Tumor mutational burden and microsatellite instability testing

Molecular Diagnostics: Current Roles in Cancer Diagnosis and Patient Management

JONATHAN NOWAK, MD PhD
Associate Pathologist, Brigham and Women's Hospital
Assistant Professor, Harvard Medical School

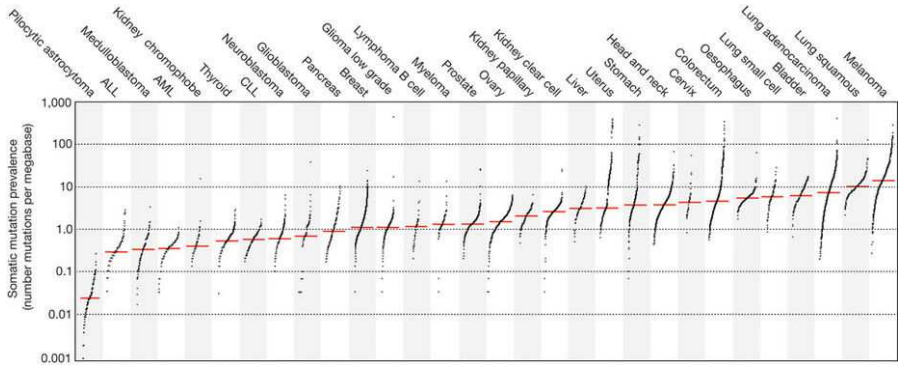
Mass General Brigham

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Outline

1. Why do tumors accumulate the mutations that they do?
2. Microsatellite instability / mismatch repair deficiency as a clinically actionable mutational signature
3. Tumor mutational burden as a clinically actionable measure of tumor immunogenicity

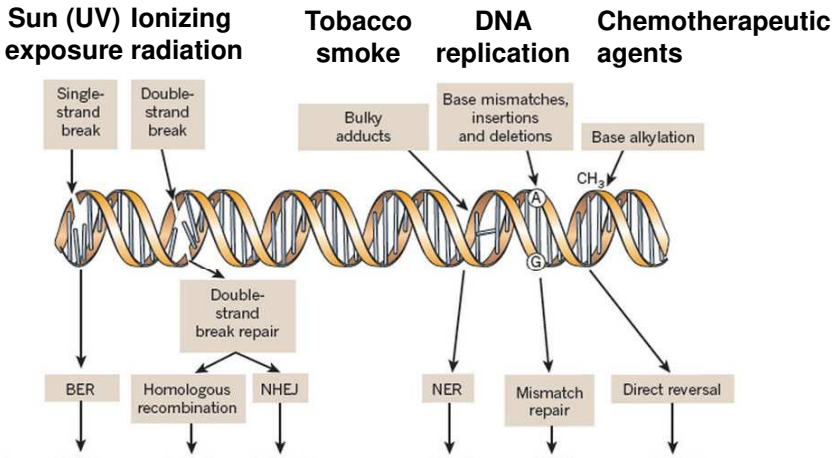
Mutational burden varies widely across tumor types



Why?

Nature 500:415-421. 2013.

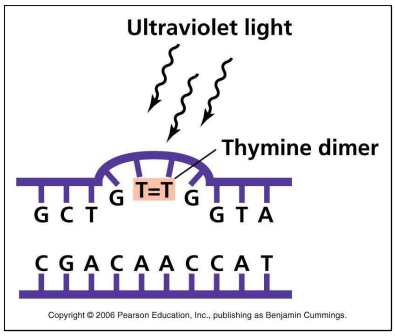
Distinct mutational processes contribute to the overall burden of mutations



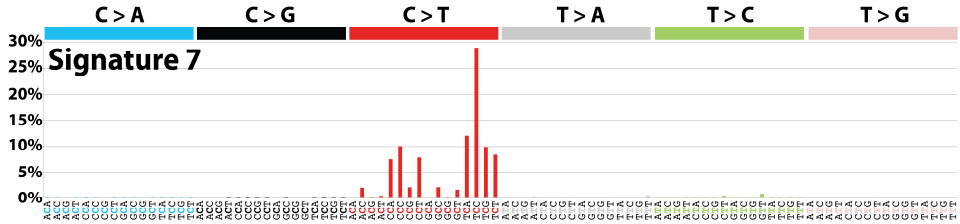
An imbalance between a mutational process and the pathway that corrects it can result in an accumulation of mutations that can be perceived as a mutational signature

Lord CJ and Ashworth A. The DNA damage response and cancer therapy. Nature 2012;481:287-294.

