Mutations, Biomarkers, and Pathways : A Molecular tour of Gastrointestinal Cancers

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Disclosures

- NONE

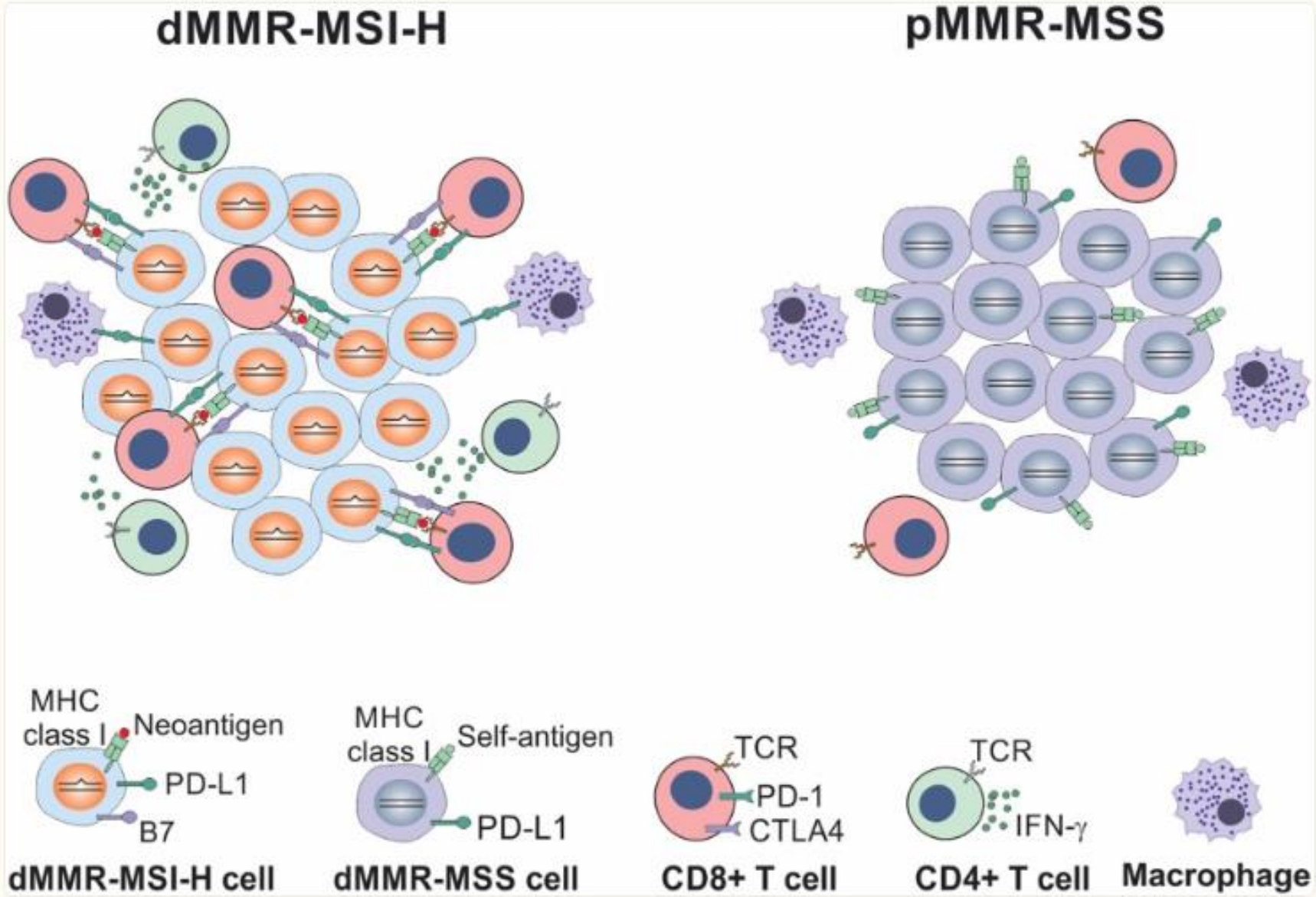
Topics for consideration

- Pan-Cancer biomarkers
- Established molecular targets for colon cancer and GEJ/stomach cancers
- Newer molecular approaches
  - ctDNA
  - Stool based DNA testing.

Pan-Cancer Biomarkers

- MMR
- TMB
- NTRK
- RET
- HER2
- BRAF

Fig. 2.



Lizardo DY, Kuang C, Hao S, Yu J, Huang Y, Zhang L. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: From bench to bedside. Biochim Biophys Acta Rev Cancer. 2020 Dec;1874(2):188447. PMID: 33035640.

Clinical trial	Design	*N*	Testing for MSI-H/dMMR	Regimen	Prior therapy
KN-016	• Investigator initiated • Prospective, single-arm • Colorectal cancer and non-colorectal cancer cohorts	28 colorectal cancer 30 non-colorectal cancer	Local PCR or IHC	10 mg/kg every 2 weeks	•–Colorectal cancer: ≥2 prior regimens •–Non-colorectal cancer: ≥1 prior regimen
KN-164	• Merck initiated • Prospective, single-arm • Patients with colorectal cancer	61	Local PCR or IHC	200 mg every 3 weeks	Prior FP, oxaliplatin, and irinotecan ± anti-VEGF/EGFR biologic
KN-012	• Merck initiated • Patients retrospectively identified as MSI-H/dMMR in a multicohort trial • PD-L1–positive cancers	6[a]	Central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KN-028	• Merck initiated • Patients retrospectively identified as MSI-H/dMMR in a multicohort trial • PD-L1–positive cancers	5[a]	Central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KN-158	• Merck initiated • Prospective cohort of patients MSI-H/dMMR non-colorectal cancer • Retrospective identification of MSI-H in patients with 1 of 10 rare tumor types	19[a]	Local PCR or IHC (central PCR for patients in rare tumor non-colorectal cancer cohorts)	200 mg every 3 weeks	≥1 prior regimen
Total		149			

Immunotherapy for MMRd/MSI tumors

- **Colon** - KEYNOTE-177 - Phase III trial pembrolizumab (anti-PD 1) vs standard chemotherapy in MSI-H/MMRd and TMB-high metastatic CRC. **Improvement in PFS and OS in pembrolizumab arm.**
- **Colon** - CheckMate 142 – Nivolumab (anti- PD1) +/- ipilimumab (anti-CTLA-4) in MSI-H/MMRd metastatic CRC. **Higher response rate with dual immunotherapy.**
- **Gastric/GEJ** - KEYNOTE-59 trial – Phase II RCT pembrolizumab in advanced gastric/GEJ cancers. **Improved OS with MSI-H/MMRd.**
- **Gastric/GEJ** - KEYNOTE- 061 trial – Phase III RCT pembrolizumab vs. paclitaxel in advanced gastric/GEJ cancers. **Improved OS with MSI-H/MMRd over chemo.**
- **Gastric/GEJ** - KEYNOTE -649 trial – Phase III RCT nivolumab + chemotherapy vs. chemo alone in advanced gastric, GEJ, and esophageal cancers. Nivolumab + chemo. **Showed improved PFS in MSI-H/MMRd gastric/GEJ cancers.**

TMB as pan-Cancer markers for Immunotherapy

- **KEYNOTE- 158 -** Phase II basket trial pembrolizumab (anti-PD 1) vs. standard of care for **MSI-H/MMRd and TMB-high advanced solid tumors.**
  - 34% response rate across all MSI-H/MMRd tumors
  - 29% response rate for TMB-H tumors.
- **Instrumental towards pan-cancer approval of pembrolizumab for TMB-H tumors.**
  - Lack of standardization for TMB assessment remains a concern.

