CME OUTFITTERS

Tackling Barriers to Genomic and Biomarker Testing in Precision Cancer Medicine: Integrating the Latest Evidence into Practice for Community Oncology Clinicians

Supported by an educational grant from
Merck Sharp & Dohme, LLC, a subsidiary of
Merck & Co., Inc.

CME OUTFITTERS

Part 1:
Genetic Testing and Biomarkers in Cancer: A Community Oncology Approach

Supported by an educational grant from
Merck Sharp & Dohme, LLC, a subsidiary of
Merck & Co., Inc.

CME OUTFITTERS

Banu K. Arun, MD, FASCO

Professor, Department of Breast Medical Oncology
Co-Medical Director, Clinical Cancer Genetics Program
Section Chief, Breast Genetics, Prevention and Screening
University of Texas MD Anderson Cancer Center
Houston, TX

CME OUTFITTERS

Kurt Schalper, MD, PhD

Associate Professor of Pathology and Medicine (Medical Oncology)

Yale School of Medicine

Director, Translational Immuno-Oncology Laboratory

Yale Cancer Center

New Haven, CT

CME OUTFITTERS

Learning Objective

Implement best practices in genetic and biomarker testing for cancer immunotherapy

Biomarkers in Oncology

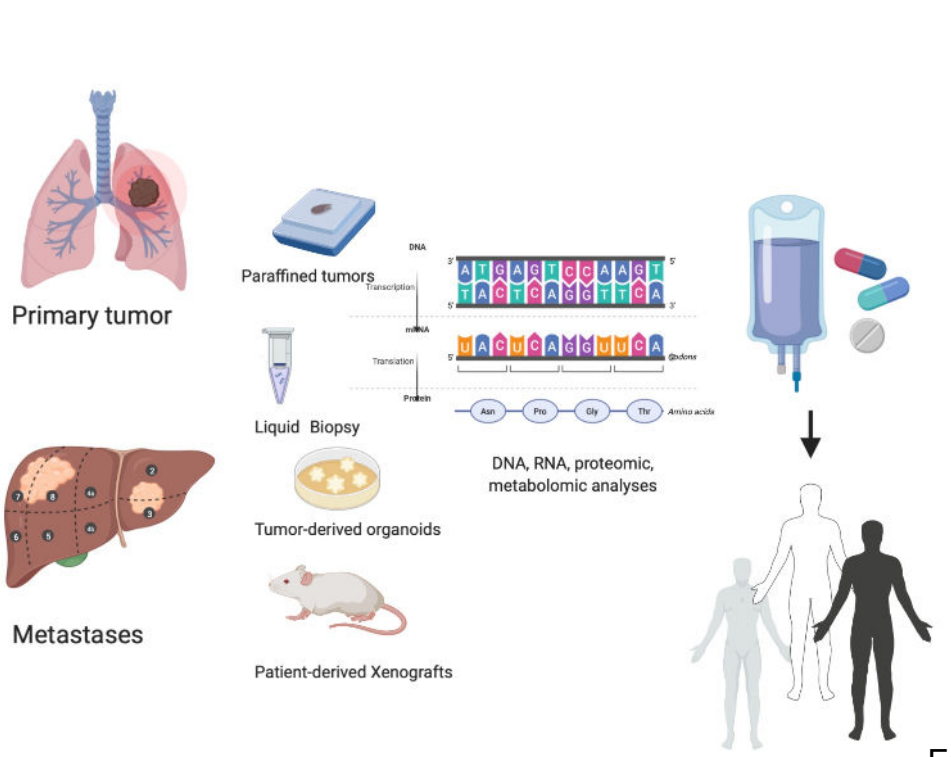
- Concept: “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”

- Classification:
  - Sample type: Tissue, tumor, circulating, body fluids, other
  - Analyte: Genomic, transcriptomic, proteomic, epigenetic, other
  - Aim: Diagnostic, pharmacodynamic, prognostic, predictive*

Biomarkers Definitions Working Group. *Clin Pharmacol Ther*. 2001;69(3):89-95.

CME OUTFITTERS

Landscape of Predictive Oncology Biomarkers



Bambardella V, et al. *Cancers (Basel)*. 2020;12(4).

CME OUTFITTERS

Biomarkers for Common Solid Tumors

Targeted Therapies & PARPi	Immunotherapy
Lung: EGFR, KRAS, ALK, BRAF, HER2, ROS1, RET, MET, NTRK1-3	**Lung**: PD-L1, TMB
Colorectal: KRAS, NRAS, BRAF, HER2, NTRK1-3	**Colorectal**: MSI, TMB
Melanoma: BRAF, NRAS, KIT, ROS1, NTRK1-3.	**Melanoma**: PD-L1, TMB
Breast: HER2, PIK3CA, ESR1, BRCA1/2, NTRK1-3, RET	**Breast**: PD-L1, TMB
Head & neck: HPV, NTRK1-3	**Head & neck**: PD-L1, TMB
Gastric: HER2, NTRK1-3	**Gastric**: PD-L1, MSI, TMB
Prostate: BRCA1/2, HRD, AR	**Prostate**: TMB, MSI

National Comprehensive Cancer Network. Detection, Prevention, and Risk Reduction. https://www.nccn.org/guidelines/category_2. Accessed March 5, 2023.

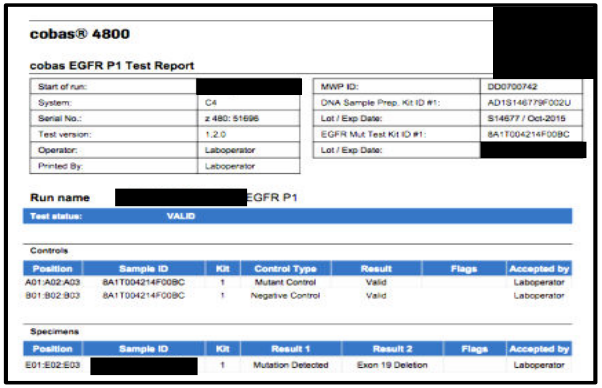
CME OUTFITTERS

Genomic Testing in Tumor and Plasma Samples: EGFR

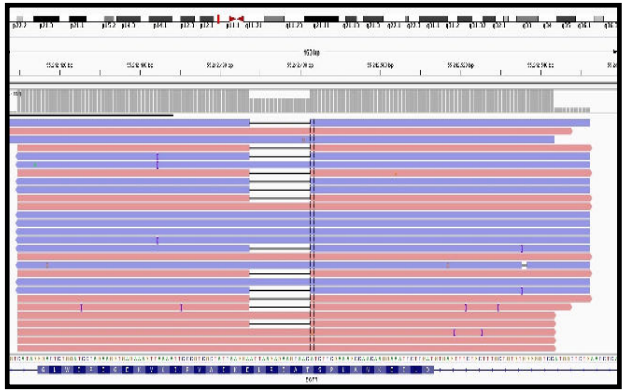
- Multiple platforms for genomic testing available
- Different analytical properties and throughput
- Different cost, availability and reporting

Sanger sequencing

qPCR (Cobas)



Next Gen Sequencing



Multiplex Genomic Testing

- Multiplex panel testing recommended, particularly for small samples
- TMB (only available in specific tests/assays)
- In selected cases, molecular testing can be done using liquid biopsy (ctDNA)
- Commercial panels available

