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Society for Immunotherapy of Cancer

Advances in Cancer Immunotherapy™

Tumor Mutation Burden as a Biomarker

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Disclosures

Research Support:

AbGenomics
Actuate
Amgen
MedImmune
Novocure
Nucana
Seattle Genetics
Surface Oncology

Objectives

ACI Series Learning Objectives

- Identify the common approaches to cancer immunotherapy and their related mechanisms.
- Summarize recent updates in cancer immunotherapy and toxicity management for key disease states.
- Implement cancer immunotherapy for key disease states into clinical practice appropriately.

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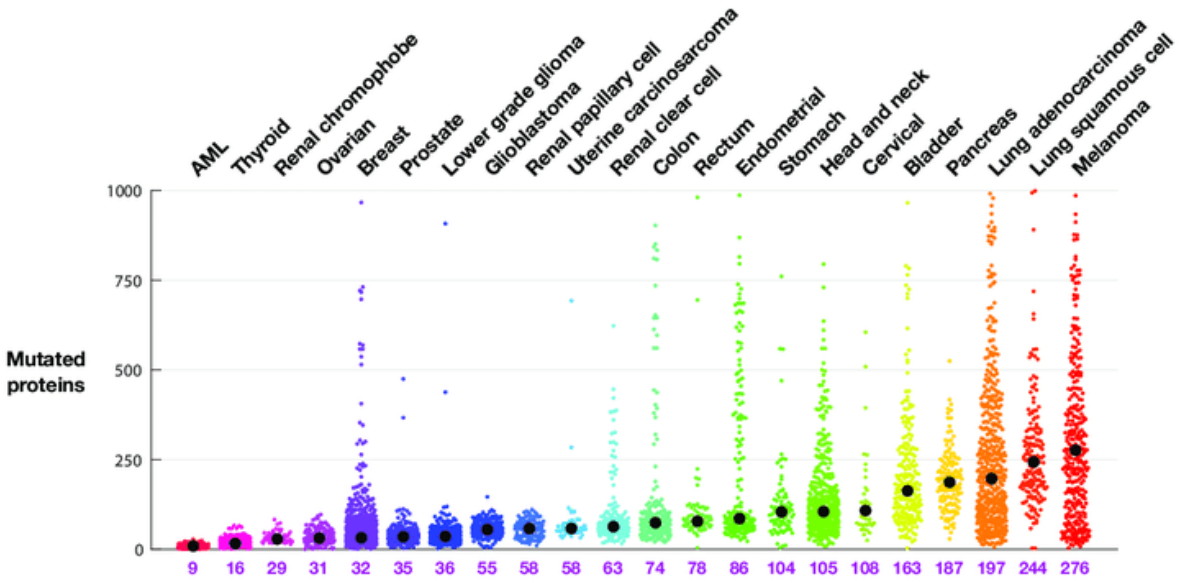
My Learning Objectives

- Why measure TMB
- How to measure TMB
- Issues with TMB
- MSI-H vs TMB

Objectives



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Castle et al. Frontiers in Immunology 2019; 10.1856.

1.16 Tumor Mutational Burden-High Cancer

PEMBRO is indicated for the treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥10 mutations/megabase (mut/Mb)] solid tumors, as determined by an FDA-approved test [see Dosage and Administration (2.1)], that have progressed following prior treatment and who have no satisfactory alternative treatment options.

This indication is approved under accelerated approval based on tumor response rate and durability of response [see Clinical Studies (14.16)]. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

Limitations of Use: The safety and effectiveness of PEMBRO in pediatric patients with TMB-H central nervous system cancers have not been established.

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125514s071s090lbl.pdf

TMB Definition

Tumor mutational burden (TMB) is defined as the number of somatic mutations per megabase of interrogated genomic sequence.

And it demonstrates predictive biomarker potential for the identification of cancer patients most likely to respond to immune checkpoint inhibitors.

Average TMB High: 13.3% of Solid Tumors

- Highest TMB
  - Melanoma
  - NSCLC
  - SCC
- GI – High TMB Percentage
  - 12% Small Bowel
  - 5% CRC
  - 5% Gastric
- Intermediate and Lower
  - Breast
  - Uterine
  - Ovarian
  - Kidney
  - Prostate
  - Bladder
  - Head and Neck
  - Some Sarcomas

Per FoundationOne

TMB

- TMB reflects the overall somatic genomic burden of mutations within a given tumor.
- TMB differs across tumor types, and variability within tumor types has been observed
  - Prostate TMB 0.03 - 14.13 mutations/Mb
  - Bladder TMB 0.04 - 99.68 mutations/Mb
- 10 mut/Mb as the lower bound of an “equivocal zone”, or a cut-off point that would identify patients with TMB-high tumors.

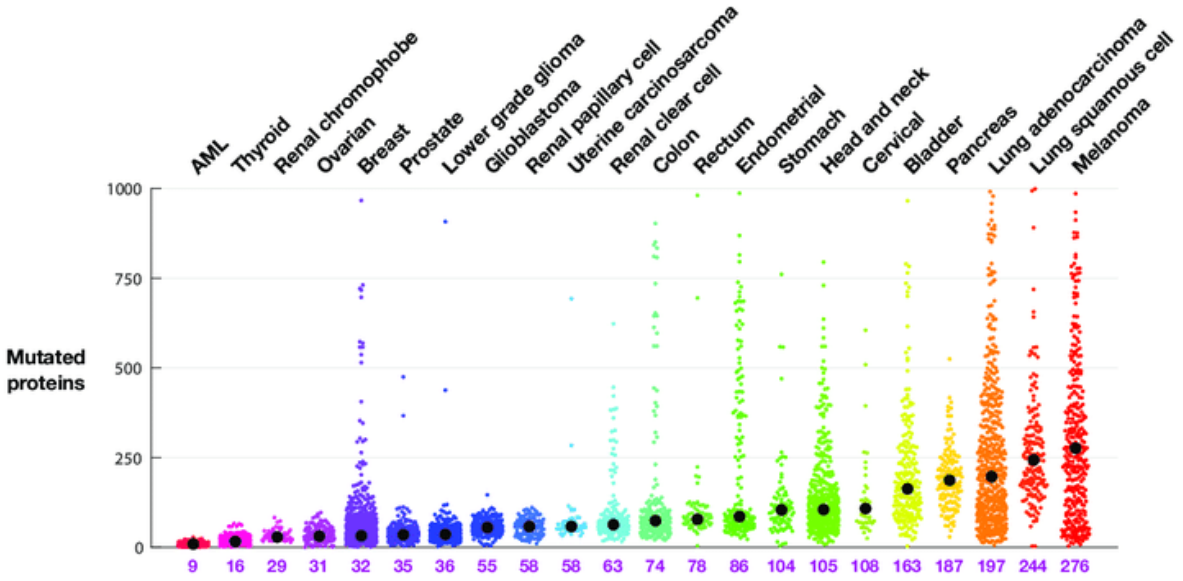
Why was it approved?

Overall response rate:	29% (95%, 21–39)
Duration of Response:	57% lasting ≥ 12 months

The efficacy of pembrolizumab was supported by the results of whole-exome sequencing (WES) analyses of TMB in additional patients enrolled across multiple pembrolizumab clinical trials.