UV light exposure as an example mutational process

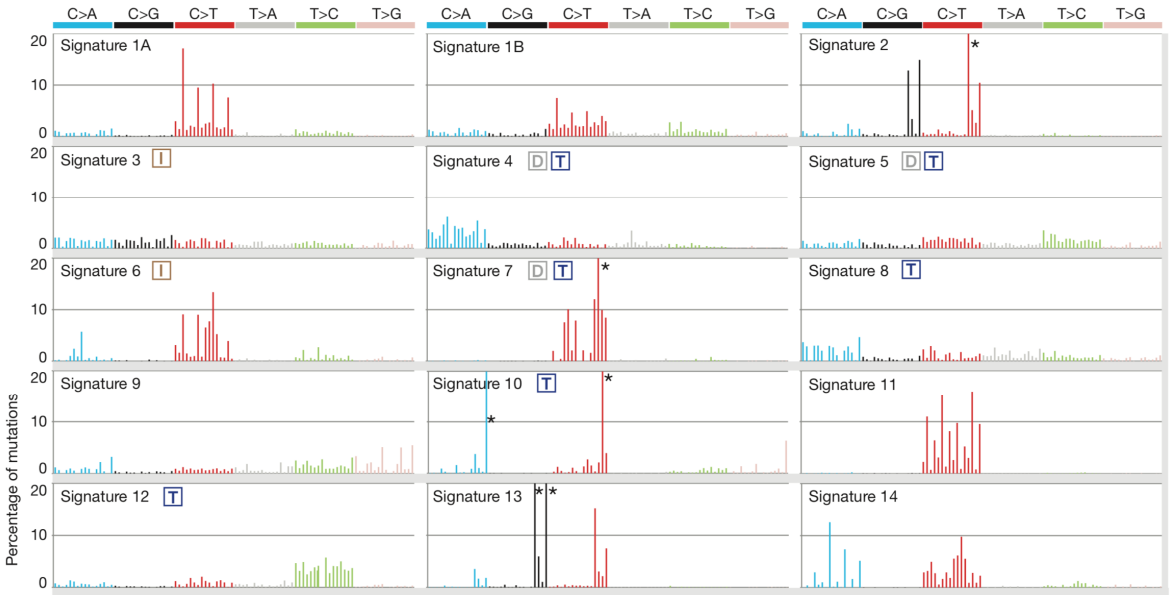


- **Associated with large numbers of CC>TT dinucleotide mutations at dipyrimidines**
- **Predominantly found in skin cancers and in cancers of the lip categorized as head and neck or oral squamous cancers**
- **Based on its prevalence in ultraviolet exposed areas and the similarity of the mutational pattern to that observed in experimental systems exposed to ultraviolet light, Signature 7 is likely due to ultraviolet light exposure**



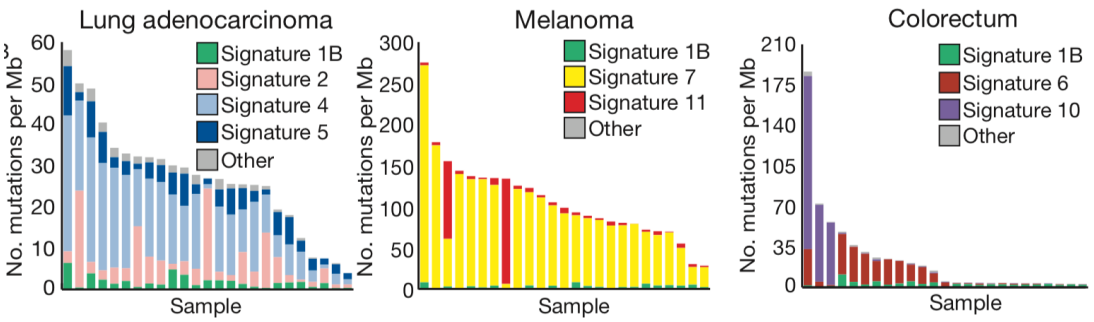
Alexandrov LB, et al. Signatures of mutational processes in human cancer. Nature 2017;500:415-421.
http://cancer.sanger.ac.uk/cosmic/signatures

Different mutational processes exhibit distinct signatures



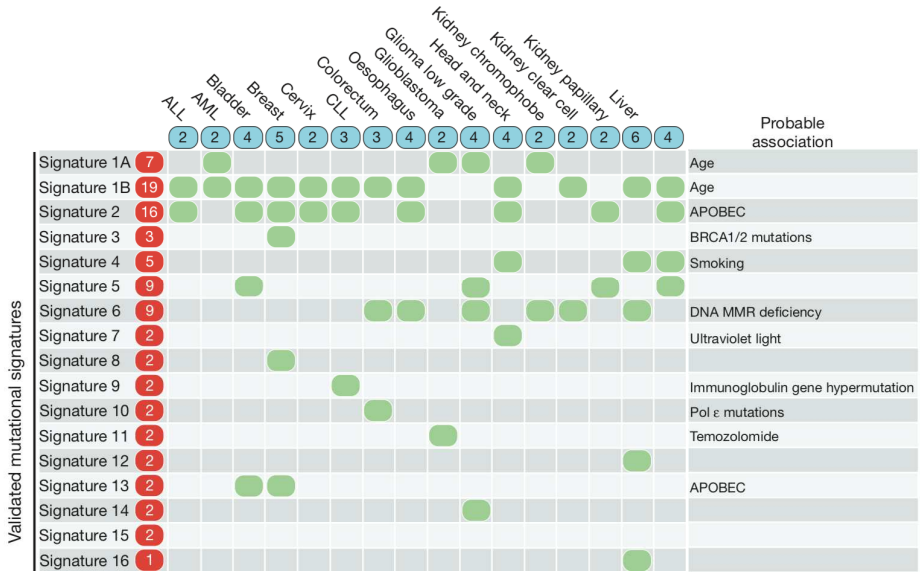
Alexandrov LB, et al. Signatures of mutational processes in human cancer. Nature 2017;500:415-421.

Any given tumor or tumor type can harbor multiple signatures



Alexandrov LB, et al. Signatures of mutational processes in human cancer. Nature 2017;500:415-421.

Not all mutational signatures have recognized causes



Alexandrov LB, et al. Signatures of mutational processes in human cancer. Nature 2017;500:415-421.

What is the value in detecting mutational signatures?

- Diagnosis: Since some mutational signatures are specific for environmental exposures, they can aid in classifying the probable anatomic site of origin for a tumor
    - A squamous cell carcinoma in the lung that exhibits a UV signature may represent metastasis from a sun-exposed cutaneous primary tumor
- Prognosis: Some mutational processes are associated with better outcomes
    - *POLE* mutant tumors in both the colon and endometrium have better prognoses compared to *POLE* wild-type tumors
- Therapeutic response: Some mutational processes indicate that a tumor may be differentially sensitive to a particular therapy (either targeted OR non-targeted)
    - Colon cancer with an MMR deficiency signature responds poorly to 5-FU based chemotherapy but responds well to immune checkpoint inhibition