Hotspot genes			
AKT1	*ESR1*	*KIT*	*PDGFRB*
AKT2	*EZH2*	*KNSTRN*	*PIK3CB*
AKT3	*FGFR1*	*KRAS*	*PIK3CA*
ALK	*FGFR2*	*MAGOH*	*PPP2R1A*
AR	*FGFR3*	*MAP2K1*	*PTPN11*
ARAF	*FGFR4*	*MAP2K2*	*RAC1*
AXL	*FLT3*	*MAP2K4*	*RAF1*
BRAF	*FOXL2*	*MAPK1*	*RET*
BTK	*GATA2*	*MAX*	*RHEB*
CBL	*GNA11*	*MDM4*	*RHOA*
CCND1	*GNAQ*	*MED12*	*ROS1*
CDK4	*GNAS*	*MET*	*SF3B1*
CDK6	*H3F3A*	*MTOR*	*SMAD4*
CHEK2	*HIST1H3B*	*MYC*	*SMO*
CSF1R	*HNF1A*	*MYCN*	*SPOP*
CTNNB1	*HRAS*	*MYD88*	*SRC*
DDR2	*IDH1*	*NFE2L2*	*STAT3*
EGFR	*IDH2*	*NRAS*	*TERT*
ERBB2	*JAK1*	*NTRK1*	*TOP1*
ERBB3	*JAK2*	*NTRK2*	*U2AF1*
ERBB4	*JAK3*	*NTRK3*	*XPO1*
ERCC2	*KDR*	*PDGFRA*	

Full-length genes		
ARID1A	*FBXW7*	*PTEN*
ATM	*MLH1*	*RAD50*
ATR	*MRE11*	*RAD51*
ATRX	*MSH6*	*RAD51B*
BAP1	*MSH2*	*RAD51C*
BRCA1	*NBN*	*RAD51D*
BRCA2	*NF1*	*RNF43*
CDK12	*NF2*	*RB1*
CDKN1B	*NOTCH1*	*SETD2*
CDKN2A	*NOTCH2*	*SLX4*
CDKN2B	*NOTCH3*	*SMARCA4*
CHEK1	*PALB2*	*SMARCB1*
CREBBP	*PIK3R1*	*STK11*
FANCA	*PMS2*	*TP53*
FANCD2	*POLE*	*TSC1*
FANCI	*PTCH1*	*TSC2*

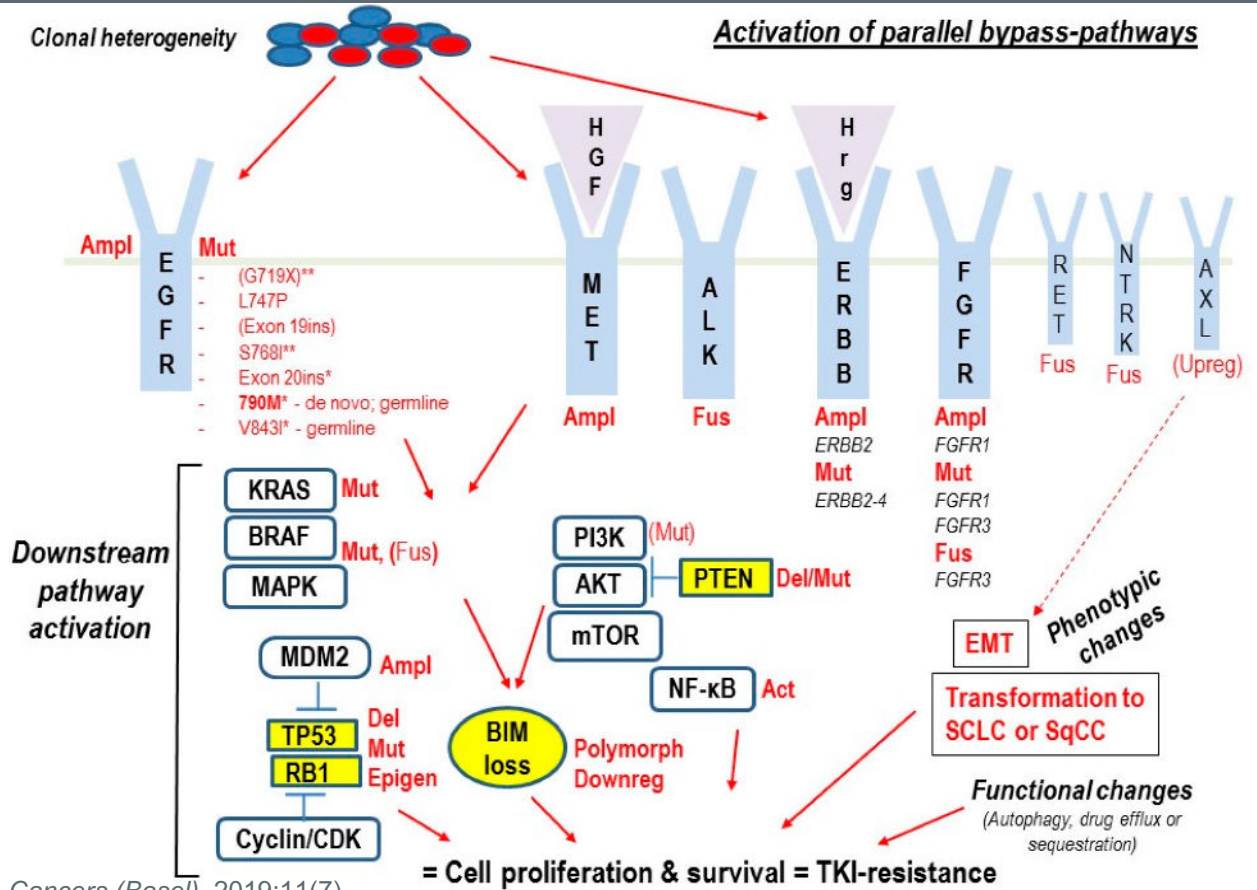
Copy number genes	
AKT1	*FGFR4*
AKT2	*FLT3*
AKT3	*IGF1R*
ALK	*KIT*
AXL	*KRAS*
AR	*MDM2*
BRAF	*MDM4*
CCND1	*MET*
CCND2	*MYC*
CCND3	*MYCL*
CCNE1	*MYCN*
CDK2	*NTRK1*
CDK4	*NTRK2*
CDK6	*NTRK3*
EGFR	*PDGFRA*
ERBB2	*PDGFRB*
ESR1	*PIK3CB*
FGF19	*PIK3CA*
FGF3	*PPARG*
FGFR1	*RICTOR*
FGFR2	*TERT*
FGFR3	

Gene fusions (inter- and intragenic)		
AKT2	*FGFR2*	*NUTM1*
ALK	*FGFR3*	*PDGFRA*
AR	*FGR*	*PDGFRB*
AXL	*FLT3*	*PIK3CA*
BRCA1	*JAK2*	*PRKACA*
BRCA2	*KRAS*	*PRKACB*
BRAF	*MDM4*	*PTEN*
CDKN2A	*MET*	*PPARG*
EGFR	*MYB*	*RAD51B*
ERBB2	*MYBL1*	*RAF1*
ERBB4	*NF1*	*RB1*
ERG	*NOTCH1*	*RELA*
ESR1	*NOTCH4*	*RET*
ETV1	*NRG1*	*ROS1*
ETV4	*NTRK1*	*RSPO2*
ETV5	*NTRK2*	*RSPO3*
FGFR1	*NTRK3*	*TERT*

Vestergaard LK, et al. *Cancers (Basel)*. 2021;13(20).