Marcus, L, et al;.Clin Cancer Res 2021;27:4685–9

Objectives



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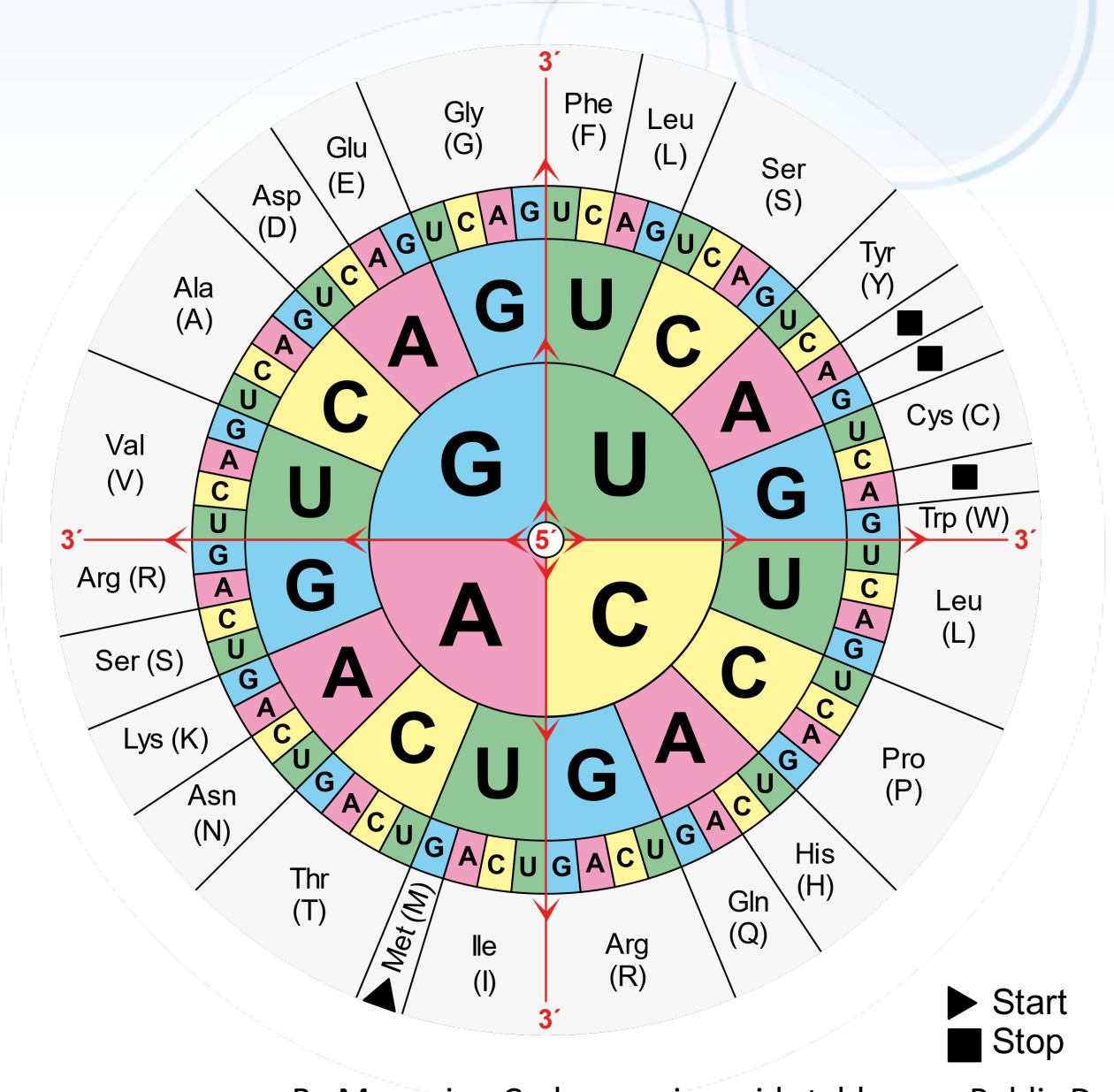
Methods for calculating TMB are inconsistent, especially the inclusion or exclusion of synonymous mutations.



By Mouagip - Codons aminoacids table.png, Public Domain, https://commons.wikimedia.org/w/index.php?curid=5986132

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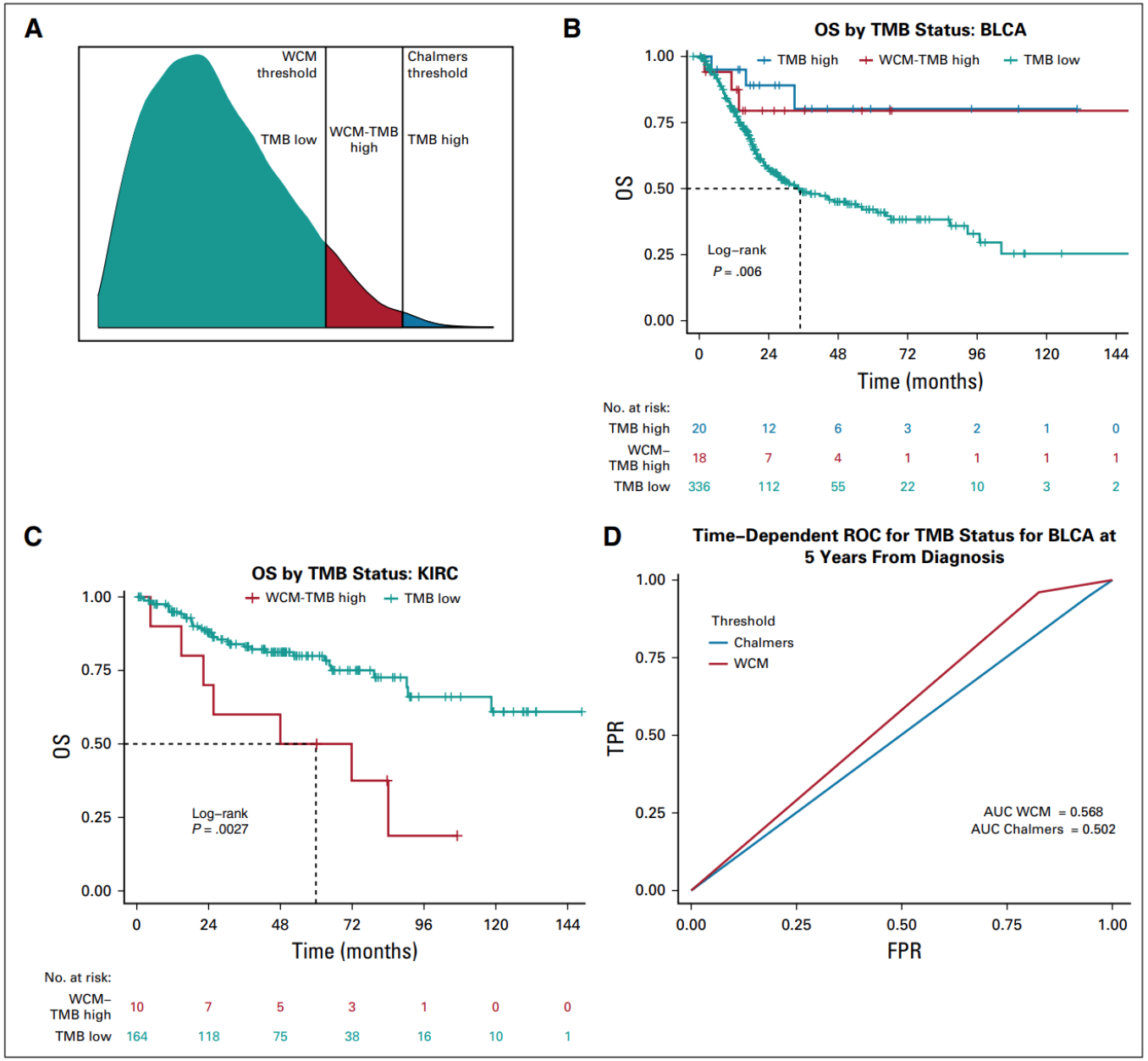
- WCM = Weill Cornell Med
- BLCA = bladder
- KIRC = Kidney RCC (clear)

Metastatic Samples had higher TMB.



Fernandez et al, JCO Precision Oncology 2019 :3, 1-12:

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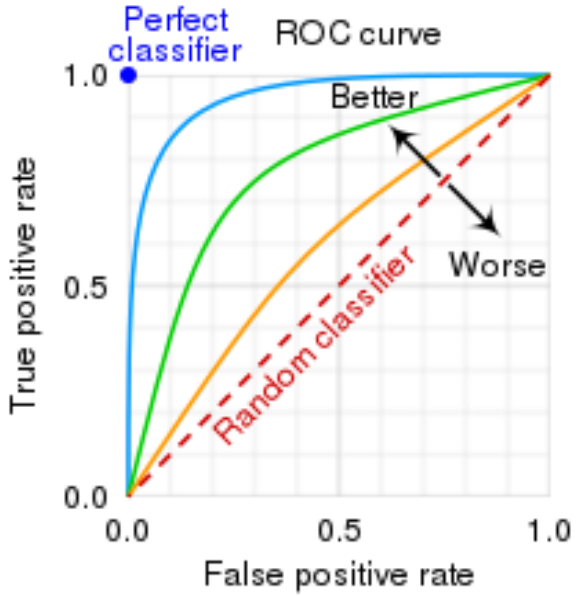
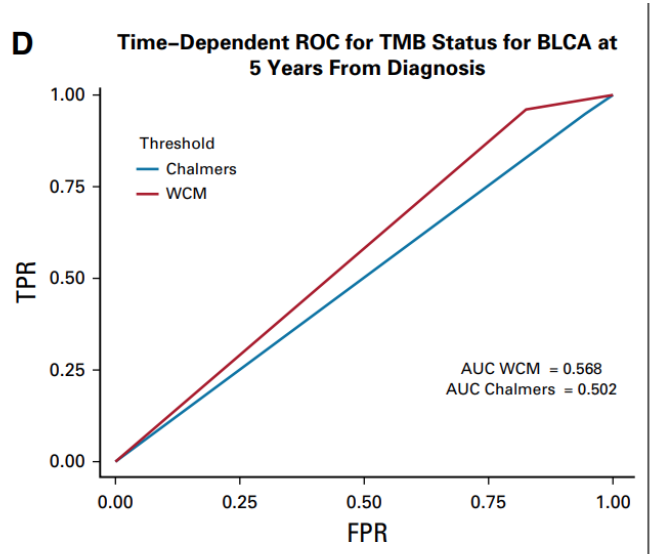
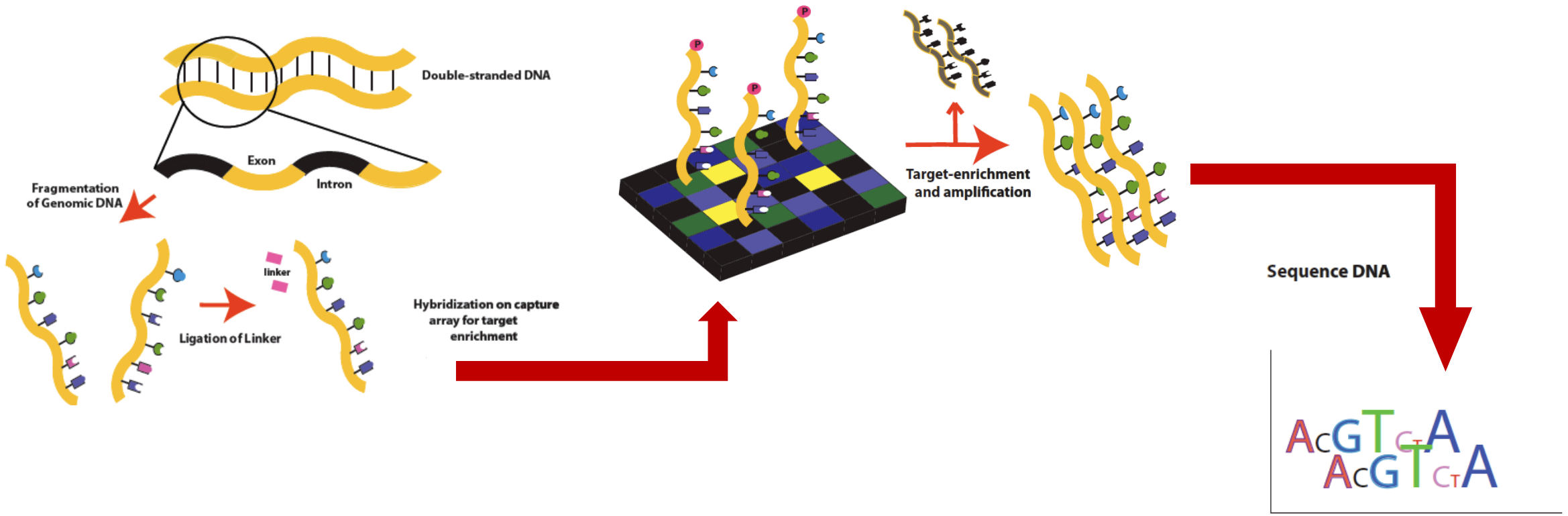