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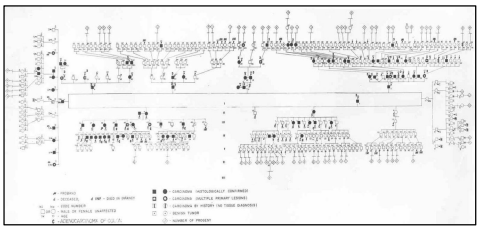
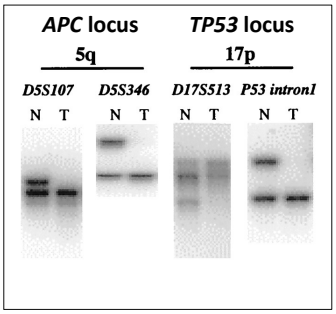
Lynch syndrome: From description to mechanism

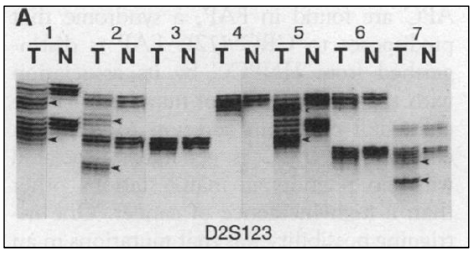
Warthin's Family G: Colon, uterus, stomach cancer



Loss of heterozygosity (LOH) at CRC-associated tumor suppressor loci



Classical linkage studies on familial CRC clusters search for LOH in order to identify a novel tumor suppressor gene…



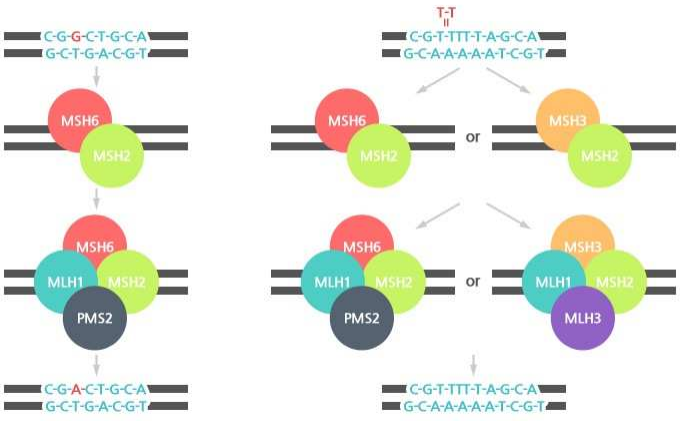
Thibodeau / Peltomaki / Aaltonen

…and instead discover microsatellite instability.

Yashiro M, et al. Cancer Res 2001. 61(6):2676-2683.
Boland, CR, Lynch, HT. The history of Lynch syndrome. Fam Cancer. 12(2):145-157. 2013.

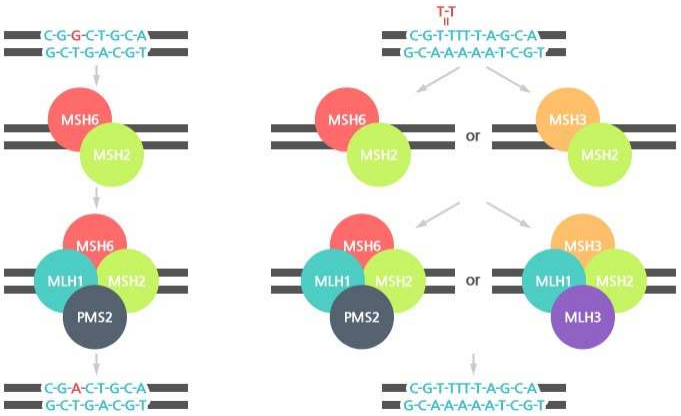
The mismatch repair system targets two types of DNA replication errors

Functional MMR system



Adapted from http://www.edgc.com/riskcare/lynch-syndrome-hnpcc

The mismatch repair system targets two types of DNA replication errors



Functional MMR system

Deficient MMR system

Increased number of single nucleotide variants (SNVs)

Increased number of insertions/deletions (indels), often in repeat regions

Adapted from http://www.edgc.com/riskcare/lynch-syndrome-hnpcc

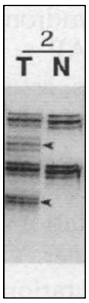
Evaluation of MMR status by interrogation of microsatellite length ("MSI testing") via PCR

- Microsatellite are short, repeated sequences of DNA

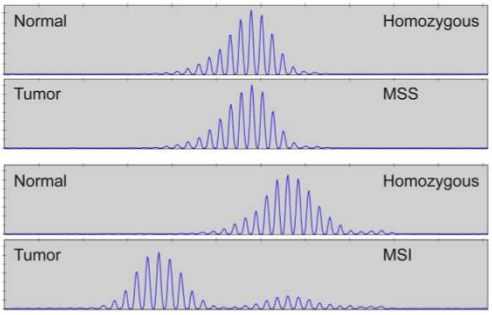
Microsatellite	Repeat
BAT-25	A_{25}
BAT-26	A_{26}
NR-21	A_{21}
NR-24	A_{24}
MONO-27	A_{27}

- Repeat units are 1-6 base pairs in length
- Prone to slippage during DNA replication
- MMR pathway is typically robust enough to efficiently repair errors before they are further replicated
- Defective MMR causes microsatellite instability (MSI)

Gel electrophoresis

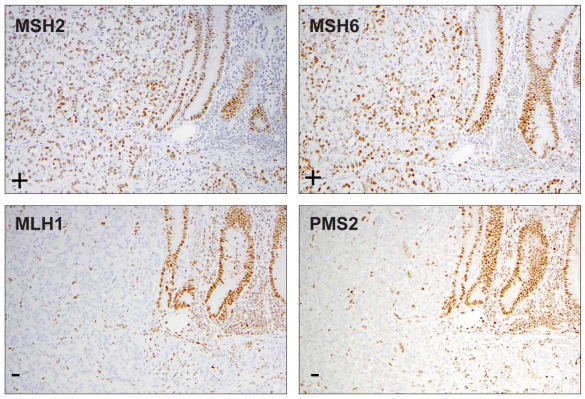


Capillary electrophoresis



Nowak JA, Hornick JL. Surg Path Clin 2016;9(3):427-439.