CME OUTFITTERS

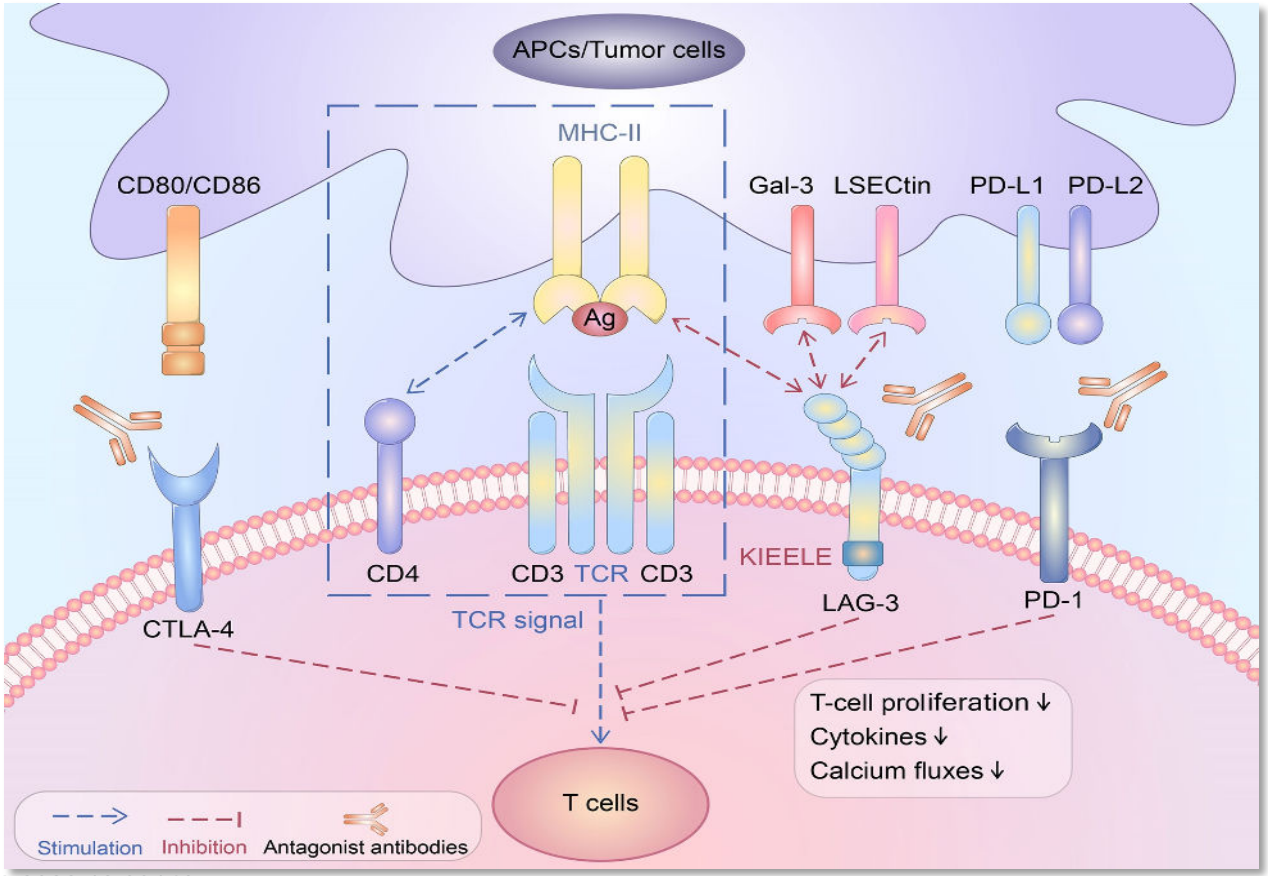
EGFR Mutations and TKI Resistance



Santoni-Rugiu E, et al. *Cancers (Basel)*. 2019;11(7).

CME OUTFITTERS

Immune Checkpoint Inhibitors: T-Cell Inhibitory Signals

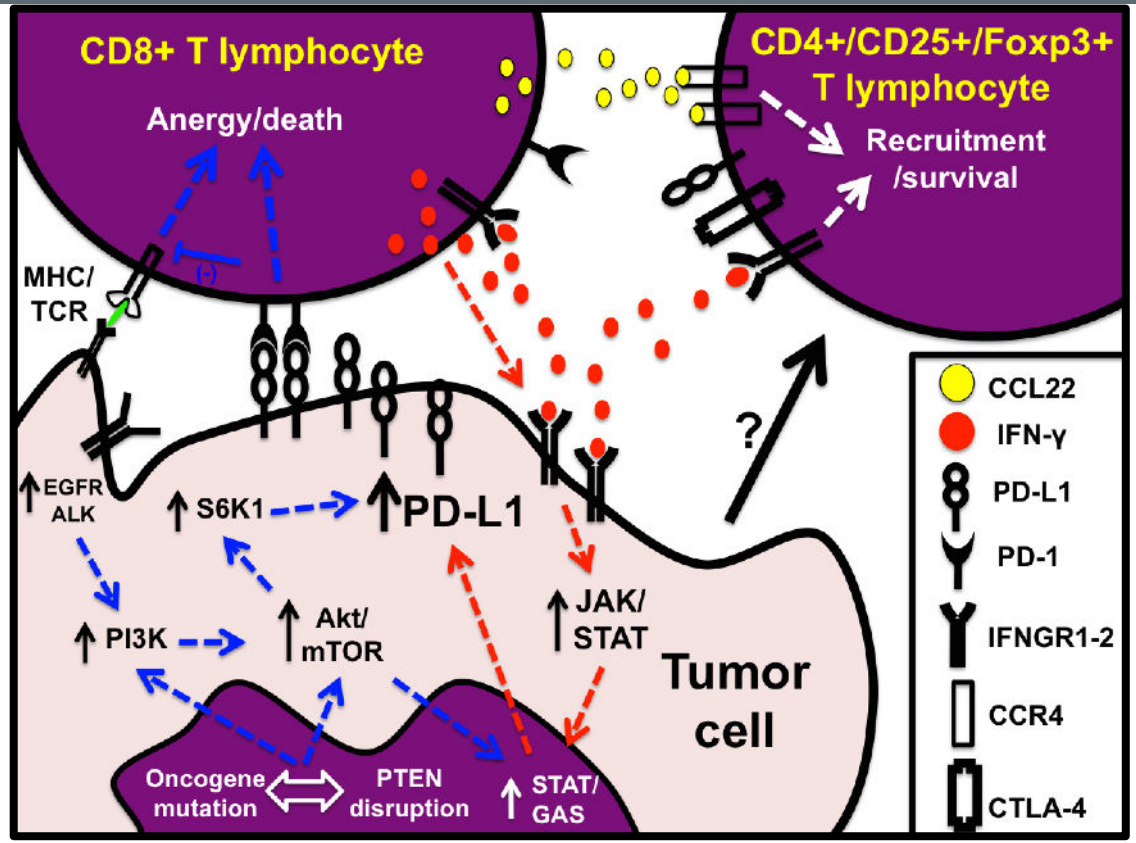


Wei Y, et al. *Front Oncol*. 2022;12:831407.