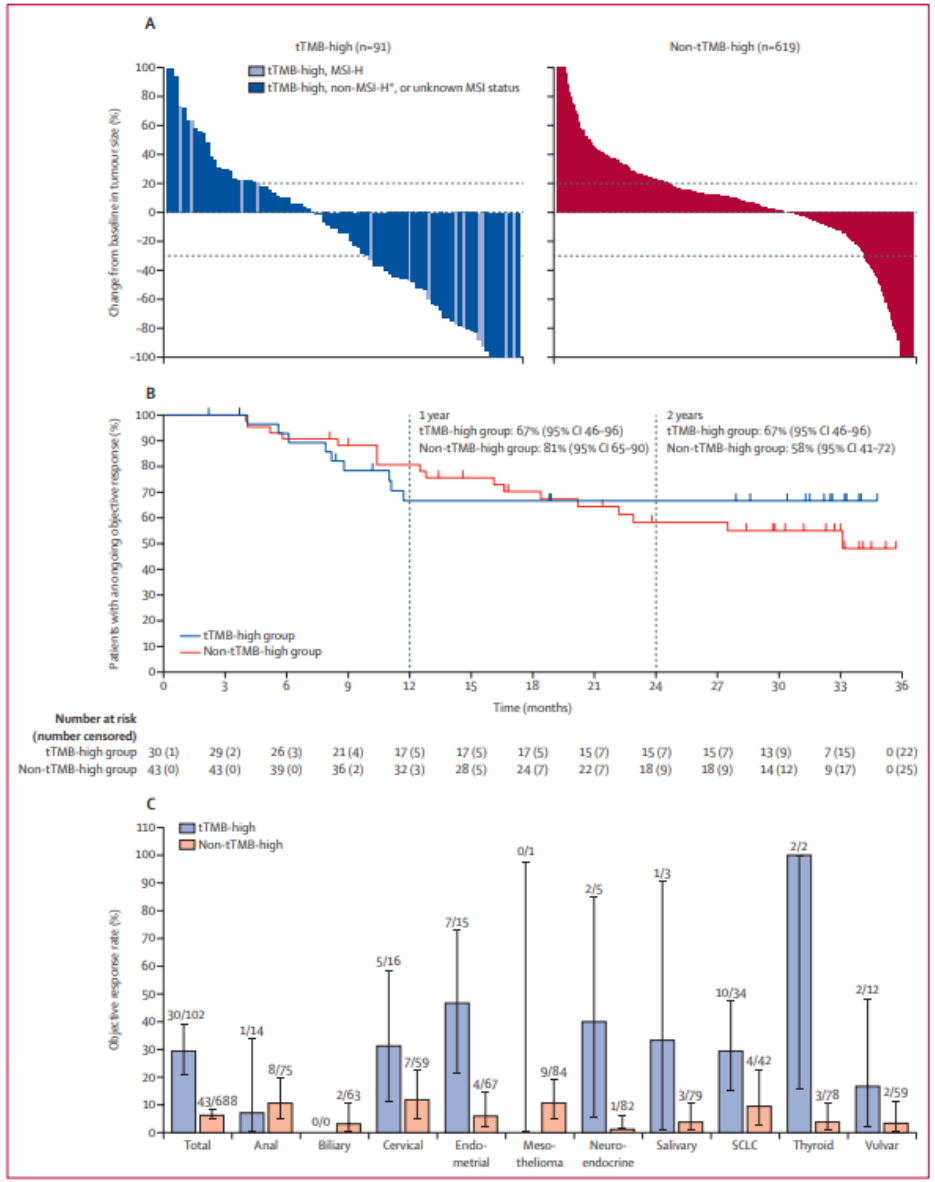
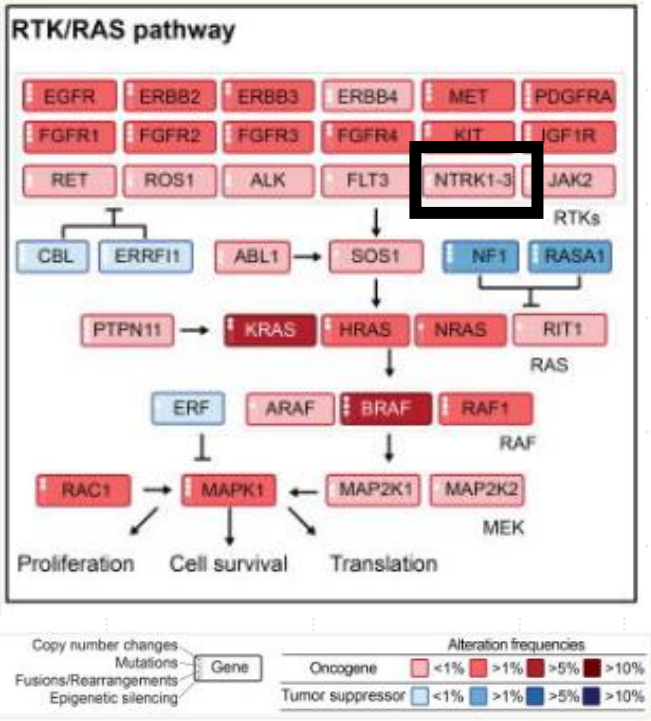


Figure 2: Responses as per RECIST version 1.1, assessed by independent central review, in the efficacy population

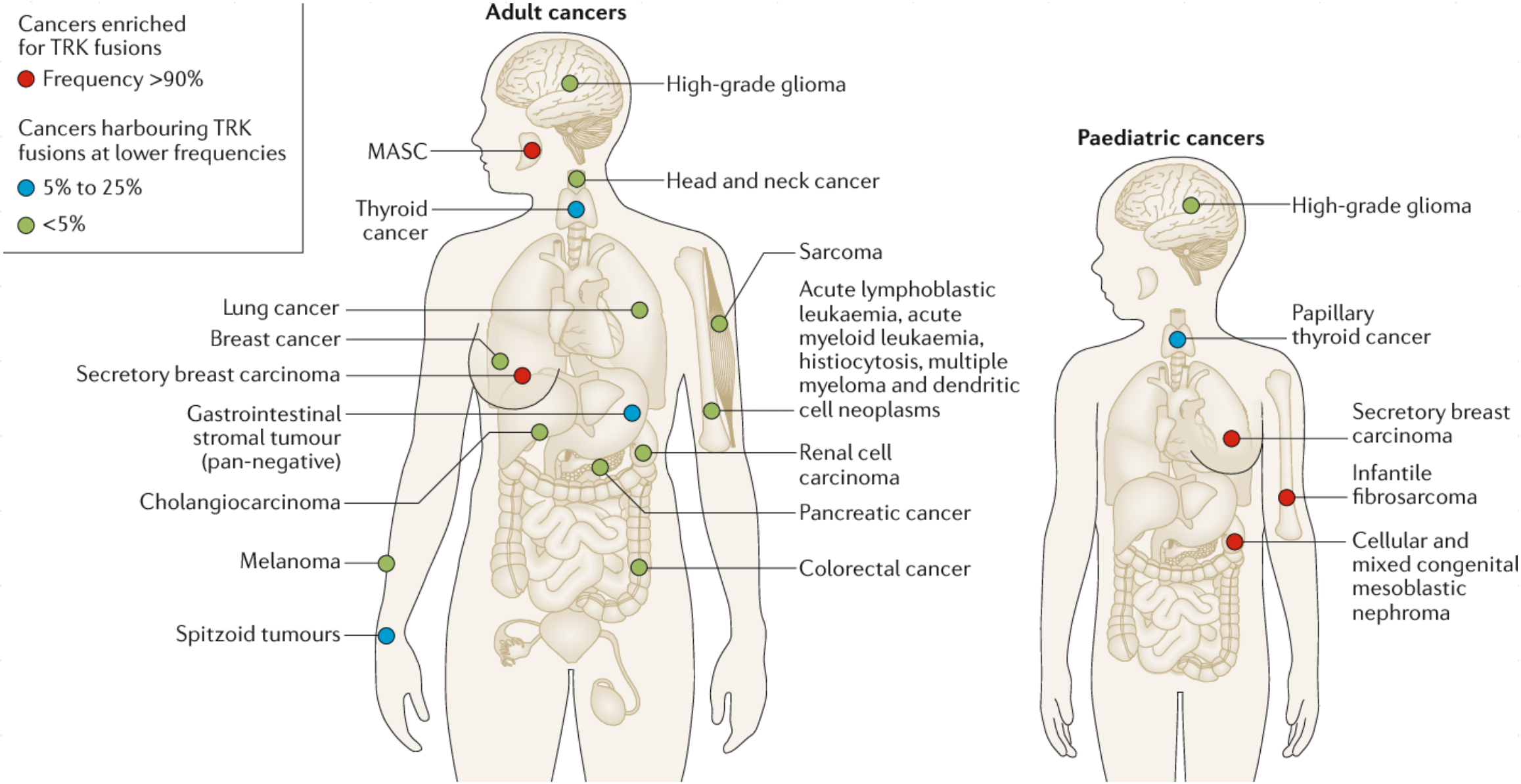
Marabelle, Aurélien et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. The Lancet Oncology, Volume 21, Issue 10, 1353 - 1365