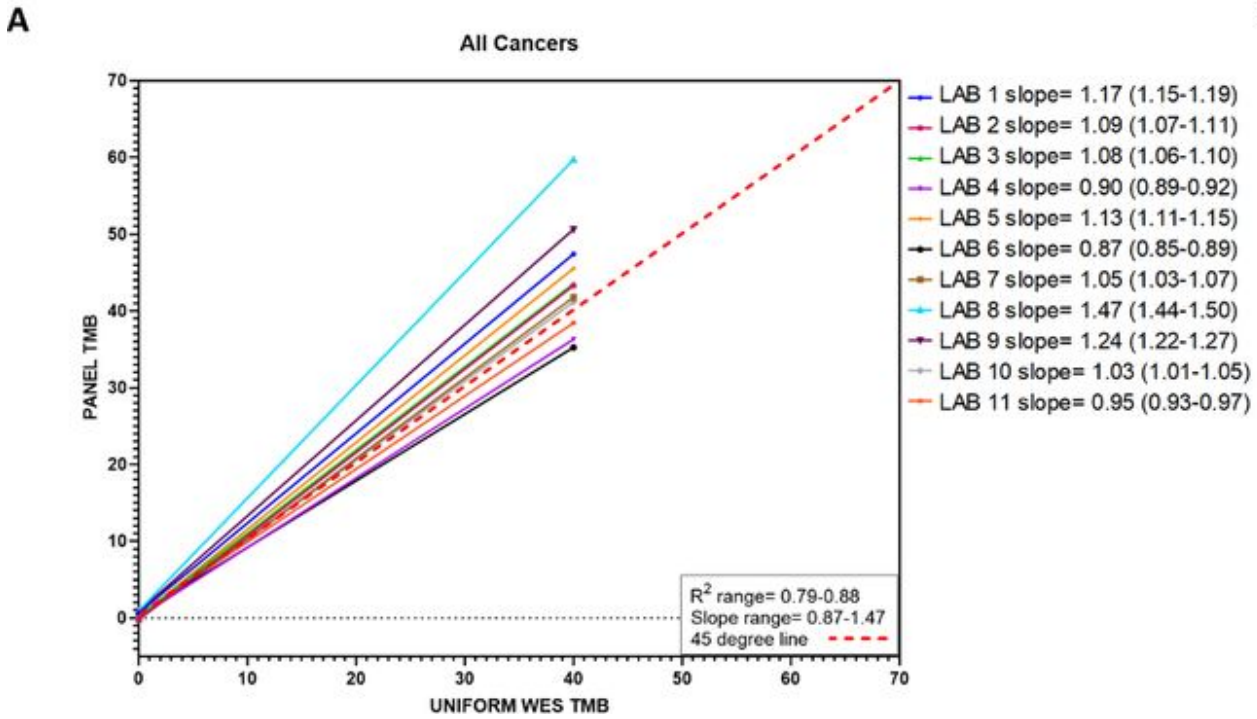
Wikipedia. Fernandez et al, JCO Precision Oncology 2019 :3, 1-12:

TMB is optimally calculated by Whole Exome Sequencing (WES)
Next Generation Sequencing (NGS) provide TMB estimates