CME OUTFITTERS

PD-L1 and Peripheral Immune Tolerance

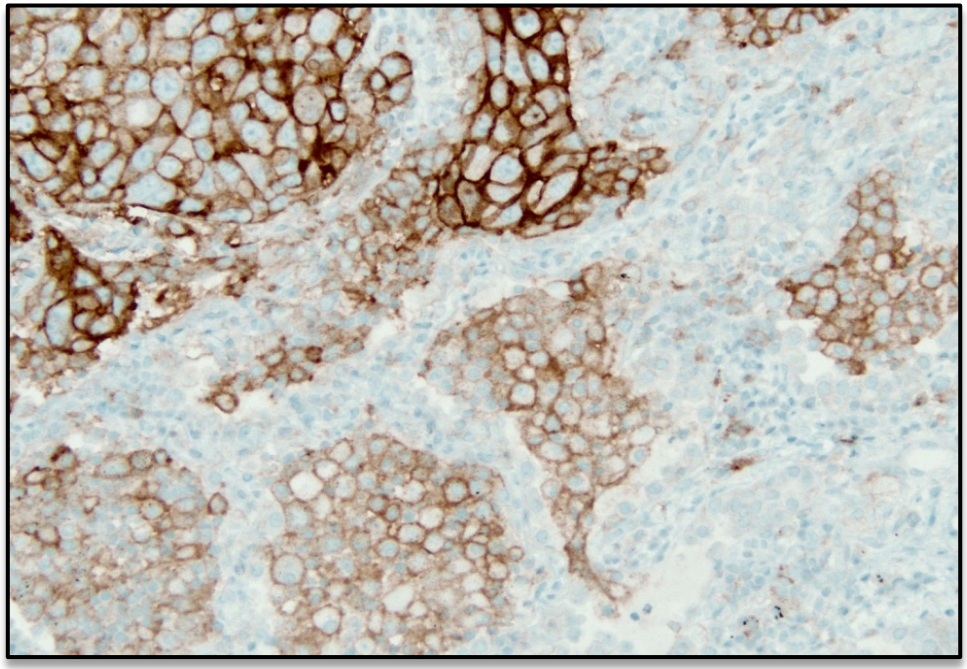
- Extrinsic pathway
  - IFNγ
  - Tumor/immune cells
  - T-cell inflamed tumors
- Intrinsic pathway
  - Oncogenic signaling
  - PI3K/PTEN pathway
  - T-cell “cold” tumors

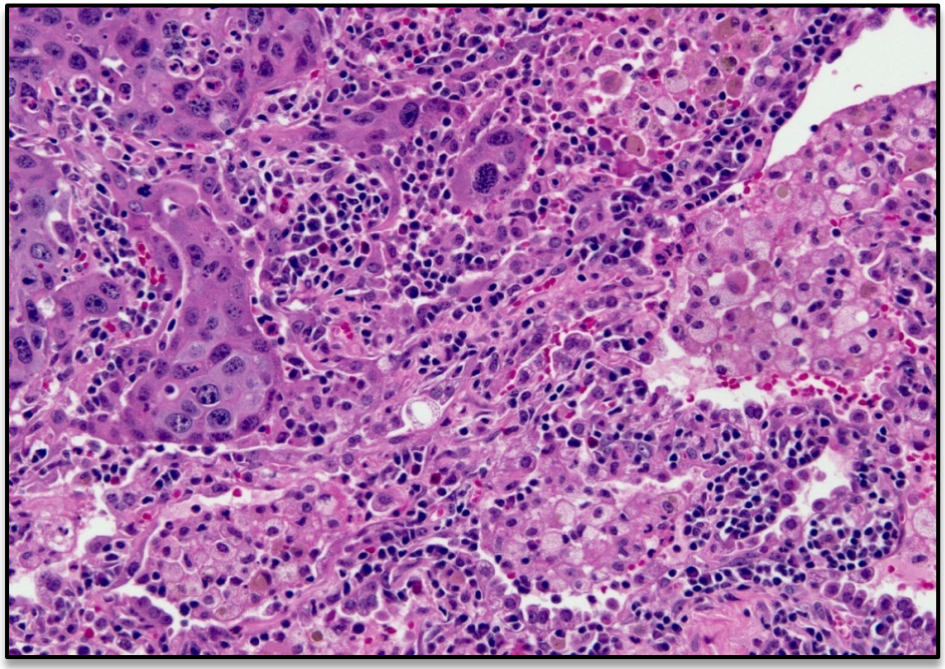


Schalper KA. *Oncoimmunology*. 2014;3:e29288.