NTRK fusions as pan-Cancer biomarker

- **Larotrectinib (FDA approved 2018)**
  - NAVIGATE – Phase II basket trial in patients with advanced NTRK fused solid tumors.
  - SCOUT – Phase I/II evaluation of pediatric patients with advanced NTRK fused solid tumors.
  - Phase I trial (NCT02122912) – Adult patients with advanced NTRK fused solid tumors.
  - Combined results – 55 patients- **75% response rate with 22% with CR.**
- **Entrectinib (FDA approved 2019)**
  - ALKA-372-001 – Phase I trial.
  - STARTRK -1 – Phase I trial.
  - STARTRK – 2 – Phase II basket trial – NTRK fused solid tumors.
  - Combined results – 54 patients – **57% response rate with 7% with CR.**



Sanchez-Vega F, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell. 2018 Apr 5;173(2):321-337.e10. PMID: 29625050



Cocco, E., Scaltriti, M. & Drilon, A. *NTRK* fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol* **15**, 731–747 (2018)

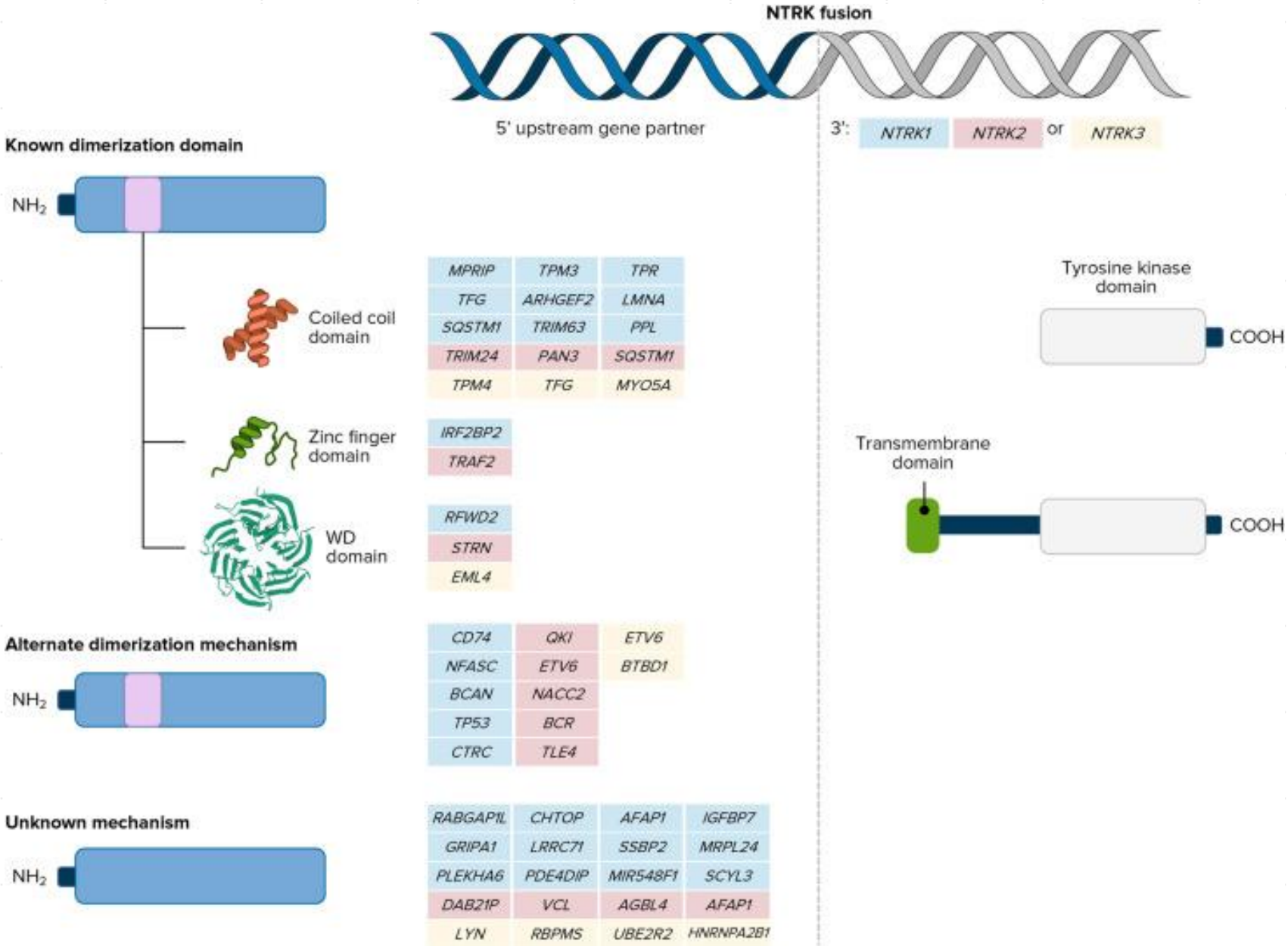
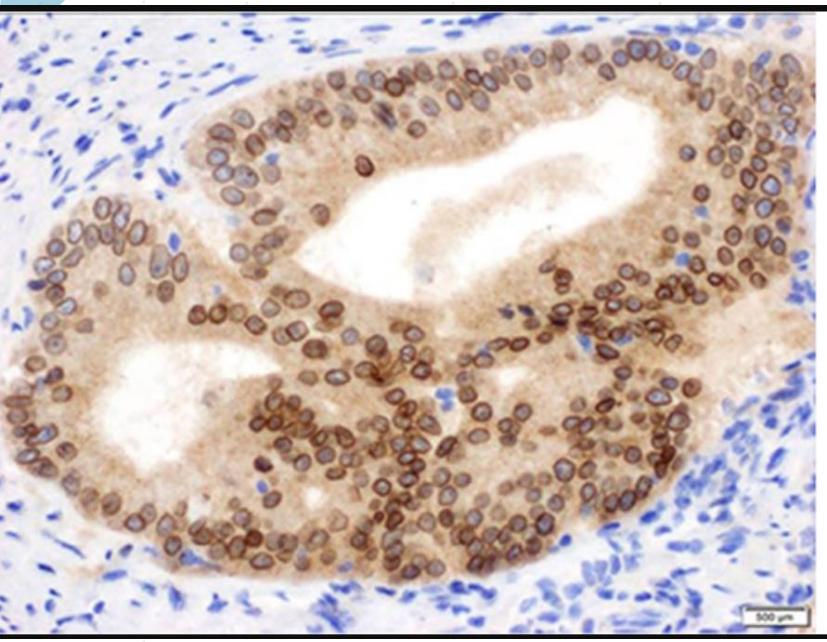
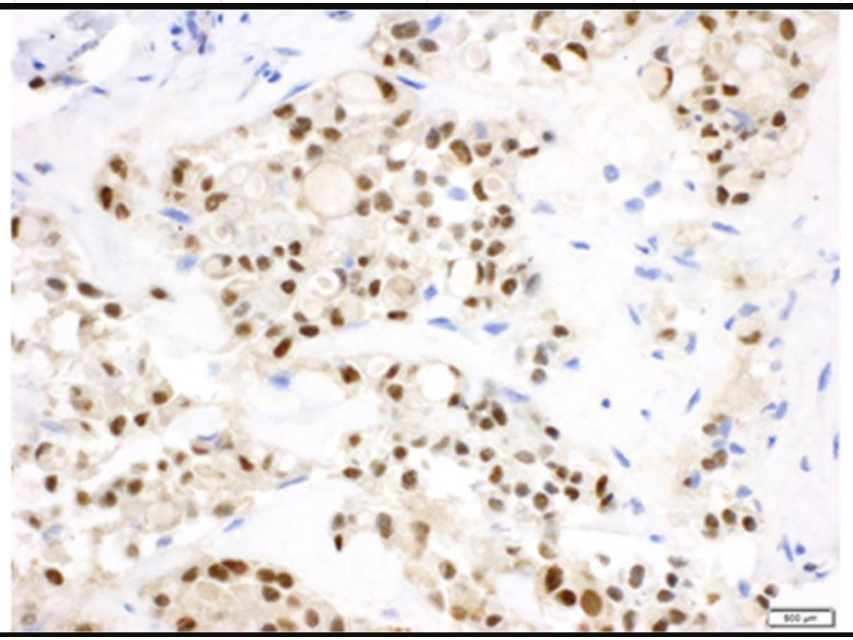