Evaluation of MMR status by IHC for MMR protein expression



MLH1	PMS2	MSH2	MSH6	In CRC, pattern suggests…
+	+	+	+	Intact MMR pathway, rare germline point mutations or other gene mutations
-	-	+	+	Somatic *MLH1* promoter methylation or, rarely, *MLH1* germline mutation
+	+	-	-	*MSH2* germline mutation
+	-	+	+	*PMS2* germline mutation
+	+	+	-	*MSH6* germline mutation

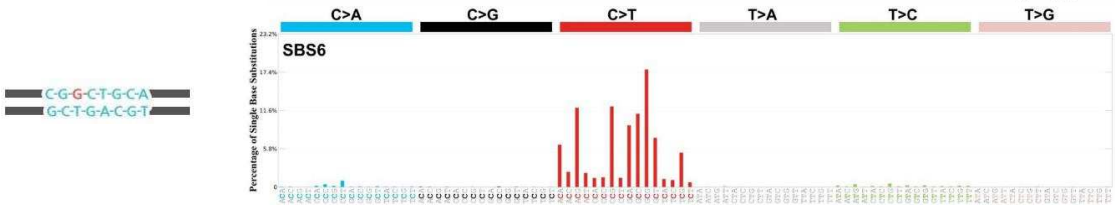
Lack of expression of one or more MMR proteins is a very good surrogate test for MSI

Nowak JA, Hornick JL. Surg Path Clin 2016;9(3):427-439.

NGS approaches to detecting MMR deficiency

Microsatellite evaluation by NGS

SNV / missense mutation evaluation by NGS

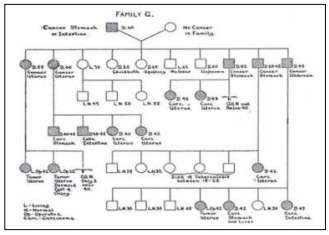
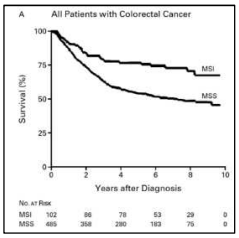
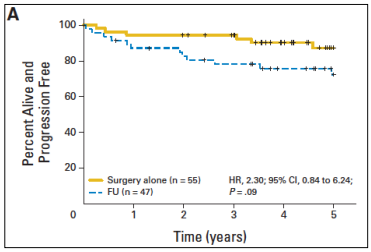
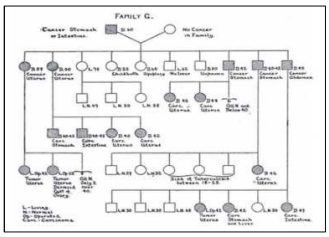
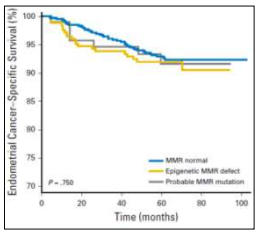


Cortes-Ciriano I, et al. Nat Comm. 2017;8:15180.
COSMIC Mutational Signatures v3.2 (March 2021)

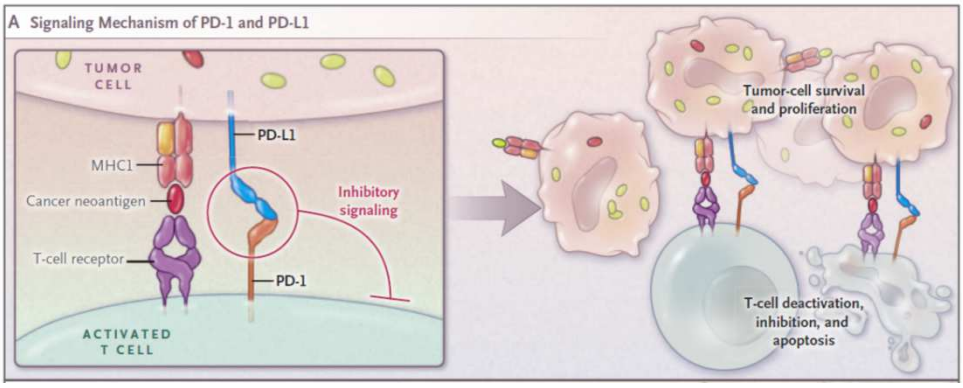
Terminology

- Tumors may have either a proficient or deficient mismatch repair system
- "Microsatellite instability" (MSI) status is a consequence of MMR deficiency
  - Microsatellite instability high (MSI-H) → MMR deficient
  - Microsatellite stable (MSS) → MMR proficient
- MMR protein expression is a correlate of MMR system status
  - Absent expression → MMR deficient
  - Retained expression → MMR proficient

Historical indications for MMR testing

	Diagnostic (Lynch syndrome)	Prognostic	Predictive
Colorectal cancer	 LS patients are often missed by other screening strategies	 Patients with MSI-H tumors have better overall survival	 Relevant for adjuvant therapy decisions in stage II and III cancers
Endometrial cancer			

A much broader indication for MMR testing…



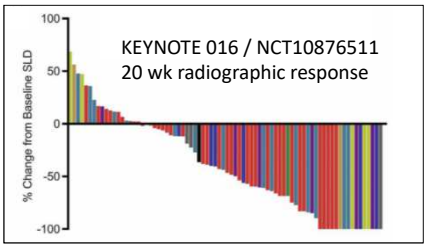
Lemery S, et al. NEJM. 2017;377(15)-1409-1412.