CME OUTFITTERS

Tumor and Immune/Stromal Cells Can Express PD-L1

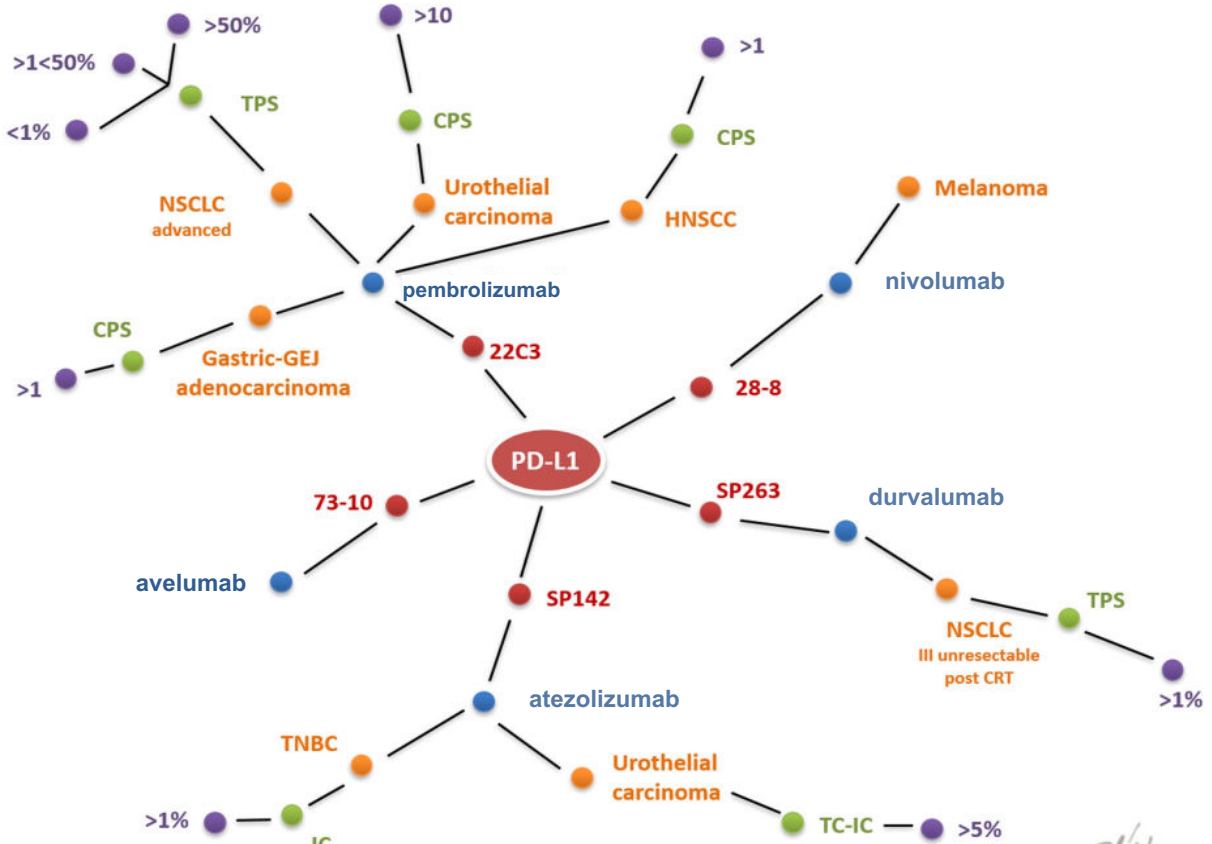




Schalper KA, unpublished images

CME OUTFITTERS

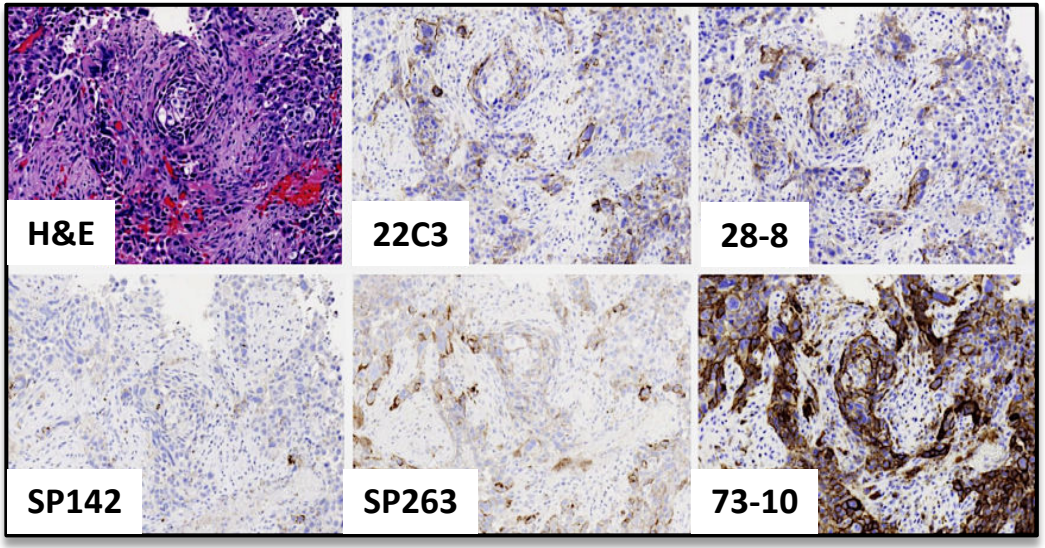
PD-L1 Expression and PD-1 Axis Blockers Across Tumors

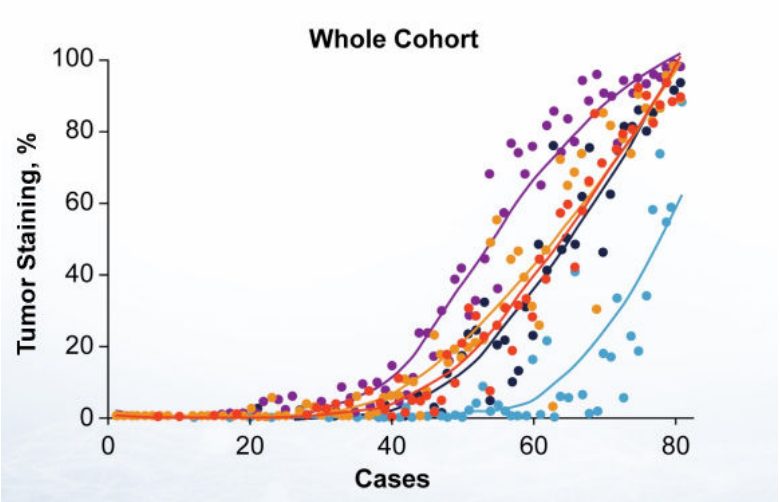


Paolino G, et al. *Pathol Res Pract*. 2021;226:153605.

CME OUTFITTERS

Blueprint 2 Study: PD-L1 Staining



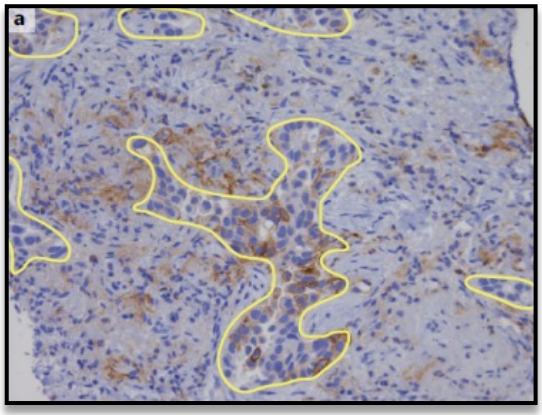


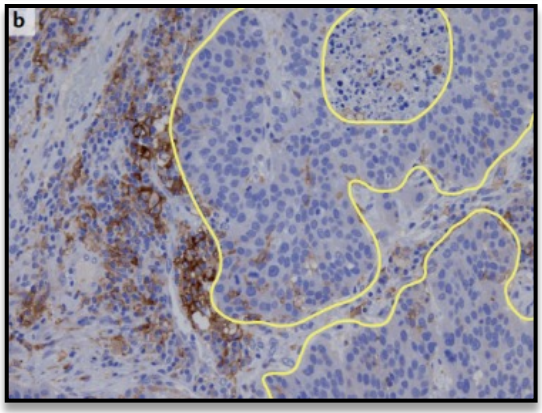
- High concordance in tumor scoring (Correlation 0.81-0.91)
- Low concordance stromal-cell scoring (Correlation **0.17-0.36**)

Tsao MS, et al. *J Thorac Oncol*. 2018;13(9):1302-1311.

CME OUTFITTERS

PD-L1 Scoring Systems





$$\text{TPS (\%)} = \frac{\text{Number of PD-L1–stained tumor cells}}{\text{Total number of viable tumor cells}} \times 100\% \text{ (for 22C3 or SP263)}$$

$$\text{CPS (\%)} = \frac{\text{Number of PD-L1–stained cells (tumor cells, lymphocytes, and macrophages)}}{\text{Total number of viable tumor cells}} \times 100\% \text{ (for 22C3)}$$

$$\text{TC (\%)} = \frac{\text{Number of PD-L1–stained tumor cells}}{\text{Total number of viable tumor cells}} \times 100\% \text{ (for SP142)}$$

$$\text{IC (\%)} = \frac{\text{Area of tumor infiltrated by PD-L1–stained immune cells}}{\text{Total tumor areas}} \times 100\% \text{ (for SP142)}$$

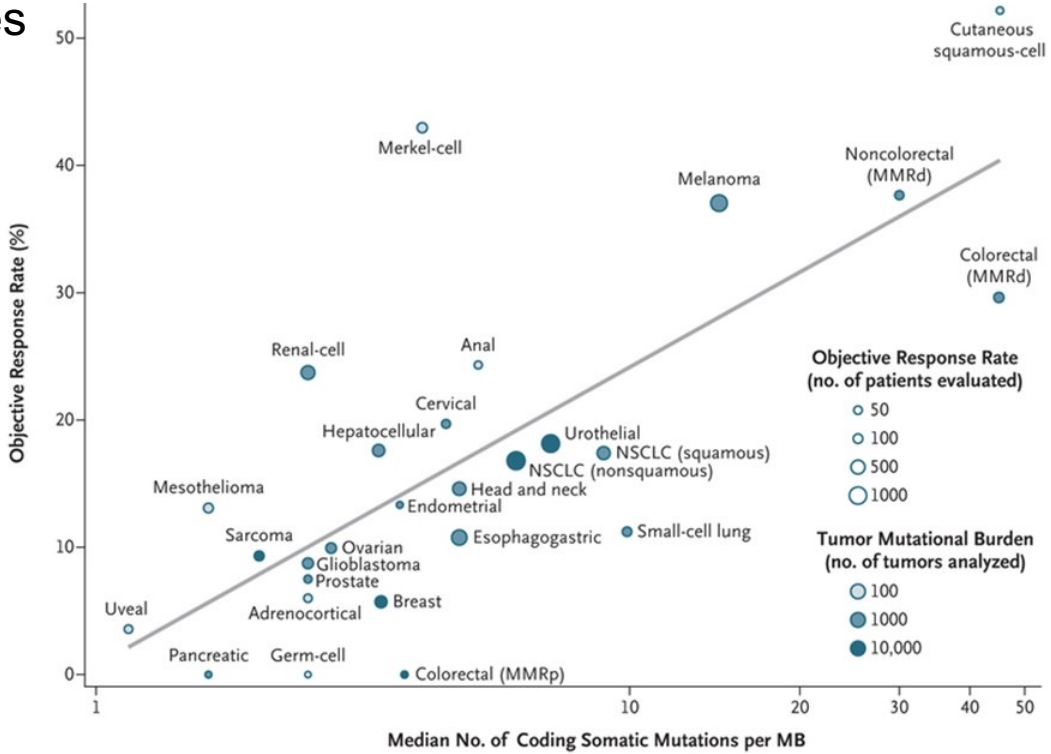
CPS = combined positive score; IC = tumor-infiltrating immune cell score; TPS = tumor proportion score