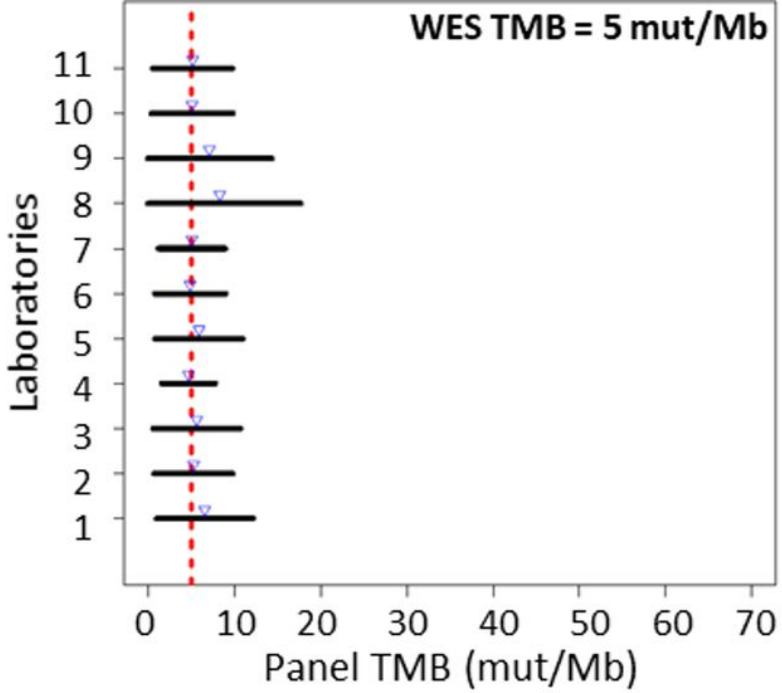
- Time Effective and Cost Effective

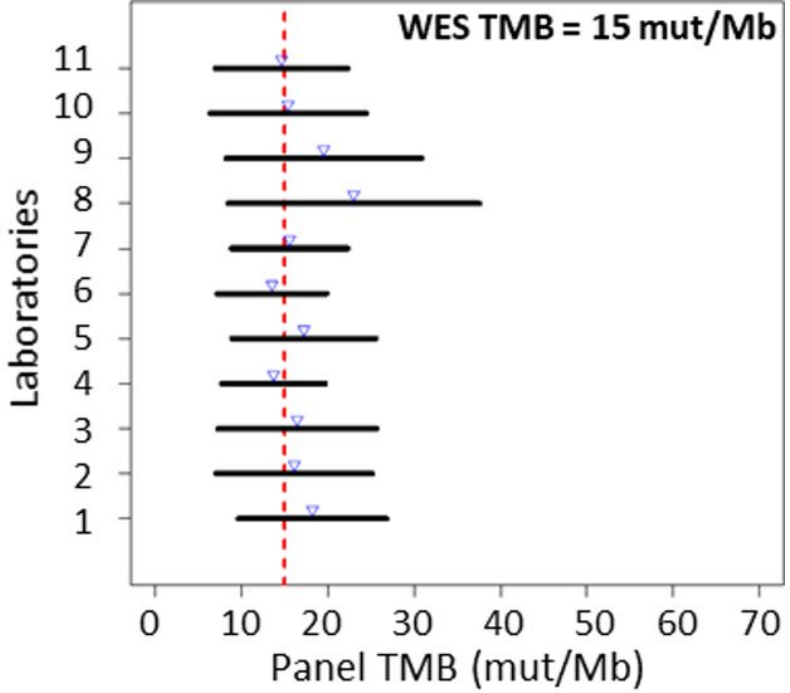


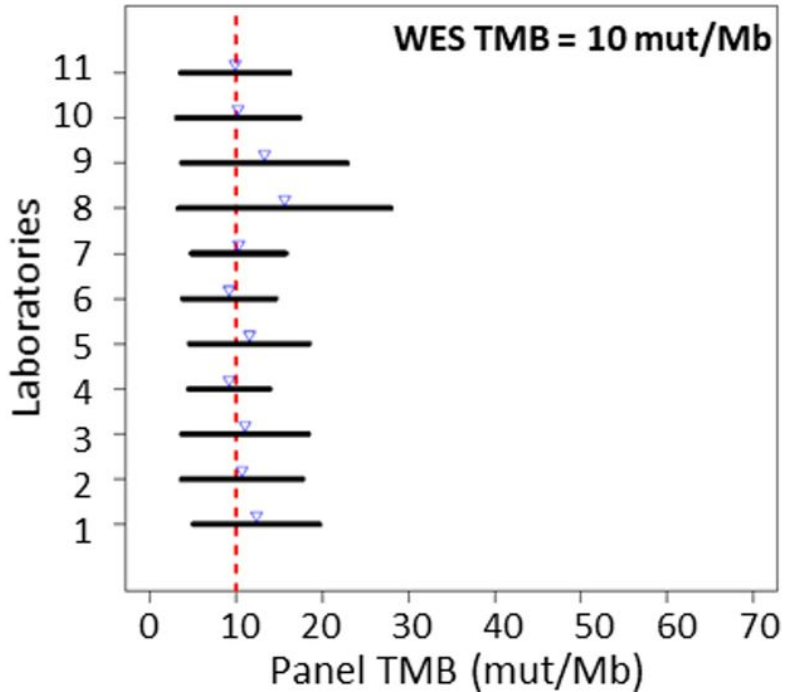
NGS TMB vs WES TMB

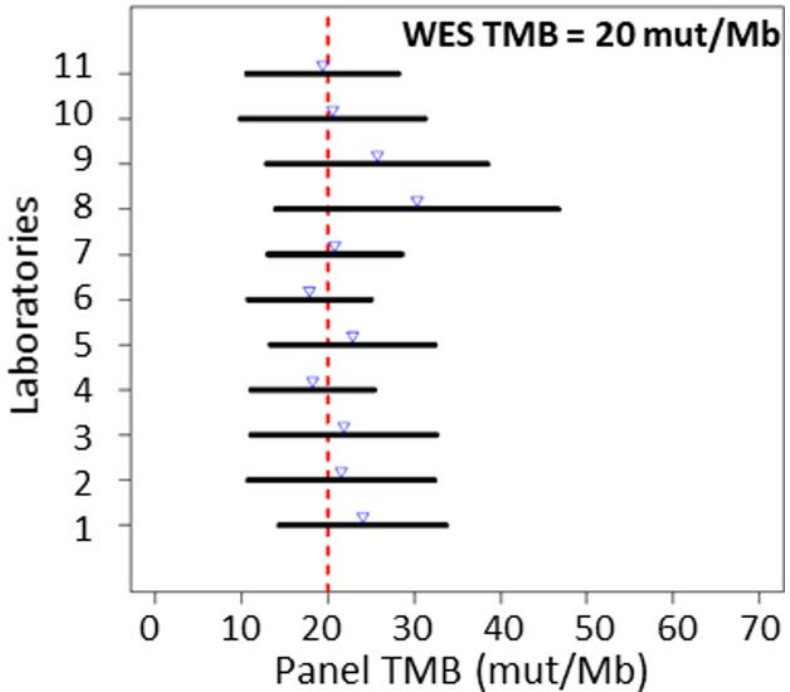


Journal for ImmunoTherapy of Cancer
Diana M Merino et al. J Immunother Cancer 2020;8:e000147









So TMB is TMB?

Company 1	Company 2
1.9Mb of sequencing coverage	0.8 Mb of sequencing coverage (793kb)
filter out germline alterations	filter out germline variants according to published databases and somatic-germline/zygosity
non-synonymous alterations	synonymous and non-synonymous variants present at 5% or greater
Do not filter out pathogenic variants they believe size of sequencing coverage minimizes the potential impact for TMB overestimation from alterations in single driver genes	Known and likely driver mutations are filtered out to exclude bias in the data set

Table 1. Characteristics of 16 participating diagnostic NGS panel assays

Factor type	ACT	AZ	BWH	Caris	FMI	ILLUM	IPG	JHU	MSKCC	NeoGenomics	OmniSeq	PGDx	Q2	QIAGEN	Thermo_OCA	Thermo_OTMLA
Panel assay characteristics																
Name of panel assay	ACTOnco+	AZ650	OncoPanel v3.1	SureSelectXT	F1 CDx	TSO500	TheraMap Solid Tumor (TSO500)	JHOP2	MSK-IMPACT	NeoTYPE Discovery Profile for Solid Tumors	Ion AmpliSeq Comprehensive Cancer Panel	PGDx elio tissue complete	TSO500	QIAseq TMB panel	Oncomine Comprehensive Assay Plus (OCA Plus)	Oncomine Tumor Mutation Load Assay (OTMLA)
Number of genes	440	649	447	592	324	523	523	432	468	372	409	505	523	486	517	409
TMB region covered	1.1 Mb	1.65 Mb	1.94 Mb	1.40 Mb	0.8 Mb	1.33 Mb	1.27 Mb	1.14 Mb	1.14 Mb	0.935 Mb	1.17 Mb	1.3 Mb	1.2 Mb	1.33 Mb	1.06 Mb	1.2 Mb
Processing																
Minimum DNA input	40 ng	100 ng	50 ng	50 ng	50 ng	40 ng	40 ng	50 ng	150 ng	20 ng	30 ng	50 ng	40 ng	40 ng	20 ng	20 ng
Quantification method	Fluorescence	Fluorescence	Fluorescence	Electrophoresis	Fluorescence	Fluorescence	Fluorescence	Electrophoresis	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Fluorescence
Technology uses UMIs	No	Yes	No	No	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	No	No
Deduplication	No	Yes, UMI based	Yes, not UMI based	Yes, not UMI based	Yes, UMI based	Yes, UMI based	Yes, UMI based	Yes, not UMI based	Yes, not UMI based	Yes, UMI based	No	Yes, UMI based	Yes, UMI based	Yes, UMI based	No	No
Sequencing																
Seq platform	Ion Torrent	Illumina	Illumina	Illumina	Illumina	Illumina	Illumina	Illumina	Illumina	Illumina	Ion Torrent	Illumina	Illumina	Illumina	Ion Torrent	Ion Torrent
Library prep/ target enrichment	Amplicon	Hybrid	Hybrid	Hybrid	Hybrid	Hybrid	Hybrid	Hybrid	Hybrid	Amplicon	Amplicon	Hybrid	Hybrid	Amplicon (single primer extension)	Amplicon	Amplicon
Sample-level minimum coverage threshold	800×	NA	30×	300×	250×	150×	300×	300×	50×	500×	125×	100×	50×	100×	500×	500×
Sample-level avg coverage for cell line exercise	1400×	1060.5×	394×	750×	982×	549×	800×	>400×	753×	>500×	314×	1517×	100×	500×	2000×	1300×
Variant-level minimum coverage	20×	50×	50×	100×	100×	50×	50×	50×	20×	100×	20×	Position-specific threshold. Determined by ML	150×	100×	60×	60×
Variant-level minimum read (ALT depth)	20	5	5	10	5	2[a]	3	3	8	10	4	6	2	4	10	10
Variant calling																
Type of variant	Non-synonymous and synonymous	Non-synonymous and synonymous	Non-synonymous only	Non-synonymous only	Non-synonymous and synonymous	Non-synonymous and synonymous	Non-synonymous and synonymous	Non-synonymous and synonymous	Non-synonymous only	Non-synonymous and synonymous	Non-synonymous only	Non-synonymous and synonymous	Non-synonymous and synonymous	Non-synonymous only	Non-synonymous only	Non-synonymous only
Germline variant filtration approach	Tumor only	Normal tissue	Tumor only	Tumor only	Tumor only	Tumor only	Tumor only	Tumor only	Normal tissue	Tumor only	Tumor only	Tumor only	Tumor only	Tumor only	Tumor only	Tumor only
Removes variants from known cancer genes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	No	Yes	No	Yes	No	No
Published performance characteristics			30,31	32	9,20,33,34				9,35-37			38				39-42