FDA approval for pembrolizumab in MMR-deficient solid tumors

- Data from 149 patients with MSI-H or MMR-D cancer across 5 clinical trials
- 90 patients had CRC, remainder had one of 14 other tumor types
- Patients identified using MMR IHC (n=47), MSI PCR (n=60), or both tests (n=42)
- Most patients had received two or more therapies for metastatic or unresectable disease
- Overall response rate 39.6% (CI 31.7-47.9%)
- Responses lasted ≥ 6 mos in 78% of patients that had a response
- 11 CRs and PRs

Pembrolizumab Response Rate by Tumor Type.*

Tumor Type	No. of Tumors	Patients with a Response	Range of Response Duration
		no. (%)	*mo*
Colorectal cancer	90	32 (36)	1.6+ to 22.7+
Endometrial cancer	14	5 (36)	4.2+ to 17.3+
Biliary cancer	11	3 (27)	11.6+ to 19.6+
Gastric or gastroesophageal junction	9	5 (56)	5.8+ to 22.1+
Pancreatic cancer	6	5 (83)	2.6+ to 9.2+
Small-intestine cancer	8	3 (38)	1.9+ to 9.1+
Breast cancer	2	2 (100)	7.6 to 15.9
Prostate cancer	2	1 (50)	9.8+
Other cancers	7	3 (43)	7.5+ to 18.2+



Lemery S, et al. NEJM. 2017;377(15)-1409-1412., Le Dt, et al. Science. 2017;357:409-413.

MSI/MMR status as an approved biomarker for immune checkpoint inhibitor therapy

Pembrolizumab

- First-line treatment of patients with unresectable or metastatic MSI-H or dMMR CRC
- MSI-H or dMMR CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan
- Unresectable or metastatic MSI-H dMMR solid tumors that have progressed following prior treatment and in patients who have no satisfactory alternative treatment options

Nivolumab

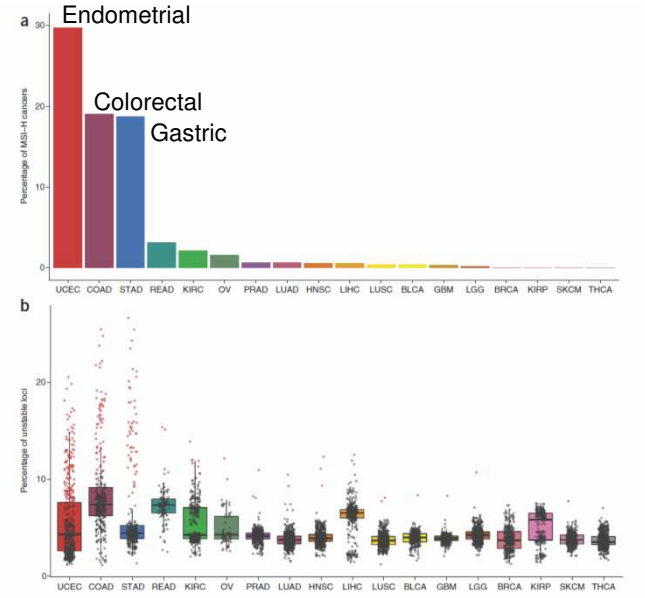
Nivolumab + Ipilimumab

- MSI-H or dMMR metastatic CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan

Rates of MMR deficiency across tumor types vary widely

Percentage of MSI-H tumors

Percentage of unstable loci



Hause RJ, et al. Nat Med. 2016;22(11):1342-1349

Outline

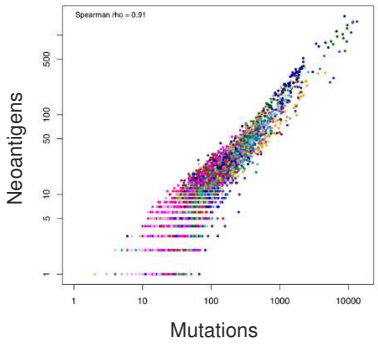
1. Why do tumors accumulate the mutations that they do?
2. Microsatellite instability / mismatch repair deficiency as a clinically actionable mutational signature
3. Tumor mutational burden as a clinically actionable measure of tumor immunogenicity

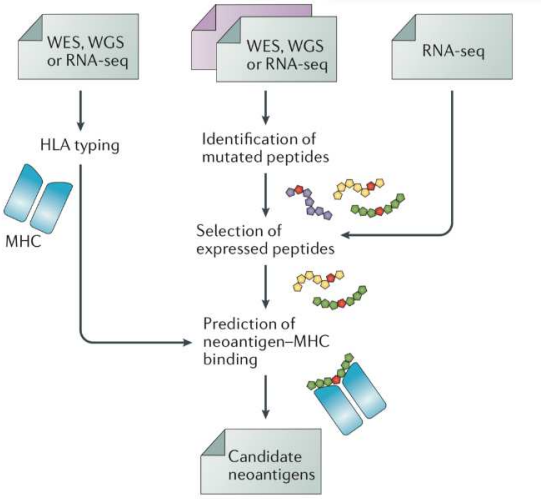
What is tumor mutational burden?

- Measurement of the number of mutations that exist within the genome of a tumor
- Generally considered to be the burden of somatic non-synonymous SNVs and small indels within exonic / coding regions
- Typically reported as mutations per megabase

TMB is a proxy for neoantigen burden

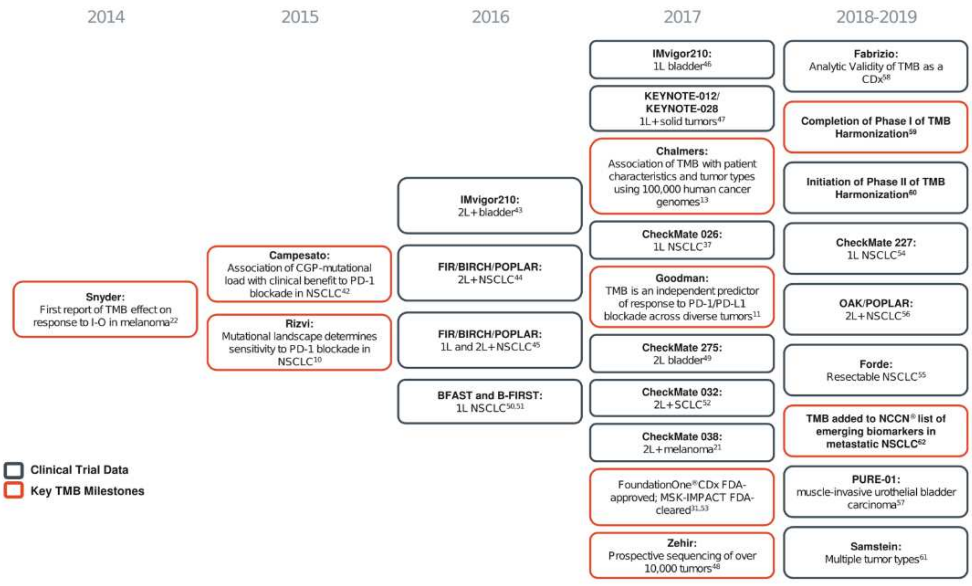
- Tumor DNA sequence data to identify mutations (TMB)
- Germline DNA sequence data for HLA typing