Figure 1: *NTRK* insights: best practices for pathologists. Hechtman, Jaclyn F. Modern Pathology, Volume 35, Issue 3, 298 - 305

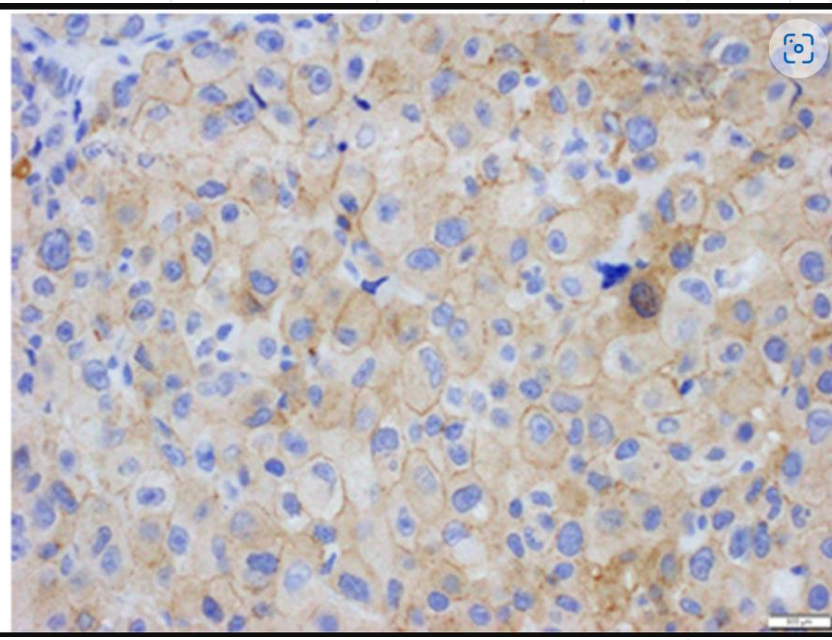
Pan-TRK IHC



LMNA::NTRK1 fusion



ETV6::NTRK3 fusion



TRAF2::NTRK2 fusion

Figure 2: NTRK insights: best practices for pathologists. Hechtman, Jaclyn F. Modern Pathology, Volume 35, Issue 3, 298 - 305

Method	Material required	Approximate turnaround time	Sensitivity	Specificity	Other considerations
IHC	1 unstained slide	1 day	· 96.2% for *NTRK1* · 100% for *NTRK2* · 79.4% for *NTRK3*	· 81.1% · Variable based on tumor type	· Relatively inexpensive · Interpretation must take tumor histology into account
FISH	3 unstained slides (1 for each *NTRK* gene)	1–3 days	· Highly sensitive · Depends on breakpoints	High specificity, yet cannot clarify structural variants of uncertain significance	· Relatively inexpensive · Useful when high suspicion of *ETV6-NTRK3* fusions
RT-PCR	1 µg of RNA (~50 000 cells)	1 week	· Variable (see notes) · Need decent RNA quality	· High	· Relatively inexpensive · Both involved genes and exons must be included in primers
DNA-based NGS	Approximately 250 ng of DNA, but depends on assay (~50 000 cells)	2–4 weeks	· 96.8% for *NTRK1* · 76.9% for *NTRK3*	· 99.86% · Dependent on whether structural variant results in transcribed fusion	· Relatively expensive, difficult to tile *NTRK3* kinase domain introns, need decent tumor purity · Can simultaneously assess point mutations, other fusions, tumor mutation burden, copy number changes, microsatellite instability status
RNA-based NGS	Approximately 200 ng of RNA, but depends on assay (~10 000 cells)	2–4 weeks	· 95.3%; dependent on RNA quality	100%	· Relatively expensive · Can assess other fusions and oncogenic transcripts across multiple genes, as well as splice variants
DNA/RNA NGS	10 ng to 40 ng of RNA (>20% tumor content)	2–4 weeks	98% to 100%	96–100%	· Relatively expensive · Can assess other aberrations listed for DNA and RNA NGS assays above

Table 2: NTRK insights: best practices for pathologists. Hechtman, Jaclyn F. Modern Pathology, Volume 35, Issue 3, 298 - 305