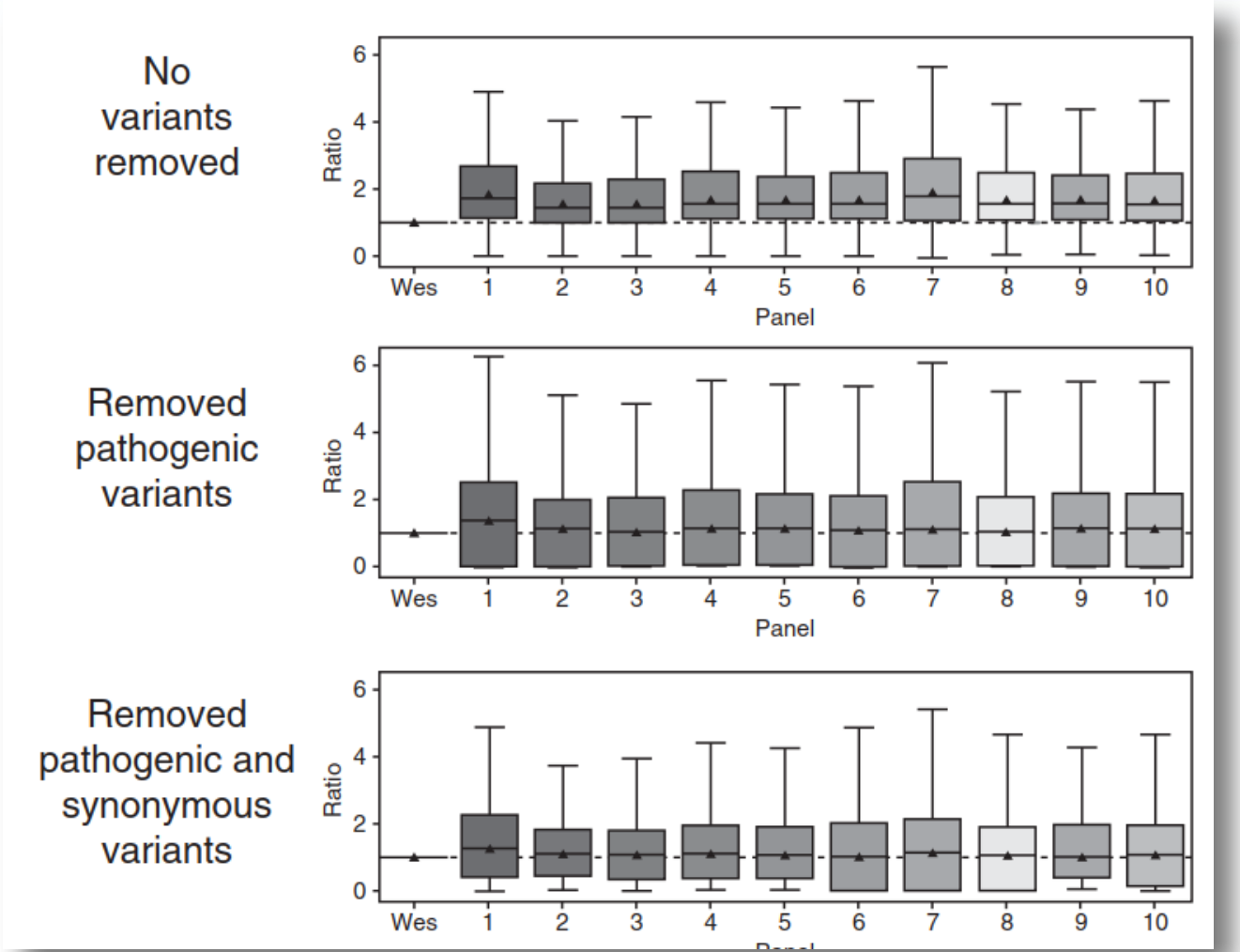
Vega et al. Annals of Oncology; v32 n12, 2021

Impact of Gene Content

Impact of gene content Including/Exclduing:

- pathogenic variants
- synonymous variants

on TMB estimate



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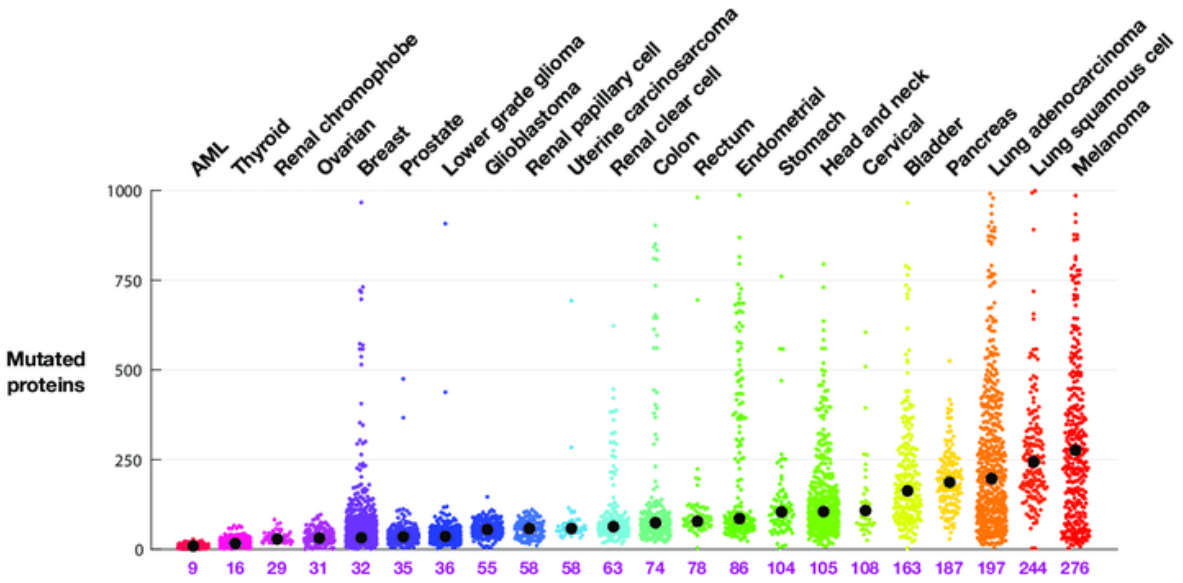

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Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/ Mismatch Repair–Deficient Cancer: Results From the Phase II KEYNOTE-158 Study

Aurelien Marabelle, MD, PhD[1]; Dung T. Le, MD[2]; Paolo A. Ascierto, MD[3]; Anna Maria Di Giacomo, MD[4]; Ana De Jesus-Acosta, MD[2]; Jean-Pierre Delord, MD, PhD[5]; Ravit Geva, MD, MSc[6]; Maya Gottfried, MD[7]; Nicolas Penel, MD, PhD[8]; Aaron R. Hansen, MBBS[9]; Sarina A. Piha-Paul, MD[10]; Toshihiko Doi, MD, PhD[11]; Bo Gao, MBBS, PhD[12]; Hyun Cheol Chung, MD, PhD[13]; Jose Lopez-Martin, MD, PhD[14]; Yung-Jue Bang, MD, PhD[15]; Ronnie Shapira Frommer, MD[16]; Manisha Shah, MD[17]; Razi Ghori, PhD[18]; Andrew K. Joe, MD[18]; Scott K. Pruitt, MD, PhD[18]; and Luis A. Diaz Jr, MD[19]

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Marabelle et al. J Clin Oncol 38:1-10

TABLE 3. Antitumor Activity for Tumor Types With Greatest Enrollment

Tumor Type	No.	CR, No.	PR, No.	ORR, % (95% CI)	Median PFS, Months (95% CI)	Median OS, Months (95% CI)	Median DOR, Months (range)
Endometrial	49	8	20	57.1 (42.2 to 71.2)	25.7 (4.9 to NR)	NR (27.2 to NR)	NR (2.9 to 27.0+)
Gastric	24	4	7	45.8 (25.6 to 67.2)	11.0 (2.1 to NR)	NR (7.2 to NR)	NR (6.3 to 28.4+)
Cholangiocarcinoma	22	2	7	40.9 (20.7 to 63.6)	4.2 (2.1 to NR)	24.3 (6.5 to NR)	NR (4.1+ to 24.9+)
Pancreatic	22	1	3	18.2 (5.2 to 40.3)	2.1 (1.9 to 3.4)	4.0 (2.1 to 9.8)	13.4 (8.1 to 16.0+)
Small intestine	19	3	5	42.1 (20.3 to 66.5)	9.2 (2.3 to NR)	NR (10.6 to NR)	NR (4.3+ to 31.3+)
Ovarian	15	3	2	33.3 (11.8 to 61.6)	2.3 (1.9 to 6.2)	NR (3.8 to NR)	NR (4.2 to 20.7+)
Brain	13	0	0	0.0 (0.0 to 24.7)	1.1 (0.7 to 2.1)	5.6 (1.5 to 16.2)	–

NOTE. Efficacy analyses included all patients who received at least one dose of pembrolizumab. Only confirmed responses are included. Response was assessed per RECIST version 1.1 by independent central radiologic review.

Abbreviations: +, no progressive disease by the time of last disease assessment; CR, complete response; DOR, duration of response; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response.

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Treatment w/ Pembro in TMB-H

TMB > 10:

- ORR 30% [95% CI, 20.8–39.3]
  - 4 patients (4%) had a CR
  - 26 patients (25%) had a PR
- Median duration of response (DOR)
  - was not reached
  - range 2.2+ to 34.8+ months
- Most (66.6%) responders had a DOR of ≥24 months

TMB < 10

- ORR of 6% (95% CI, 4.6–8.3)

TMB ≥10 mut/Mb and <13 mut/Mb

- Exploratory analysis of 32 patients
- ORR was 12% (95% CI, 4–29)
  - two CRs (6%)
  - two PRs (6%)

TMB ≥13

- ORR of 37 % (95% CI, 26–50).