Schalper KA, unpublished images. Munari E, et al. *Front Immunol*. 2022;13:954910. Pereira MA, et al. Virchows Arch. 2021;478(6):1039-1048.

CME OUTFITTERS

TMB and Response to Immunotherapy

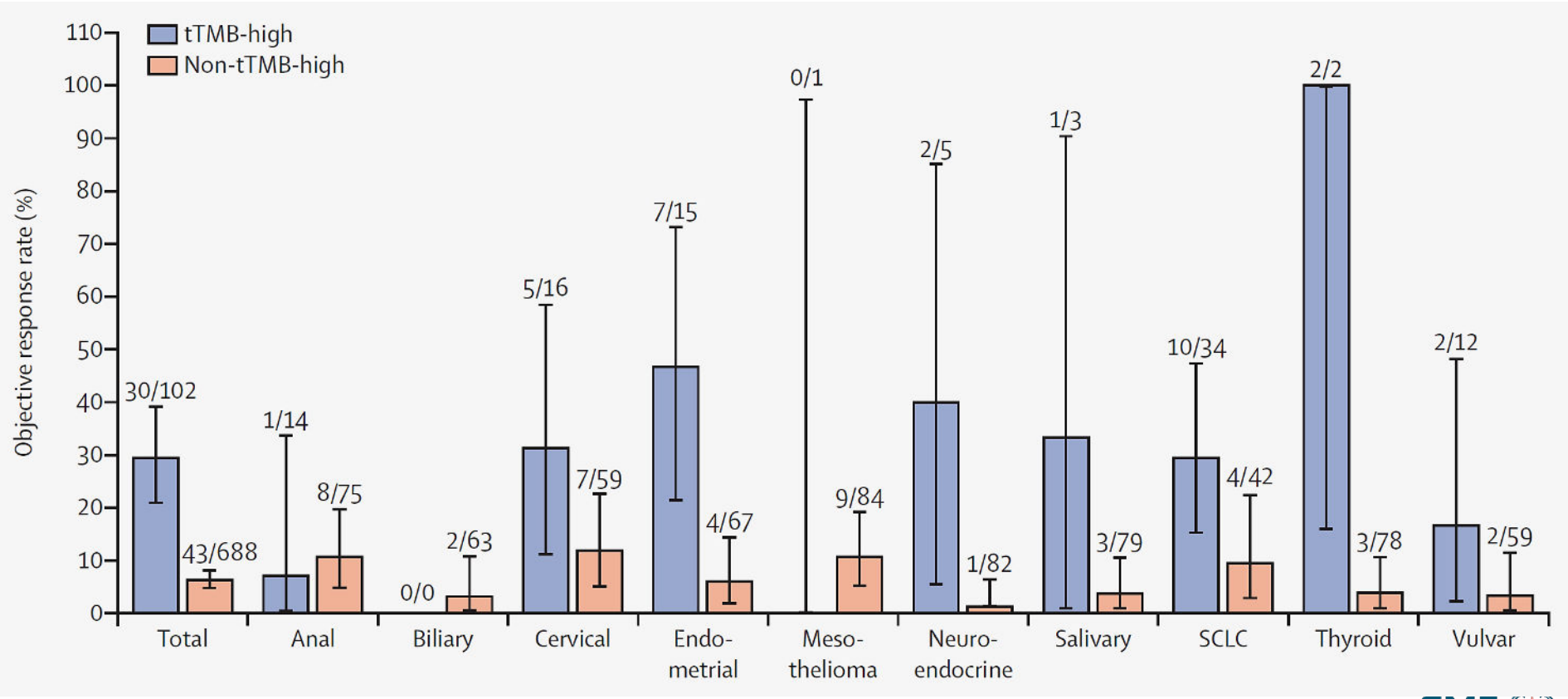
- Correlation between tumor mutational burden and ORR with anti-PD-1 or anti-PD-L1 therapy in 27 tumor types
  - Significant correlation between TMB and the ORR (P<0.001)
  - Correlation coefficient = 0.74
    - Suggests that 55% of the differences in the ORR across cancer types may be explained by the tumor mutational burden



Yarchoan M, et al. *N Engl J Med.* 2017;377(25):2500-2501.