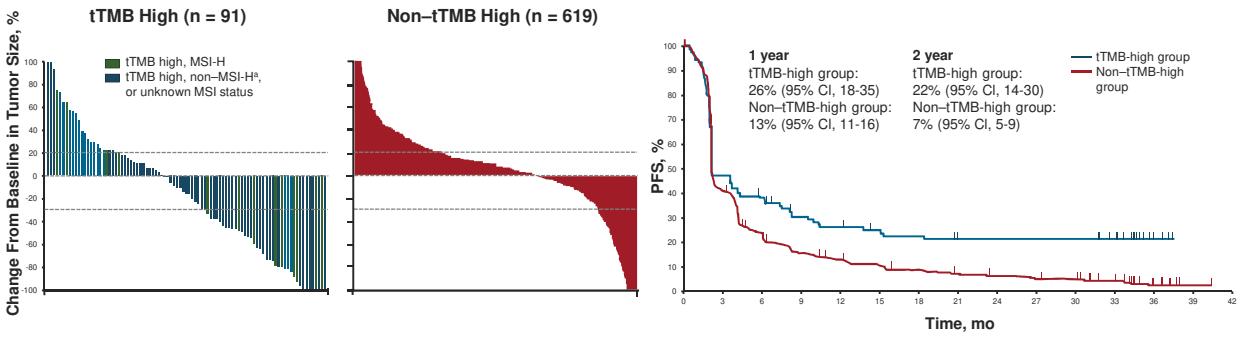
Hackl H et al. Nat Rev Genet. 2016;17:441-458.
Rooney MS et al. Cell 2015;160:48-61.

Timeline of TMB development as a biomarker



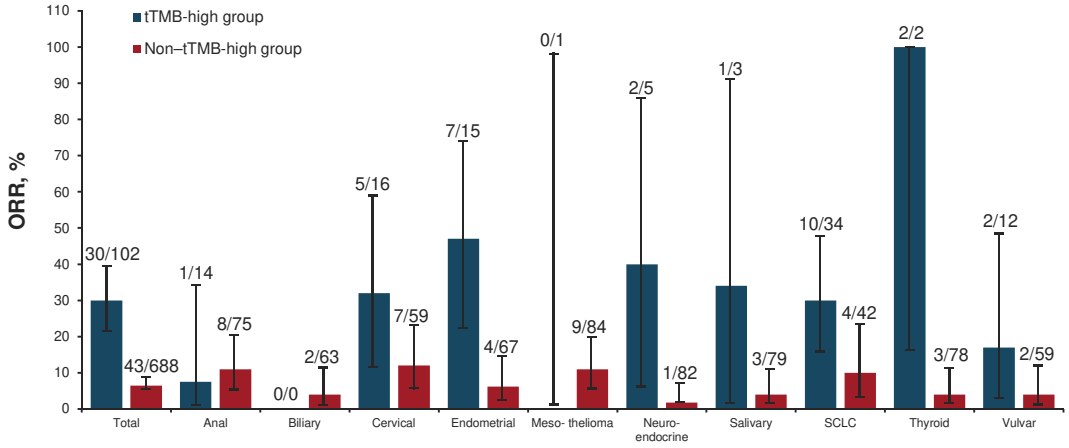
Klempner SJ et al. The Oncologist 2020;25:e147–e159.

KEYNOTE-158: TMB and Anti–PD-1 Therapy Across Solid Tumors



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

KEYNOTE-158: TMB and Anti–PD-1 Therapy Across Solid Tumors



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

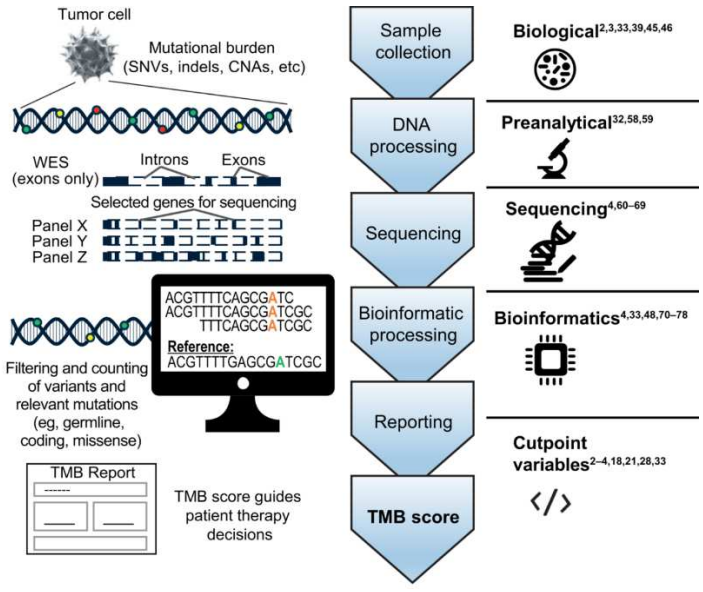
FDA Approval of Pembrolizumab Patients with TMB-H Solid Tumors

June 2020:

FDA granted accelerated approval to pembrolizumab 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, that have progressed following prior treatment and who have no satisfactory alternative treatment options

FDA also approved the FoundationOne CDx assay as a companion diagnostic for pembrolizumab

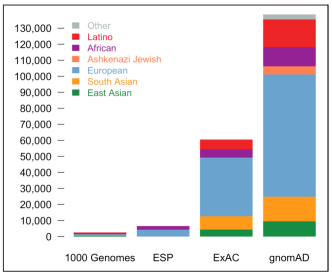
Factors that influence TMB calculation and reporting



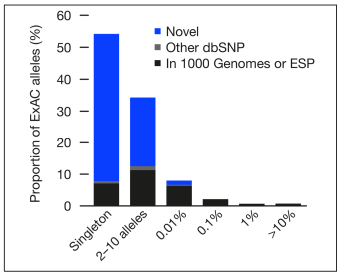
Stenzinger A et al. Genes Chromosomes Cancer. 2019;58:578–588.