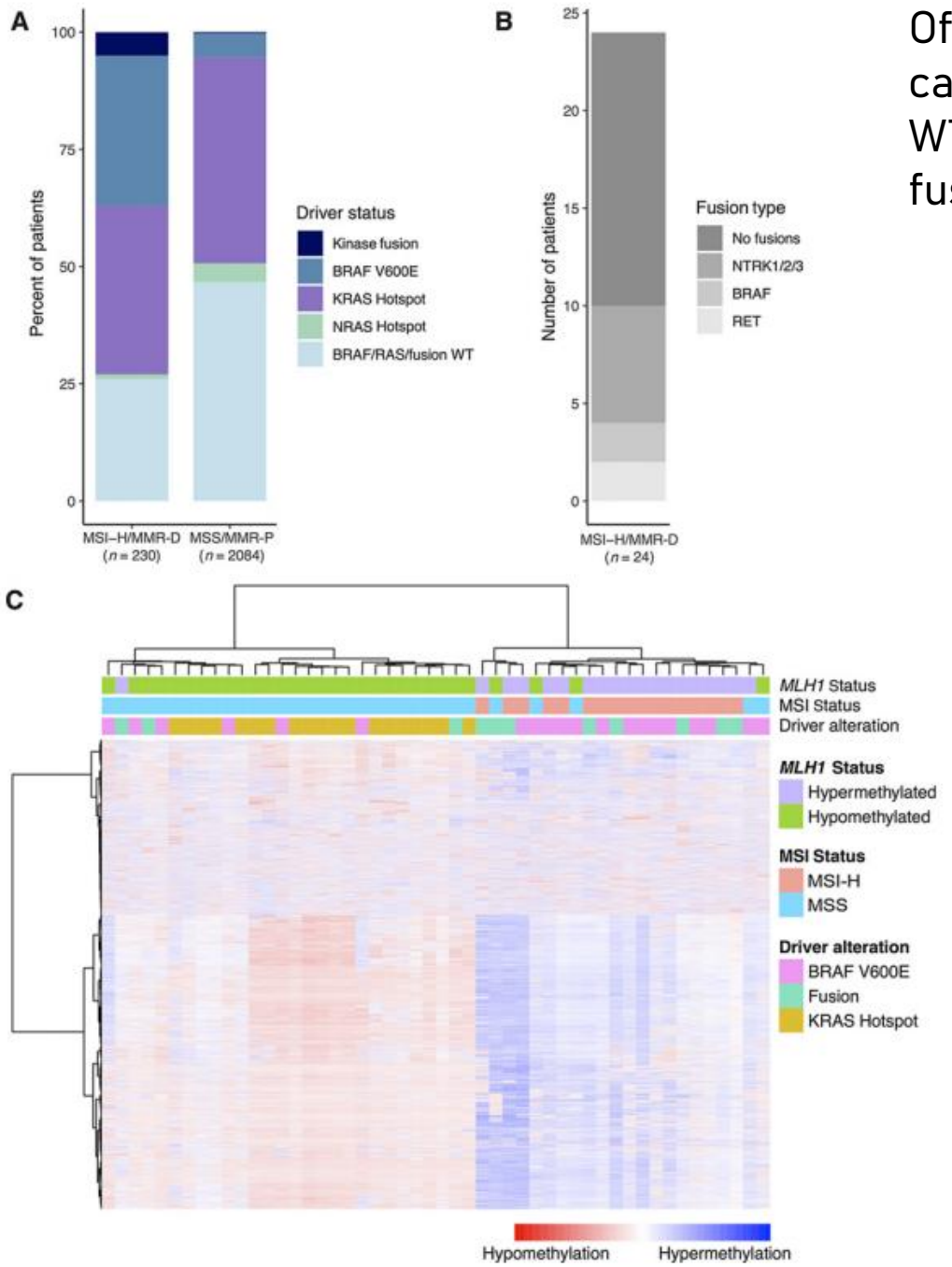


Figure 1.

Of the 10 fusions detected in the group of 24 colorectal carcinoma with MLH1 promoter hypermethylation and WT RAS/BRAF, there were 6 NTRK fusions, 2 BRAF fusions, and 2 RET fusion.

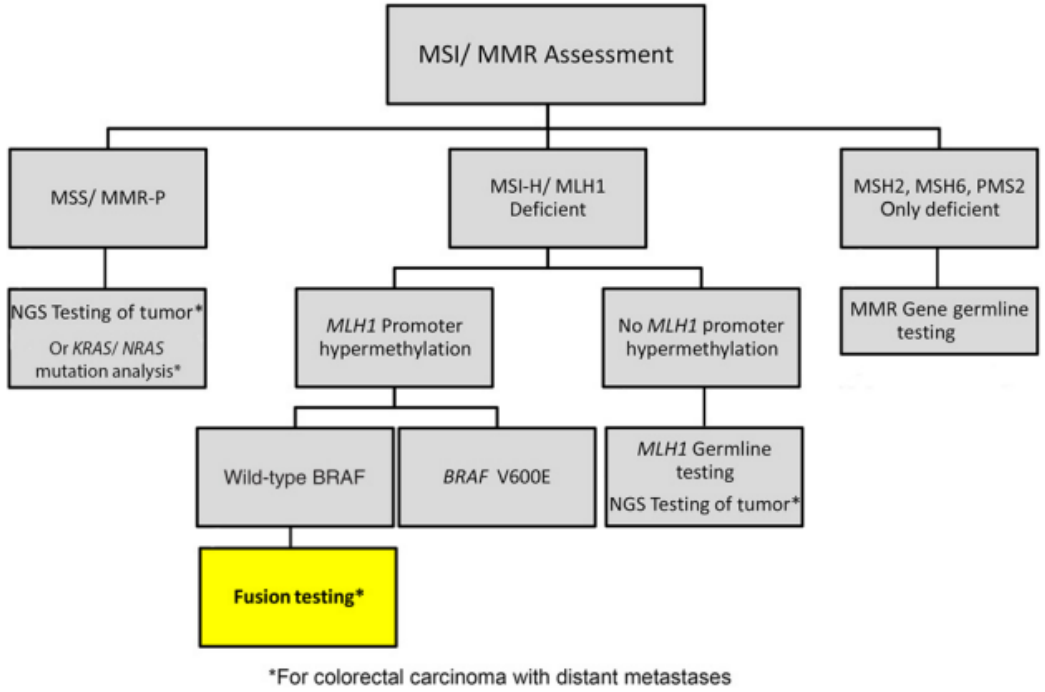
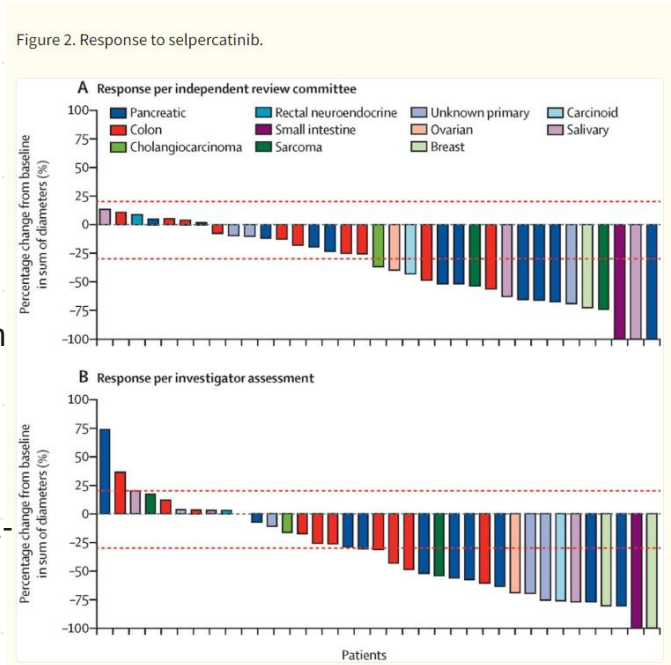


Figure 2.

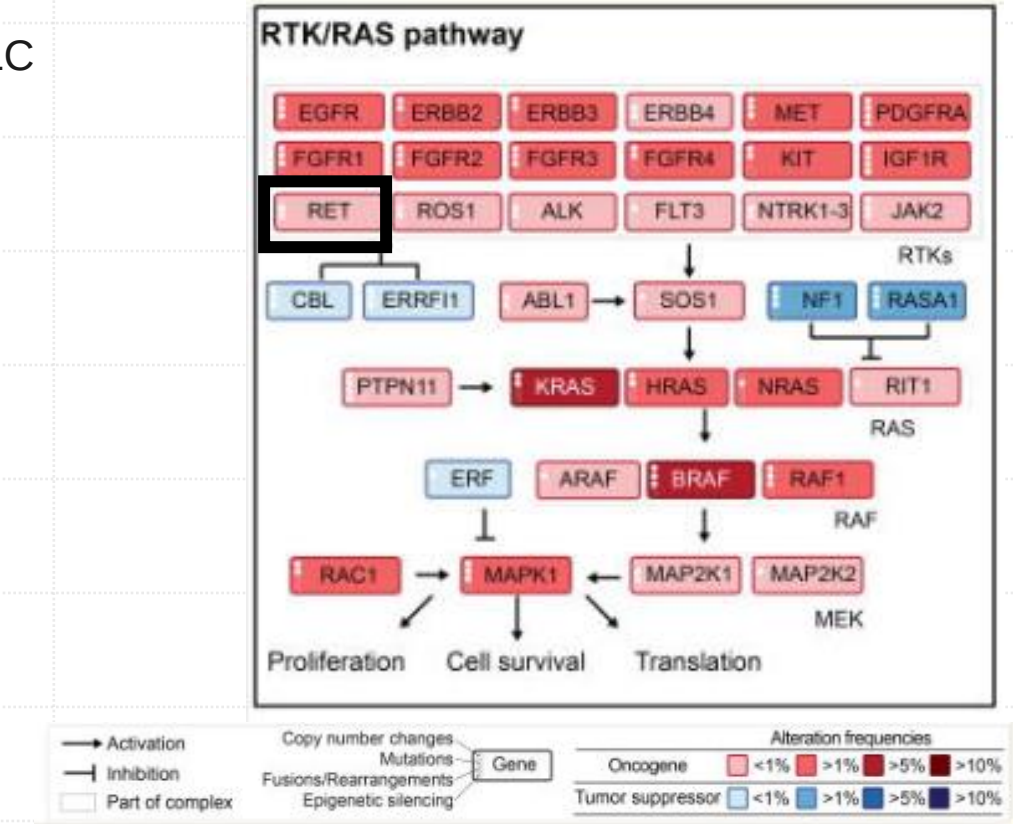
Cocco E et al. Colorectal Carcinomas Containing Hypermethylated MLH1 Promoter and Wild-Type BRAF/KRAS Are Enriched for Targetable Kinase Fusions. Cancer Res. 2019 Mar 15;79(6):1047-1053. doi: 10.1158/0008-5472.CAN-18-3126. Epub 2019 Jan 14. PMID: 30643016