CME OUTFITTERS

Keynote 158: ORR in Tumors with TMB > 10 mut/mb



Marabelle A, et al. *Lancet Oncol*. 2020;21(10):1353-1365

CME OUTFITTERS

Comparison of Select TMB Assays

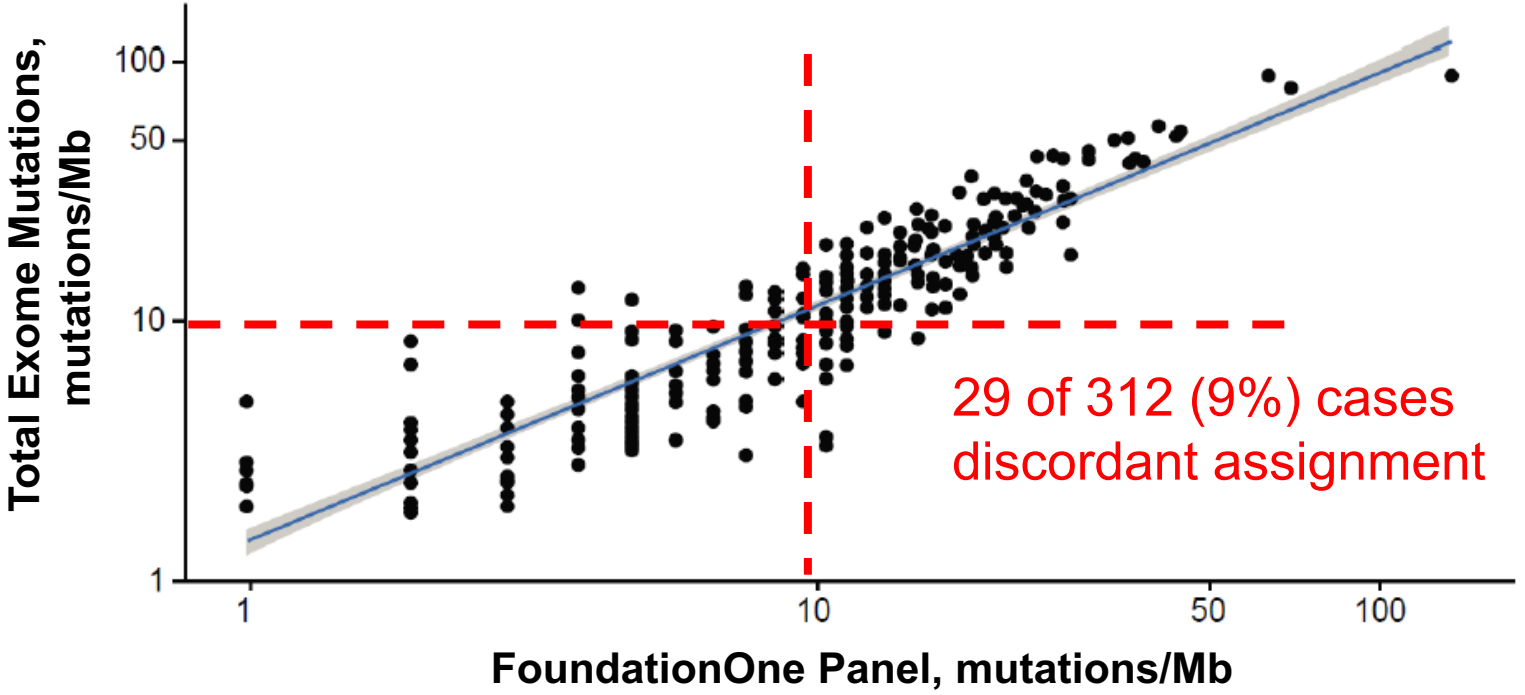
	Whole Exome	Foundation NGS	MSKCC NGS
No. of genes	~22,000	324 cancer-related genes	468 cancer-related genes
Coverage	~30 Mb	0.8 Mb	1.22 Mb
Types of mutations	Coding missense mutations	Coding, missense, and indel mutations per Mb	Coding missense mutation per Mb
Germline mutations	Subtracted using germline DNA	Estimated bioinformatically and subtracted	Subtracted using matched blood
TMB definition	No. of somatic, missense mutations in the tumor genome	No. of somatic, coding mutations (synonymous and nonsynonymous), short indels per Mb of tumor genome	No. of somatic, missense mutations per Mb of tumor genome
Turn around time	At least 4-6 weeks	2 weeks	2 weeks

Rizvi NA. https://www.nationalacademies.org/. Accessed March 5, 2023.

CME OUTFITTERS

Effect of Using Different TMB Testing Methods from Checkmate 026

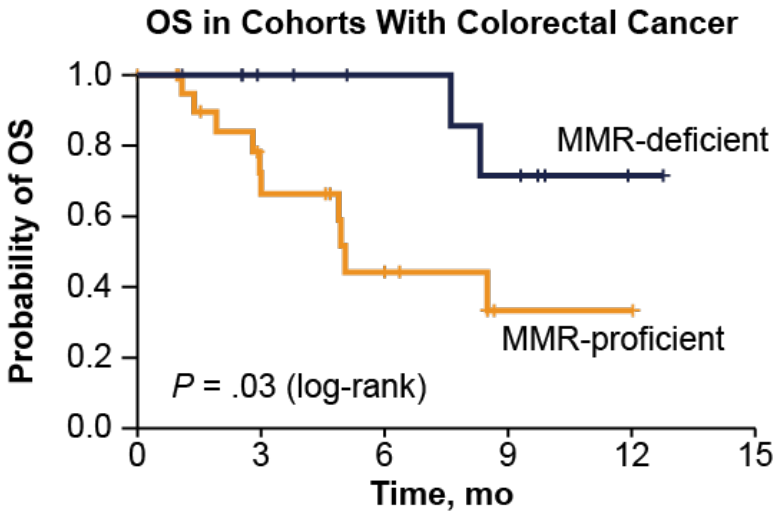
Total Exome Mutations vs Genes in FoundationOne Panel



Carbone DP, et al. *N Engl J Med.* 2017;376(25):2415-2426.