Challenges in germline variant removal using population allele frequencies

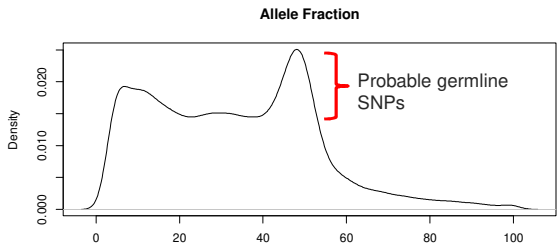
Population distribution of major germline genomic datasets



Allele frequency spectrum of variants in ExAC



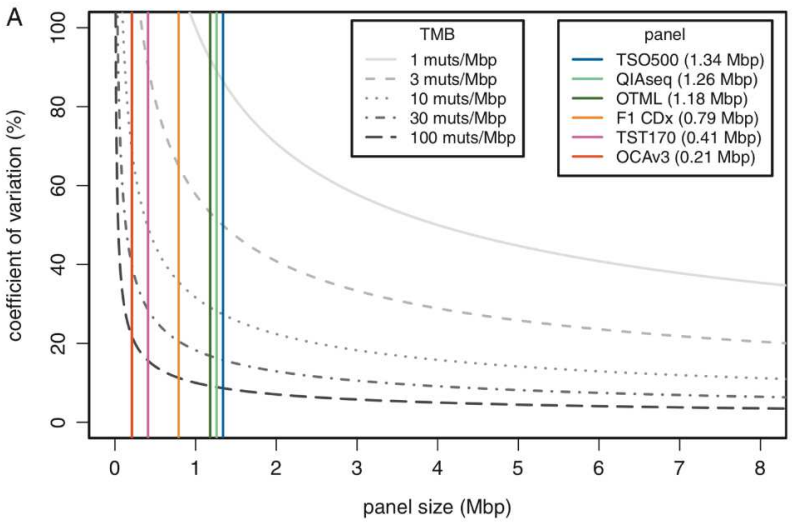
4576 BWH OncoPanel cases
64K variants post-gnomAD filtering



- Ethnic populations are unevenly represented in major germline genomic datasets
- Most genetic variants in ExAC/gnomAD are rare and novel
- Can't filter out a variant not in a database

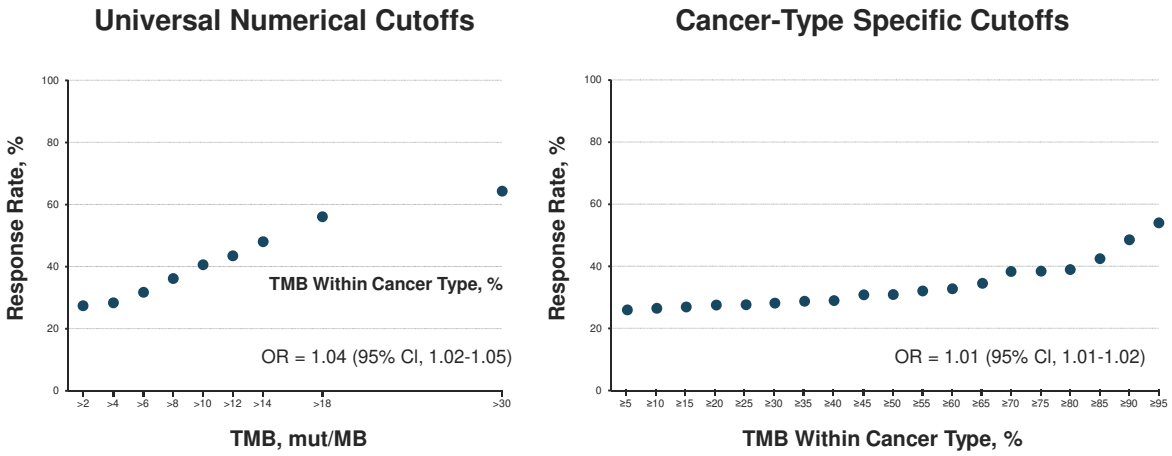
Nature 536:285-291. 2016.
DFCI Knowledge Systems Group

CV of TMB measurement as a function of panel size



Stenzinger A et al. Genes Chromosomes Cancer. 2019;58:578–588.

Universal or Cancer-Specific Cutoffs for TMB

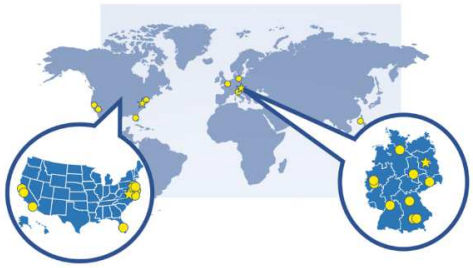


Nat Genet. 2019 Feb;51(2):202-206.