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Pooled Data

Review of Published Studies

Pooled 1732 retrospectively

TMB 10-15 is the equivocal zone.

TMB ≥ 10	30.1%
TMB < 10	13.8%

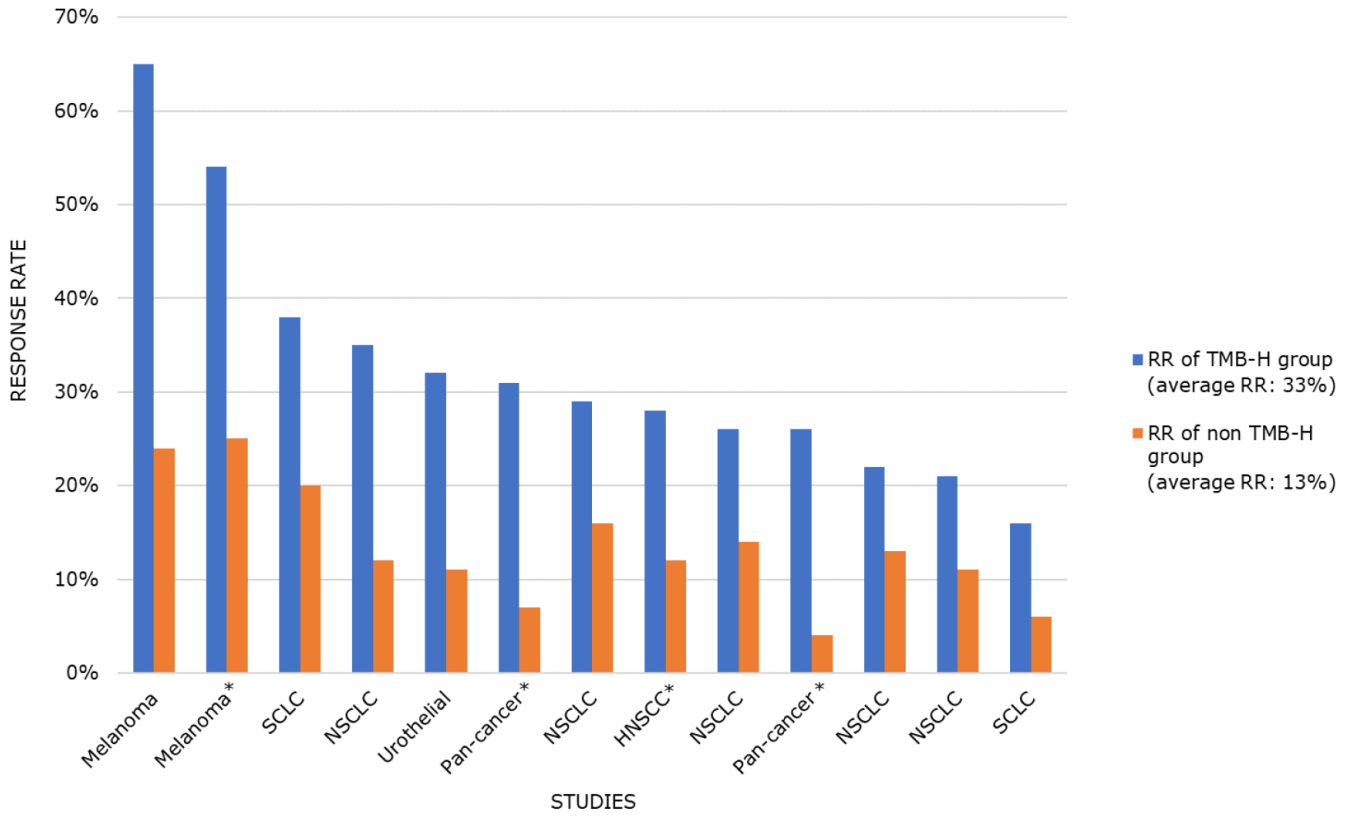

Figure 1: Response Rates for Published Studies Investigating Immunotherapies and Reporting TMB-based Response Rates.
** indicates studies that did not use TMB 10 mut/Mb as the study cut-off*

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TMB ≥ 15	37.4%
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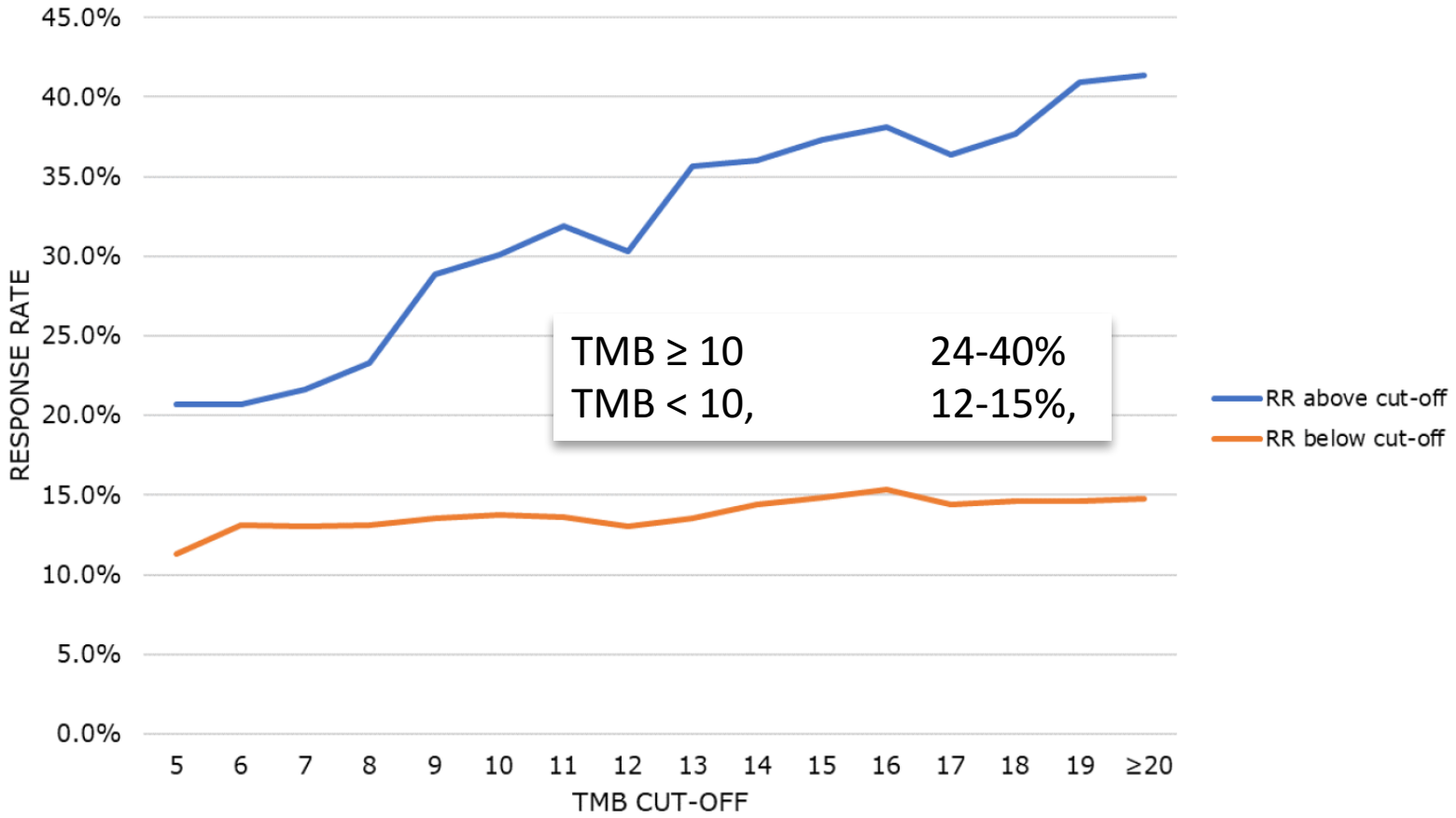

Figure 2. Average Response Rates for Patients Treated with IO Agents - Pooled Cohort