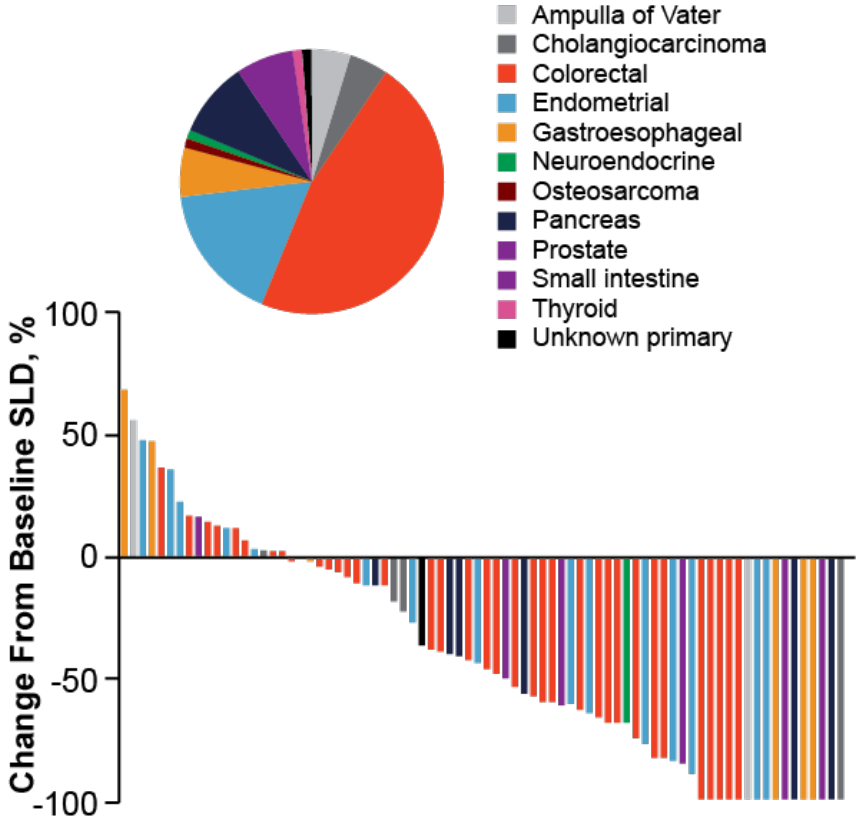
CME OUTFITTERS

Pan-Cancer Landscape of MMR Deficiency



No. at Risk

MMR-deficient	11	9	7	5	1	0
MMR-proficient	21	12	5	1	1	0

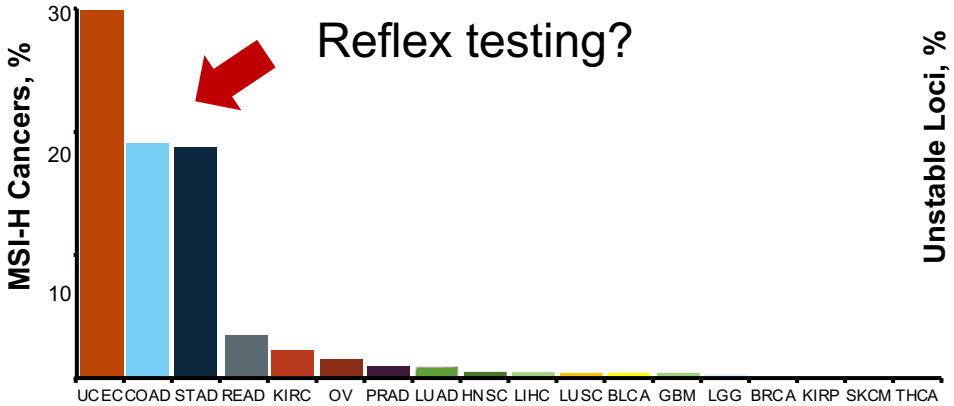


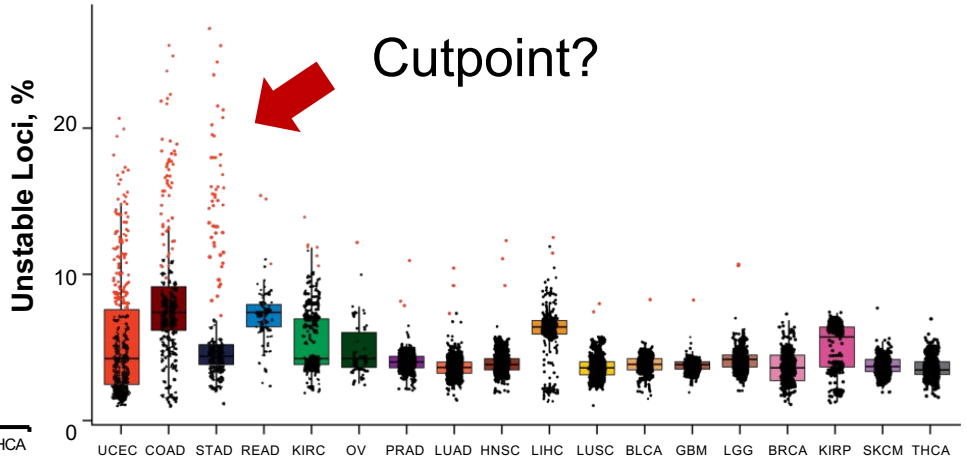
Le DT, et al. *N Engl J Med*. 2015;372(26):2509-2520.

CME OUTFITTERS

Classification and Characterization of MSI Across 18 Cancer Types

- 5,930 cancer exomes
- 18 cancer types
- >200,000 microsatellite loci





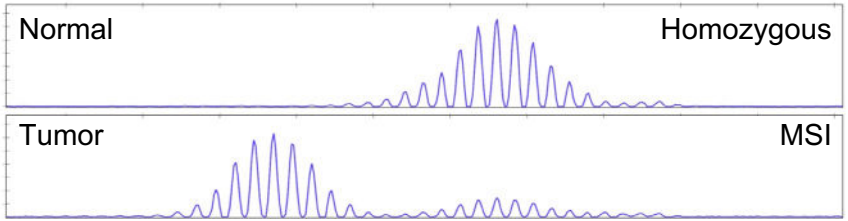
(A) Inferred proportion of MSI-H tumors identified for each cancer cohort. (B) Distributions of the overall percentages of unstable microsatellite loci identified for each cancer type. Overlaid points represent the number of unstable loci detected in individual tumor specimens; data for tumors classified as MSI-H are shown in red. UCEC, uterine corpus endometrial carcinoma (n = 437); COAD, colon adenocarcinoma (n = 294); STAD, stomach adenocarcinoma (n = 278); READ, rectal adenocarcinoma (n = 96); KIRC, kidney renal clear cell carcinoma (n = 279); OV, ovarian serous cystadenocarcinoma (n = 63); PRAD, prostate adenocarcinoma (n = 463); LUAD, lung adenocarcinoma (n = 480); HNSC, head and neck squamous cell carcinoma (n = 506); LIHC, liver hepatocellular carcinoma (n = 338); LUSC, lung squamous cell carcinoma (n = 443); BLCA, bladder urothelial carcinoma (n = 253); GBM, glioblastoma multiforme (n = 262); LGG, brain lower grade glioma (n = 513); BRCA, breast invasive carcinoma (n = 266); KIRP, kidney renal papillary cell carcinoma (n = 207); SKCM, skin cutaneous melanoma (n = 268); THCA, thyroid carcinoma (n = 484).

Hause RJ, et al. *Nat Med.* 2016;22(11):1342-1350.

CME OUTFITTERS

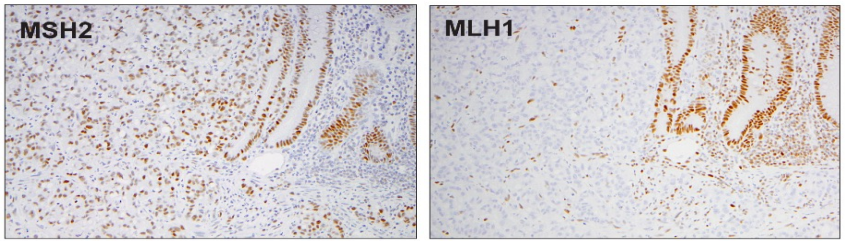
Testing for Mismatch Repair Deficiency

PCR for MSI



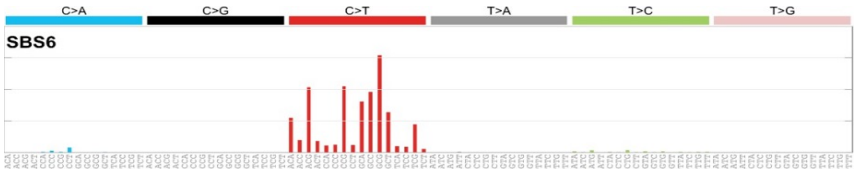
- > 20-30 ng DNA
- Requires tumor and paired normal sample
- 4-10 x 5-µm sections
- > 25%-30% tumor cells
- Not always locally available

IHC for MMR proteins



- No specific DNA requirement
- Only tumor biopsy/tissue
- 4 x 5-µm sections
- Any tumor cell content
- Widely available

Signature by NGS



- DNA requirements similar to MSI testing
- Can be tumor-only or paired tumor-normal
- Provides information beyond MMR status
- Longer TAT, expensive, not always locally available

Nowak JA, et al. *Surg Pathol Clin.* 2016;9(3):427-439.

CME OUTFITTERS

Summary

- Biomarkers can be used to support optimal cancer management
  - Different types and disease-specific context
- There are several ways to measure genomic biomarkers
  - ie, Sanger sequencing, qPCR, and next-generation sequencing
- Multi-gene panels are preferred over single-gene testing
- Immune checkpoint inhibitors block cancer-mediated immune tolerance
  - Different ICIs are supported by different PD-1 assays and scoring systems
- Biomarker tests vary
  - TMB assays can have significant differences
  - MSI is preferentially tested in tumor types with high prevalence using IHC/PCR

CME OUTFITTERS

SMART Goals

Specific, Measurable, Attainable, Relevant, Timely

- Follow guideline recommendations for biomarker testing
- Utilize multigene panels where feasible for genomic biomarker testing
- Recognize the benefits and limitations of the testing method(s) that you employ
- Understand the implications of the test results
  - Pathogenic vs non-pathogenic mutation
  - Actionable vs nonactionable mutation
  - Tumor staining vs stromal staining
  - Requirement of testing for a given therapy

CME OUTFITTERS

To Receive Credit

To receive CME/CE credit for this activity, participants must complete the post-test and evaluation online.

Participants will be able to download and print their certificate immediately upon completion.

CME OUTFITTERS

Oncology Hub

Free resources and education to educate health care providers and patients on oncology **https://www.cmeoutfitters.com/oncology-education-hub/**

Diversity and Inclusion Hub

Free resources and education to educate health care providers and patients on health-related inequities
https://www.cmeoutfitters.com/diversity-and-inclusion-hub/