RET

- Selpercatinib (FDA approval in 2022 for RET fused solid tumors)
  - LOXO-RET-17001 - 41 patients with locally advanced or metastatic *RET* fusion-positive solid tumors other than non–small cell lung cancer (NSCLC) or thyroid cancer .
    - Results – 71% response rate.
    - Supported results of LIBRETTO-001 (*RET* fusion-positive NSCLC and thyroid cancer),
    - Rare in tumors of tubal gut (<1%).
    - Preferred method of testing: RET FISH or RNA NGS



Subbiah V et al. Tumour-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): a phase 1/2, open-label, basket trial. Lancet Oncol. 2022 Oct;23(10):1261-1273. PMID: 36108661

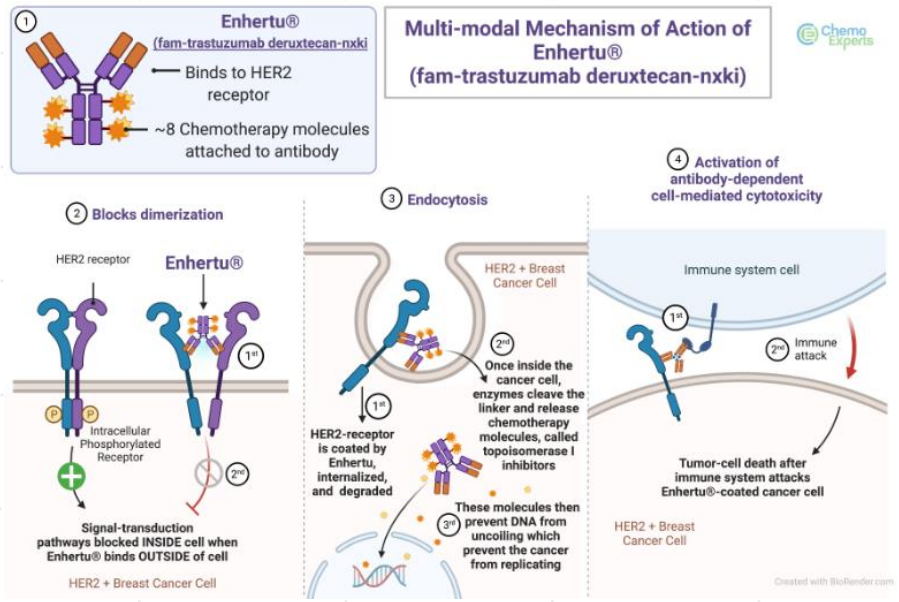


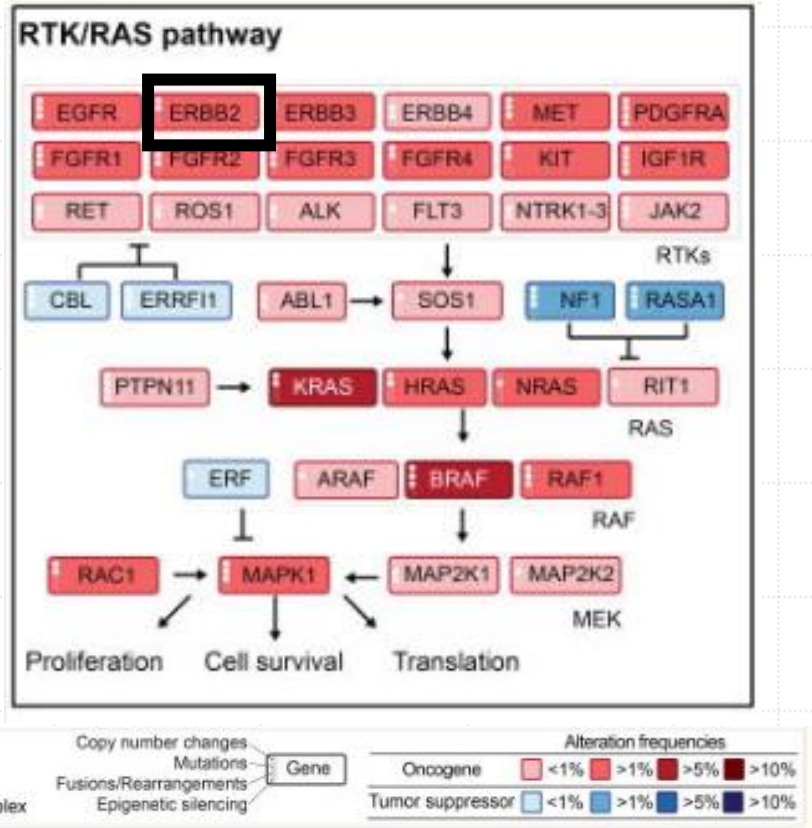
Sanchez-Vega F, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell. 2018 Apr 5;173(2):321-337.e10. PMID: 29625050

HER2 (ERBB2)

- Trastuzumab deruxtecan (FDA approved 2024)– antibody drug conjugate against HER2
  - DESTINY - Lung01
  - DESTINY – Breast04
  - DESTINY – Gastric 01 – Phase II trial Trastuzumab deruxtecan vs. physician choice chemo.
    - **Improved OS and PFS**

- **Approved for unresectable or metastatic HER2 positive solid tumor that received prior systemic therapy.**