Friends of Cancer Research and QuIP standardization and harmonization initiatives

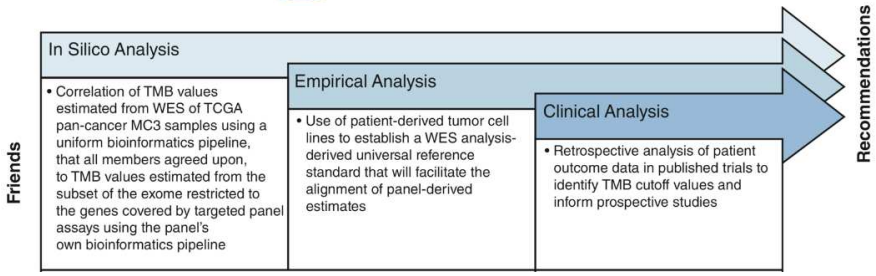
FRIENDS of CANCER RESEARCH

Patient Advocacy Organization
Washington, D.C.



QuIP Quality in Pathology

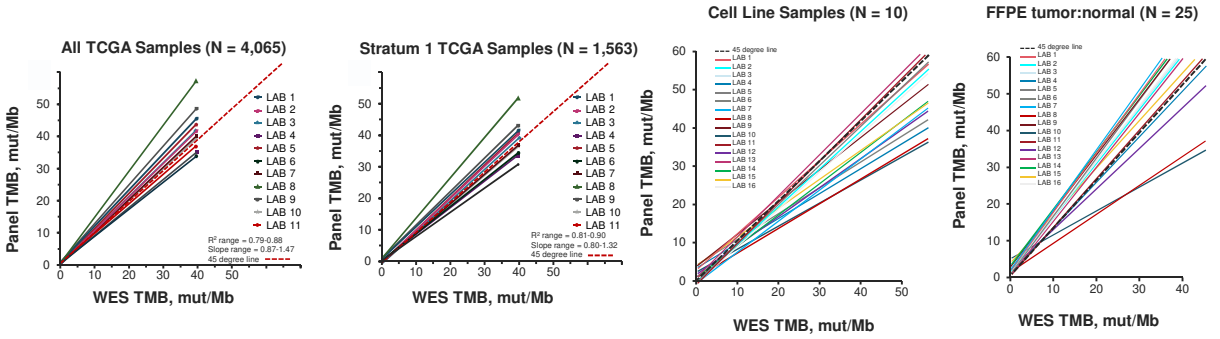
Quality Assessment Service for Pathology
Berlin, Germany



Genes Chromosomes Cancer. 2019;58:578–588.

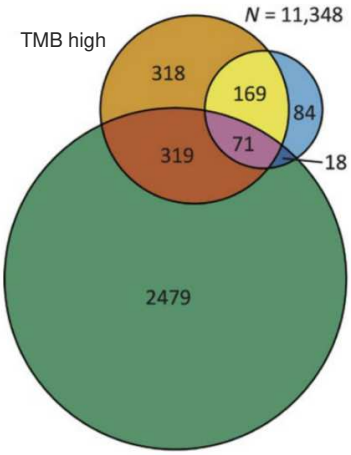
TMB Standardization Efforts

Friends of Cancer Research TMB Harmonization Project



TMB in the context of other biomarkers

- Correlation between TMB, MMR and PD-L1 expression across tumors
- 11,348 total cases across 26 tumor types
  - 3.0% MSI-H
  - 7.7% TMB high
  - 25.4% PD-L1 positive
- Only 0.6% of the cases were positive for all three markers
- 69.5% of the cases were pan-negative.



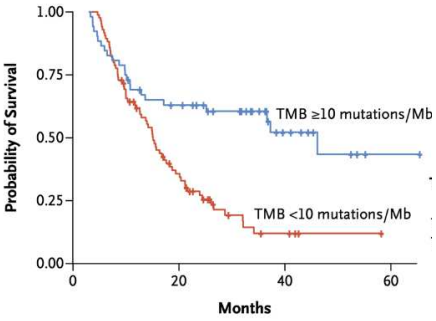
= High TMB
= MSI-H
= High PD-L1
= High TMB and MSI-H
= MSI-H and High PDL-1
= High TMB and High PD-L1
= High TMB, MSI-H, and High PD-L1

Vanderwalde A et al. Cancer Medicine. 2018;7(3):746–756.

TMB versus MMR status in colorectal cancer

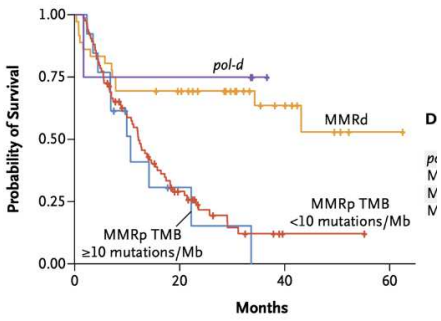
Overall survival for 137 CRC patients treated with immune checkpoint inhibition

Stratified by TMB



No. at Risk				
TMB ≥10 mutations/Mb	52	28	9	1
TMB <10 mutations/Mb	85	18	1	0

Stratified by TMB, MMR and *POLx* status

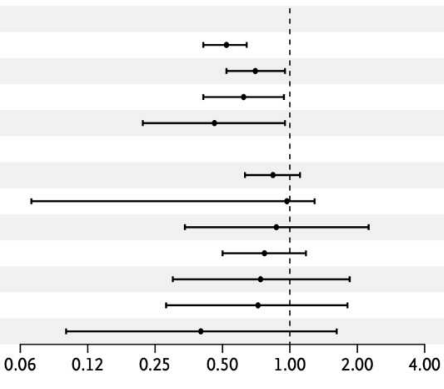


No. at Risk				
pol-d	4	3	0	0
MMRd	36	23	9	1
MMRp TMB <10 mutations/Mb	84	18	1	0
MMRp TMB ≥10 mutations/Mb	13	2	0	0

Rousseau B, et al. N Engl J Med 2021; 384:1168-1170.

Immune checkpoint inhibitor efficacy in MMR proficient tumors

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)



Rousseau B, et al. N Engl J Med 2021; 384:1168-1170.

Summary

1. Why do tumors accumulate the mutations that they do?

2. Microsatellite instability / mismatch repair deficiency as a clinically actionable mutational signature

3. Tumor mutational burden as a clinically actionable measure of tumor immunogenicity