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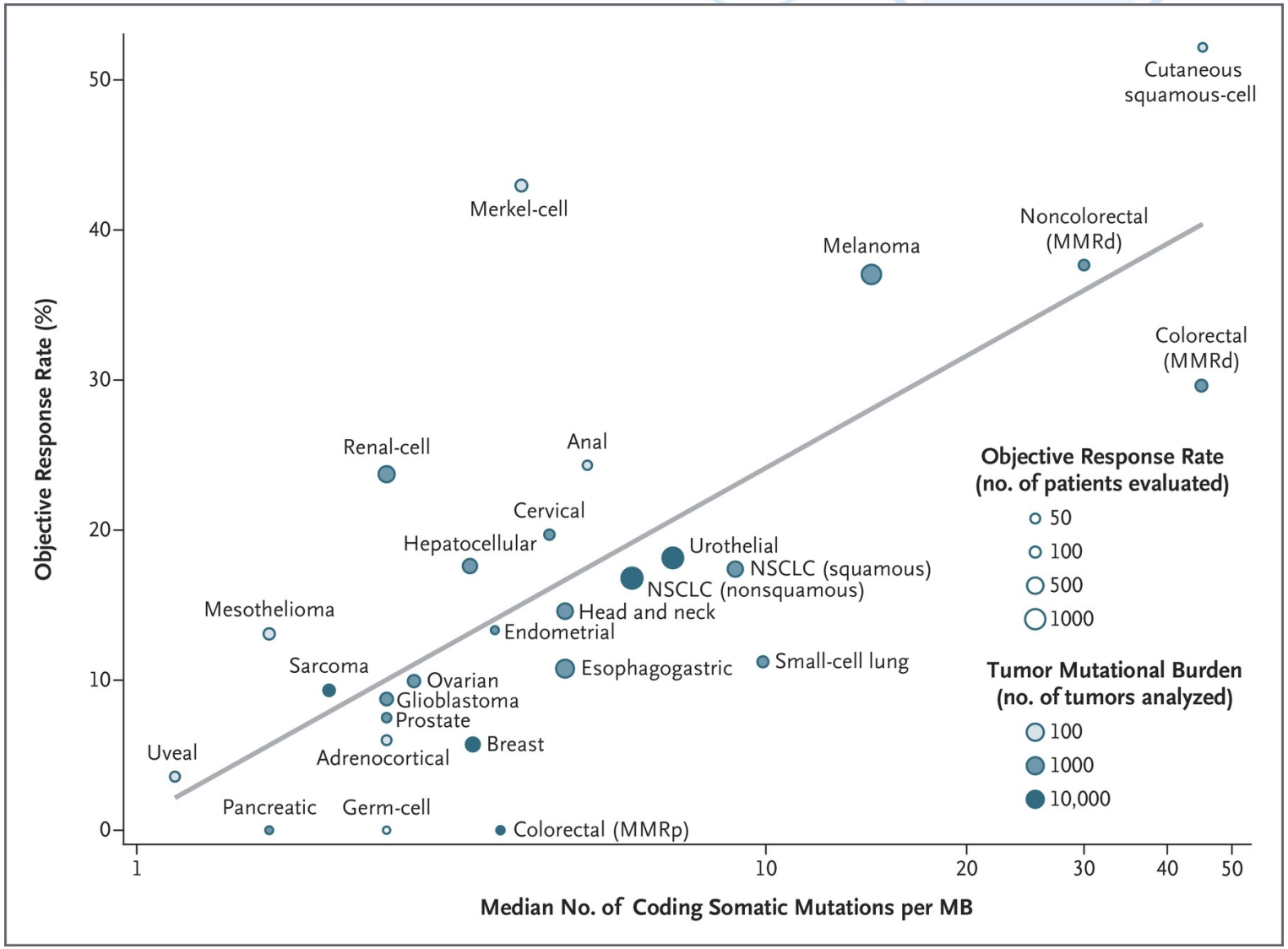
TMB and RR to PD-1 Inhibition

Correlation Coefficient: 0.74

Suggests 55% of ΔORR is TMB

Merkel Cell is better than predicted

MMRd CRC is worse than predicted



Letter to the Editor, N Engl J Med 2017; 377:2500-2501

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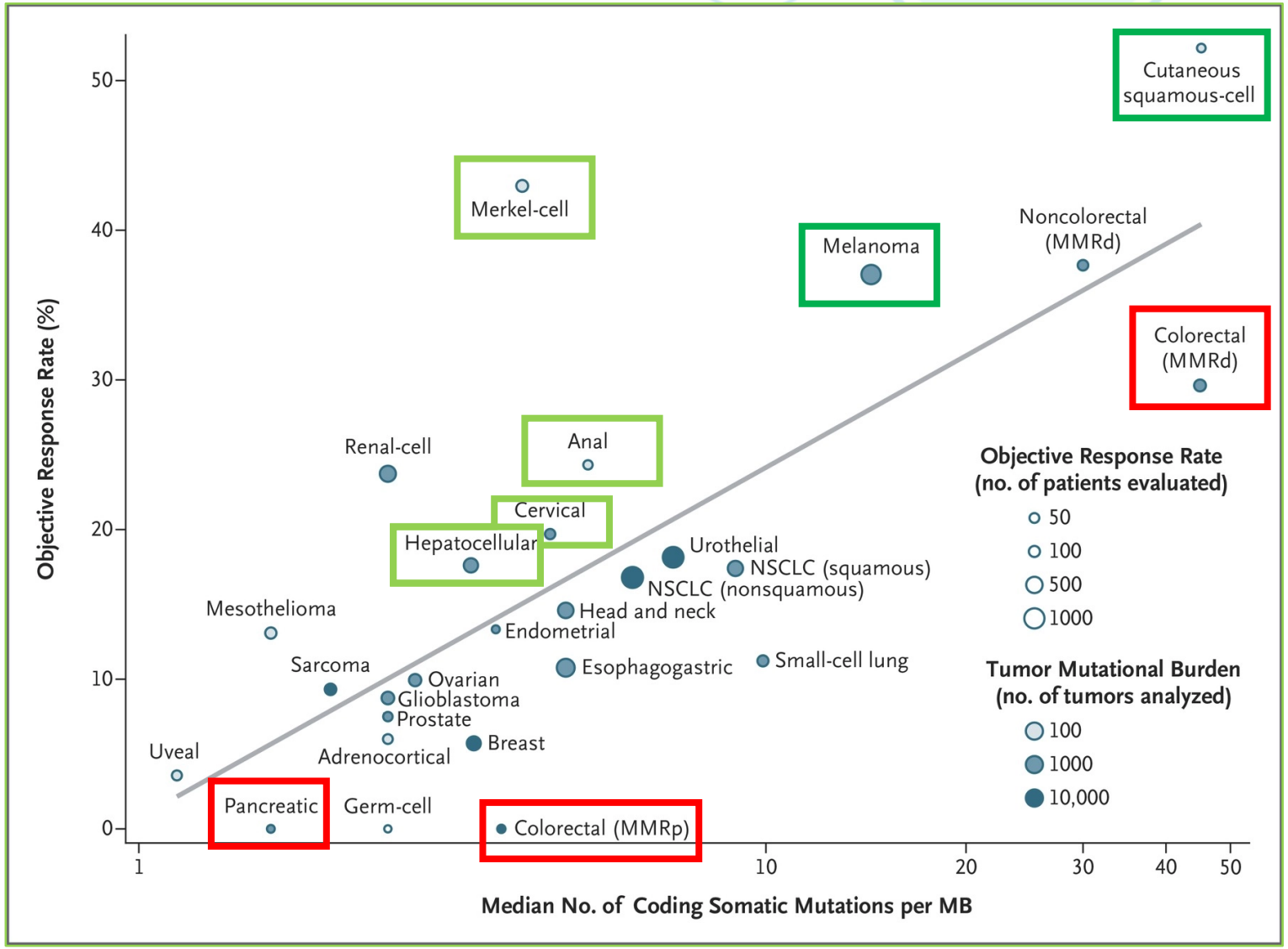

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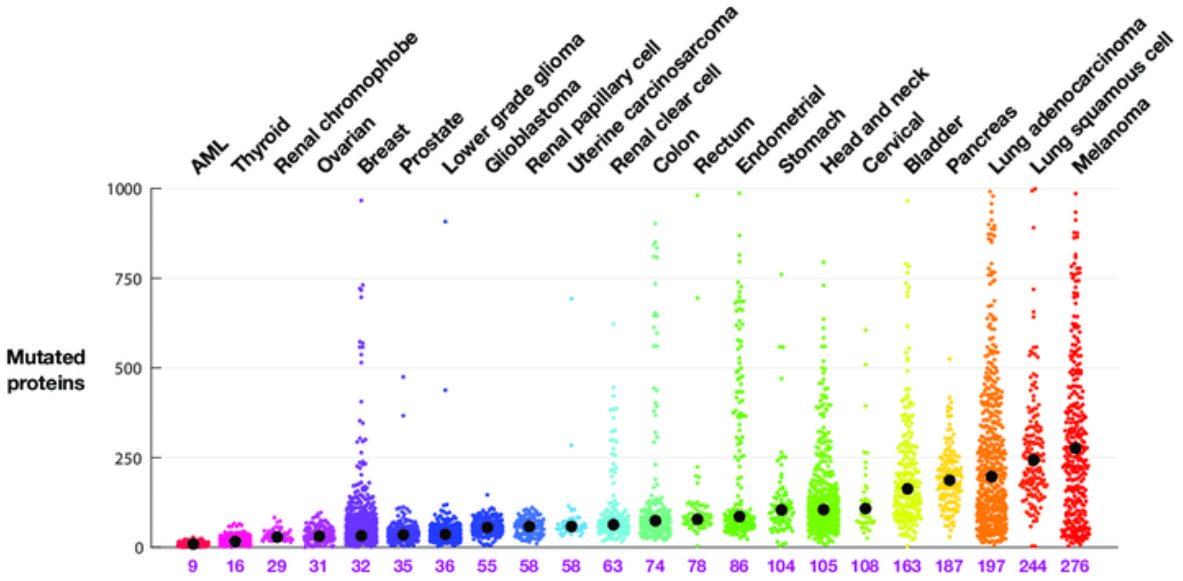