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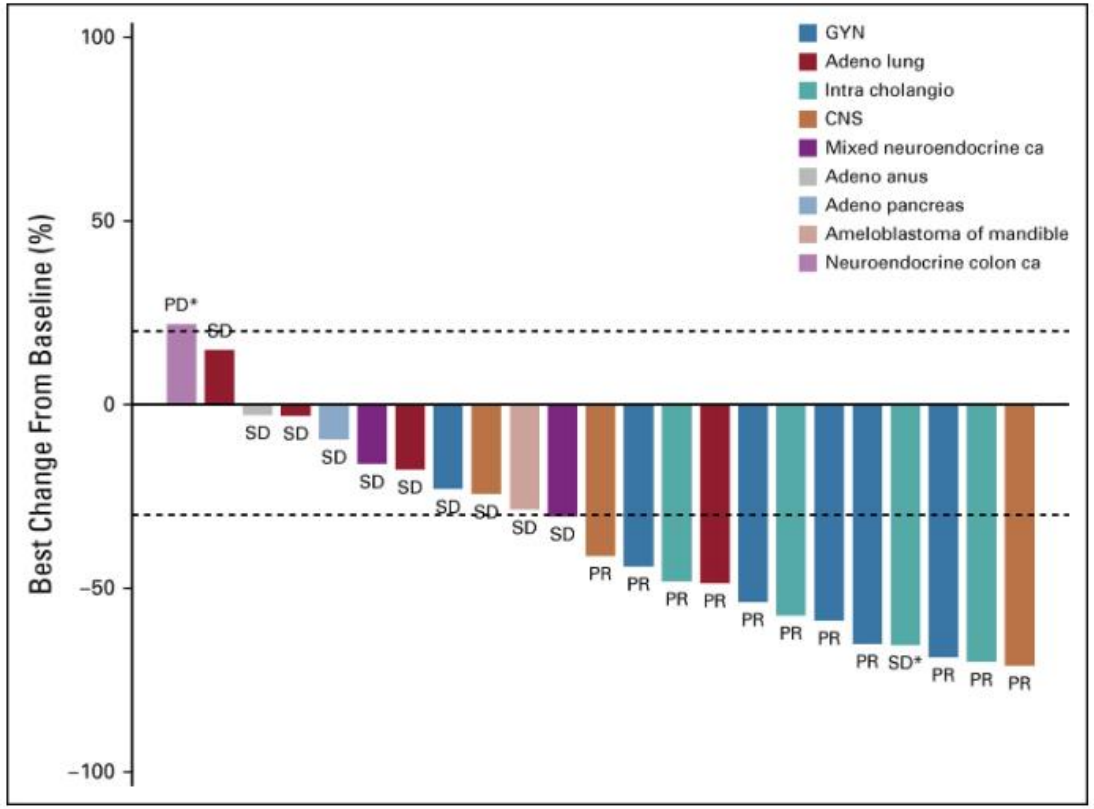
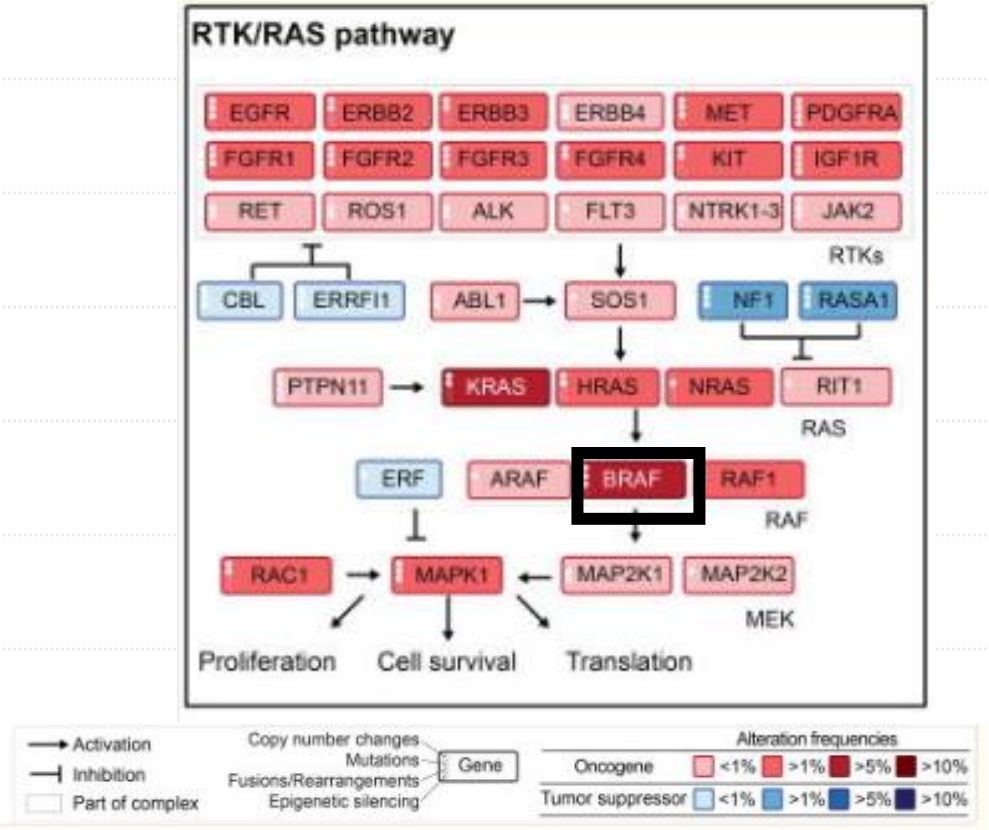
BRAF

- June 2022, FDA approved dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) for unresectable or metastatic BRAF V600E mutated solid tumors.
  - Supported by results of phase II BRF 117019 and NCI-MATCH trials.



Salama AKS, et al. Dabrafenib and Trametinib in Patients With Tumors With *BRAFV600E* Mutations: Results of the NCI-MATCH Trial Subprotocol H. J Clin Oncol. 2020 Nov 20;38(33):3895-3904.PMID: 32758030



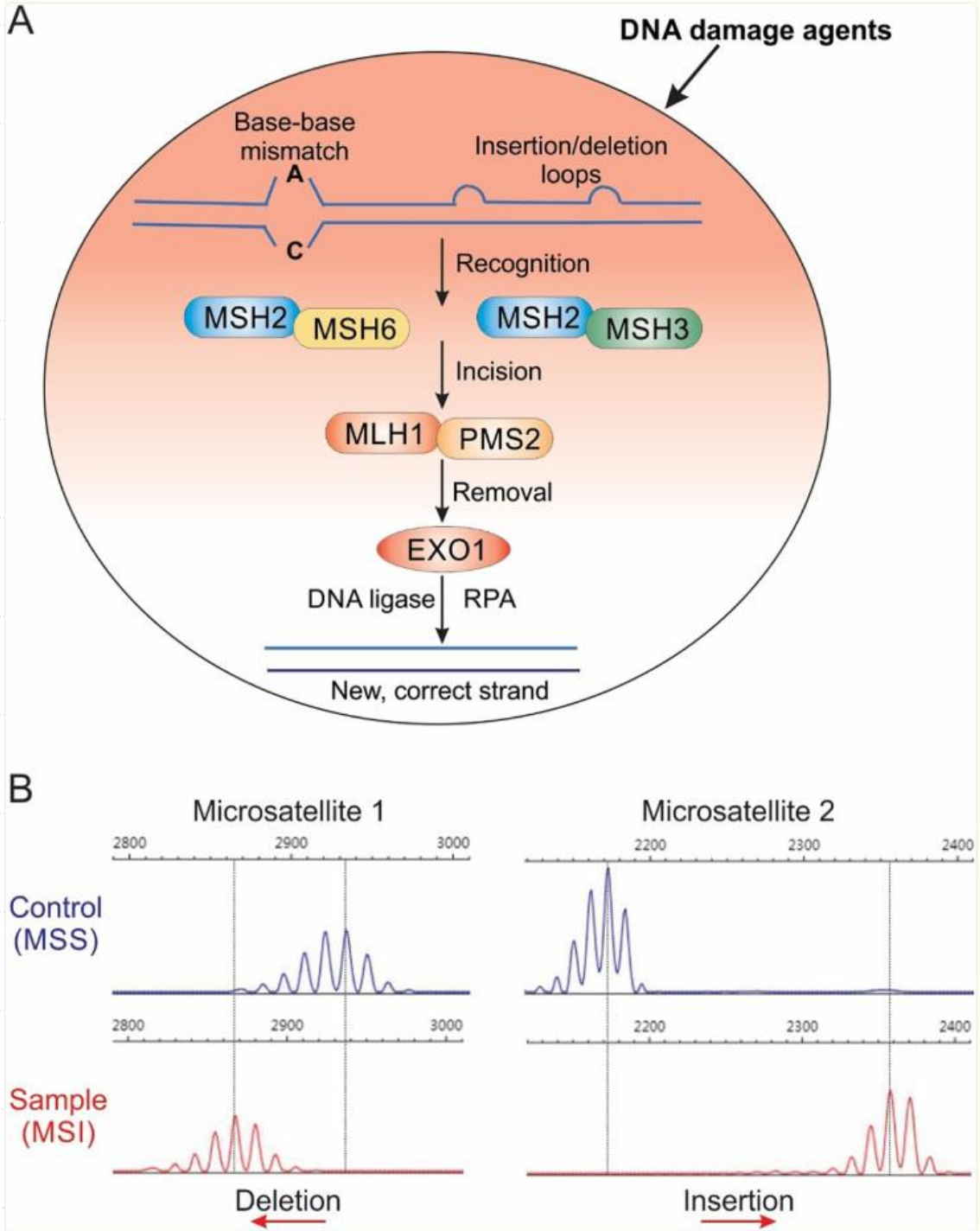
Sanchez-Vega F, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell. 2018 Apr 5;173(2):321-337.e10. PMID: 29625050

