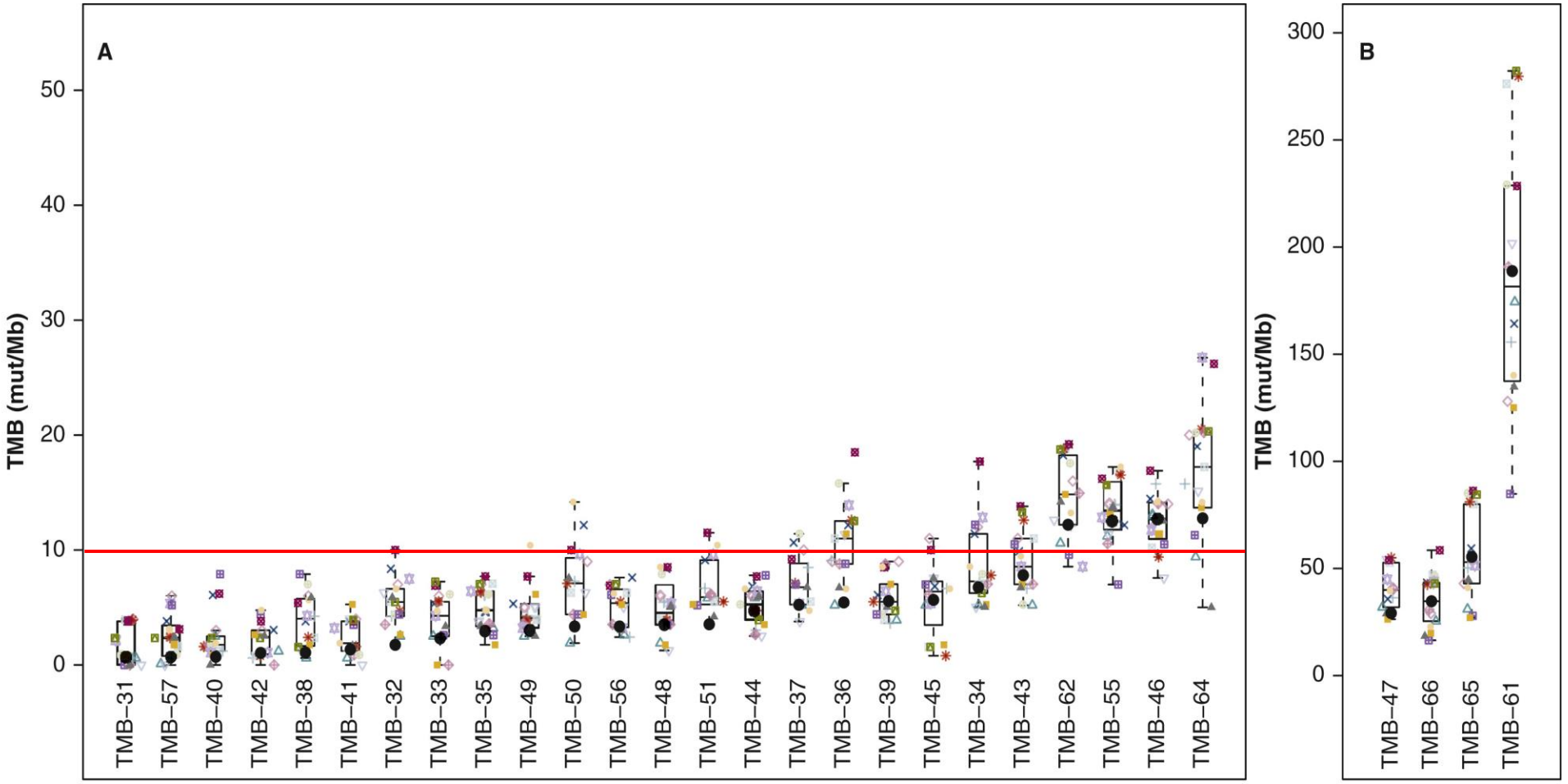
- Qualitaetsscherungs Initiative Pathologie GmbH

https://quip.eu/en_GB/2018/05/14/tumor-mutational-burden-tmb-quip-organisiert-studie-und-arbeitet-mit-focr-zusammen, last accessed 2/10/19