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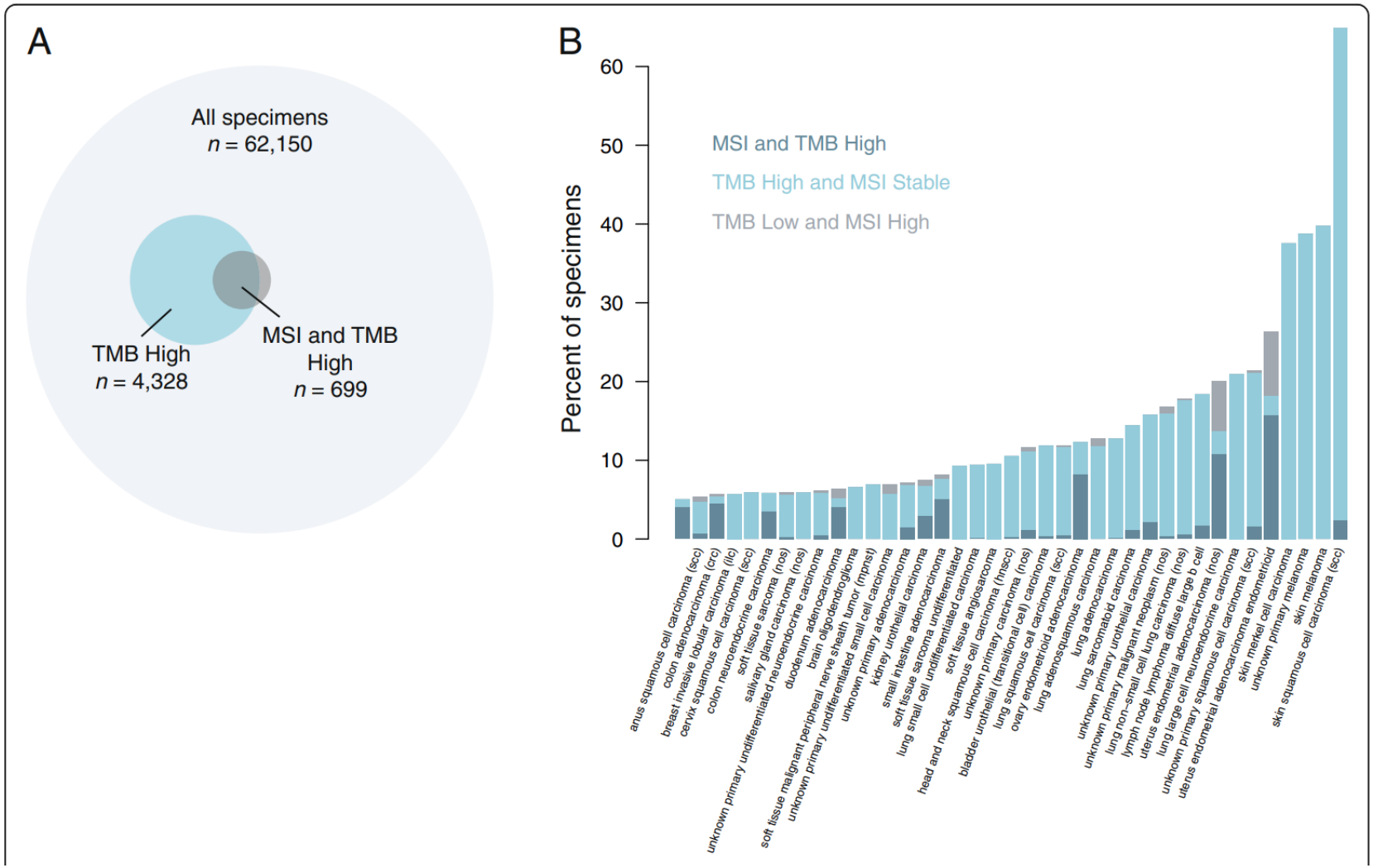
MSI and TMB

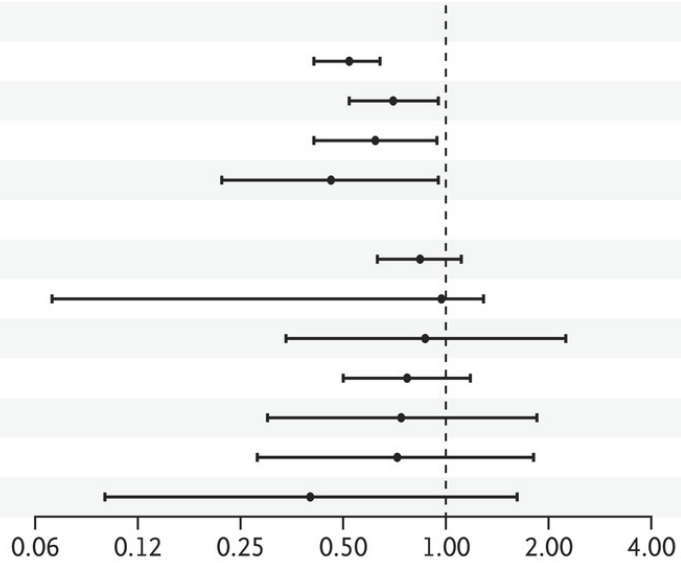


Chalmers ZR, Connelly CF, Fabrizio D, et al. Genome Med 2017; 9:34.

TMB ≥ 10 ?

C Effect of Immune Checkpoint Inhibitors, by Tumor Type

Subgroup	TMB ≥10 Mutations/Mb	TMB <10 Mutations/Mb	Hazard Ratio for Death (95% CI)
	no. of patients with MMRp tumors		
TMB-sensitive tumors			
Combined TMB-sensitive tumors	285	462	0.52 (0.41–0.64)
NSCLC	111	236	0.70 (0.52–0.95)
Melanoma	148	108	0.62 (0.41–0.94)
Head and neck	26	118	0.46 (0.22–0.95)
TMB-insensitive tumors			
Combined TMB-insensitive tumors	138	709	0.84 (0.63–1.11)
Esophageal or gastric	9	107	0.97 (0.07–1.29)
Colorectal	13	65	0.87 (0.34–2.25)
Urinary tract	77	126	0.77 (0.50–1.18)
Brain	9	108	0.74 (0.30–1.85)
Unknown primary tumor	19	44	0.72 (0.28–1.81)
Other tumors	11	259	0.40 (0.10–1.62)



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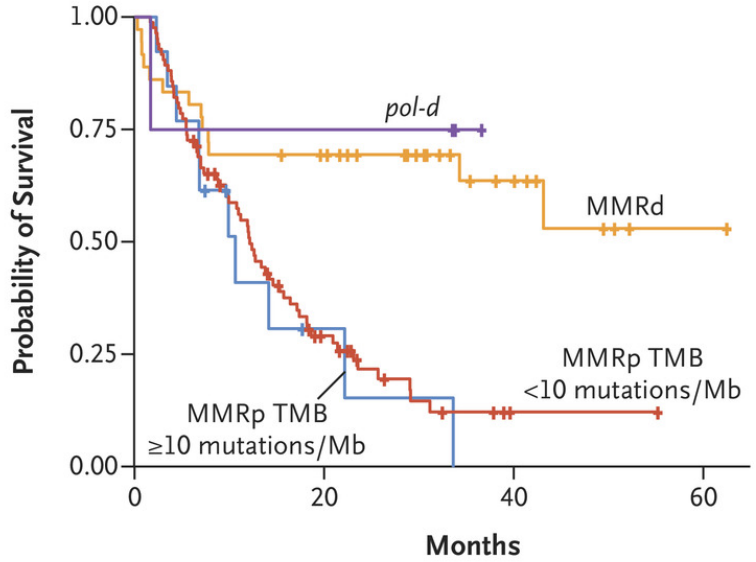
TMB ≥ 10 in Colon Cancer?

B **Overall Survival, by DNA-Repair Status**



DNA-Repair Status	No. of Deaths/ No. of Patients	Median Overall Survival (95% CI)
		mo
pol-d	1/4	NR (1.68–NE)
MMRd	13/36	NR (34.28–NE)
MMRp TMB <10 mutations/Mb	64/84	12.1 (9.61–15.3)
MMRp TMB ≥10 mutations/Mb	10/13	10.6 (4.41–22.2)

No. at Risk

pol-d	4	3	0	0
MMRd	36	23	9	1
MMRp TMB <10 mutations/Mb	84	18	1	0
MMRp TMB ≥10 mutations/Mb	13	2	0	0

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Conclusions

My Learning Objectives

- Why measure TMB
- How to measure TMB
- Issues with TMB
- MSI-H vs TMB

Pembrolizumab is approved for patients with TMB ≥ 10 tumors, but some will respond better and some worse.