FOCR: Vega, et al. Ann Oncol 2021;32:1626

- “Several factors impact variation among panel assays including **sample input, tumor content, panel size, gene content, quality control (QC), NGS platform, and bioinformatics pipeline**, which may influence TMB estimates and lead to inconsistent TMB calculation and reporting.
- “Because of these inherent differences, the standardization of clinical validation practices, harmonization of TMB assessment, and alignment across TMB panel assays are critical steps to improve consistency of results and comparability across panel assays…
- See **Table 1** for comparison of 16 different assays

FOCR phase II results



Annals of Oncology 2021 321626-1636

Genetic correlates of TMB, ICI

From Campbell *Nat Genet* 2016;48:607-616

Mutations predicted to be neoepitopes in at least 4 tumors (N=660 lung adenocarcinomas)

- PIK3CA p.E542K
- NFE2L2 (NRF2) p.E79Q
- BRAF p.G466V
- EGFR p.G719A
- TP53 p. V157F, p.G154V, p.R175G, p.P278A
- C3orf59 (MD21D2) p.Q311E

Genetic correlates of TMB

From Rizvi Science 2016;348:124-128 (reanalysis of KEYNOTE-001)

Genes harboring deleterious mutations in >=4 patients with durable clinical benefit (and not present in patients without clinical benefit)

- POLR2A
- KEAP1
- PAPPA2
- PXDNL
- RYR1
- SCN8A
- SLIT3

Further genetic considerations re: TMB

Correlates of high TMB
- Microsatellite instability
- BRCA1/2 mutations
- POLE, POLD1 mutations
- TP53 mutations

Resistance to ICI
- PTEN mutations
- STK11 mutations
- Some JAK/STAT mutations

Sensitizing to ICI
- PBRM1 mutations

Risk of hyperprogression
- MDM2 amplification

Loss-of-function PBRM1 mutations Miao et al. Science 2018
Inactivating JAK family member mutations: Zaretskyet al. N Engl J Med 2016
MDM2/4 amplification: Kato et al. Clin Cancer Res 2017
PTEN loss: Peng et al. Cancer Discov 2016
Inactivating STK11 mutations: Skoulidis et al. Cancer Discov 2018

TCGA significantly mutated genes in lung

Adenocarcinoma	Squamous cell carcinoma	Both
KRAS	HLA-A	TP53
EGFR	PTEN	PIK3CA
BRAF	MLL2	KEAP1
MET	NFE2L2	CDKN2A
STK11	NOTCH1	RB1
ARID1A		
SETD2		
RBM10		
MGA		
SMARCA4		
NF1		

- May harbor neoepitopes (Campbell 2016)
- May predict clinical benefit from ICI (Rizvi 2016)
- May predict lack of response (Peng 2016, Skoulidis 2018)

Bolded gene names represented other therapeutic targets

Future directions/possibilities for improvement of TMB

- Expressed TMB (from RNAseq data)
- Corrected TMB (for tumor purity)
- Blood TMB
- Tumor specific thresholds (e.g., Panda A, et al. JCO Precis Oncol 2017)
- Weighting of mutations by neoantigenicity
- Machine learning classifiers
- Consideration of other immune biomarkers, e.g. HLA, TCR

TMB-independent indications for ICI

Tumor type (from McGrail)	Pembrolizumab	Nivolumab
Endometrial carcinoma	MSI-H or combo w/ levantinib	
Cervical carcinoma	CPS >= 1%	
Colorectal carcinoma	MSI-H	MSI-H
Melanoma	No IVD needed	No IVD needed
Urothelial carcinoma	No IVD needed (prev CPS >= 10)	No IVD needed
Lung adenocarcinoma	TPS >= 1% No IVD needed for combo chemo	TPS >= 1% first-line No IVD needed for combo chemo

Atezolizumab: no IVD needed for NSCLC or urothelial carcinoma
Durvalumab: no IVD needed for NSCLC
Avelumab: no IVD needed for urothelial carcinoma
Cemiplimab-rwlc: TPS >= 1% (22C3) required for NSCLC