Colon

- MMR
- Extended RAS testing
- HER2
- Stool DNA testing
- ctDNA

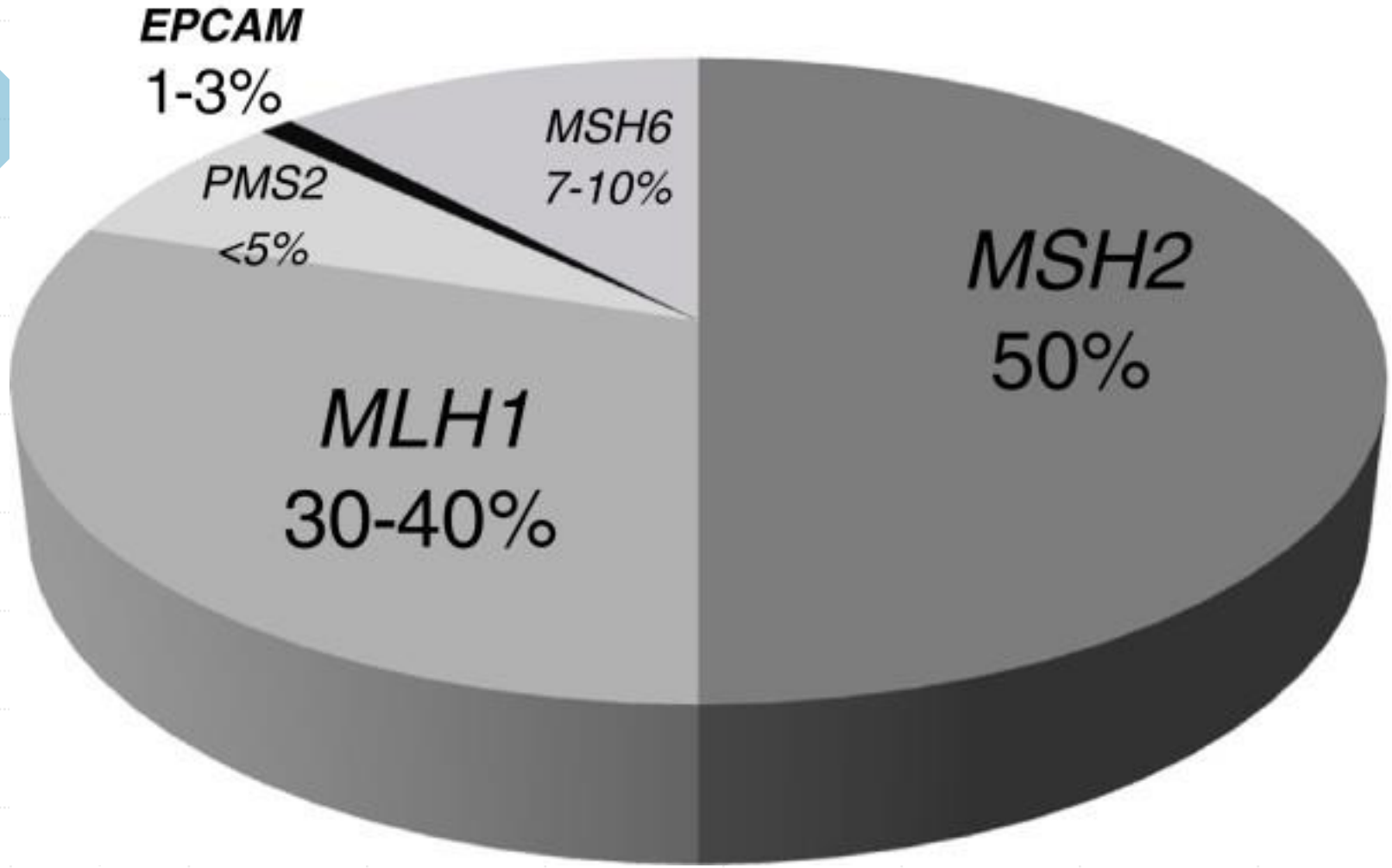
Lynch syndrome

- Most common inherited cause of CRC.
- Approximately 2–4% of all CRCs occur in patients who have Lynch syndrome.
- Germline mutations in mismatch repair genes: *MLH1*, *MSH2*, *MSH6* or *PMS2 (also EPCAM, not an MMR gene but causes MSH2 promoter methylation)*
- Autosomal dominant.
- Early onset colon cancer, depending on the MMR gene involved.
- Accelerated carcinogenesis, with adenoma to carcinoma within 2–3 years in Lynch syndrome vs. 8–10 years in the general population.
- Increased risk for malignancy at certain extracolonic sites



Lizardo DY, Kuang C, Hao S, Yu J, Huang Y, Zhang L. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: From bench to bedside. Biochim Biophys Acta Rev Cancer. 2020 Dec;1874(2):188447. PMID: 33035640.

LYNCH SYNDROME MUTATIONS



Tutlewska, K., Lubinski, J. & Kurzawski, G. *Hered Cancer Clin Pract* **11**, 9 (2013).

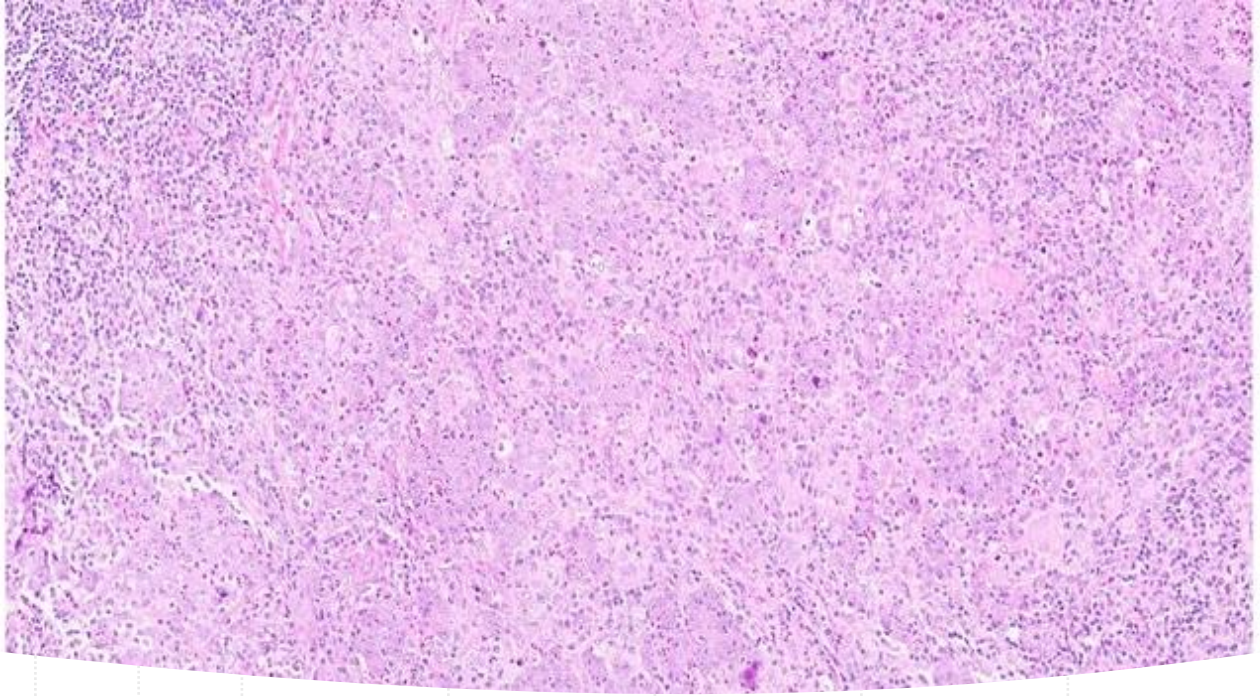
MSH6 and PMS2 alterations may be more prevalent based on colon cancer family registries but less penetrant.

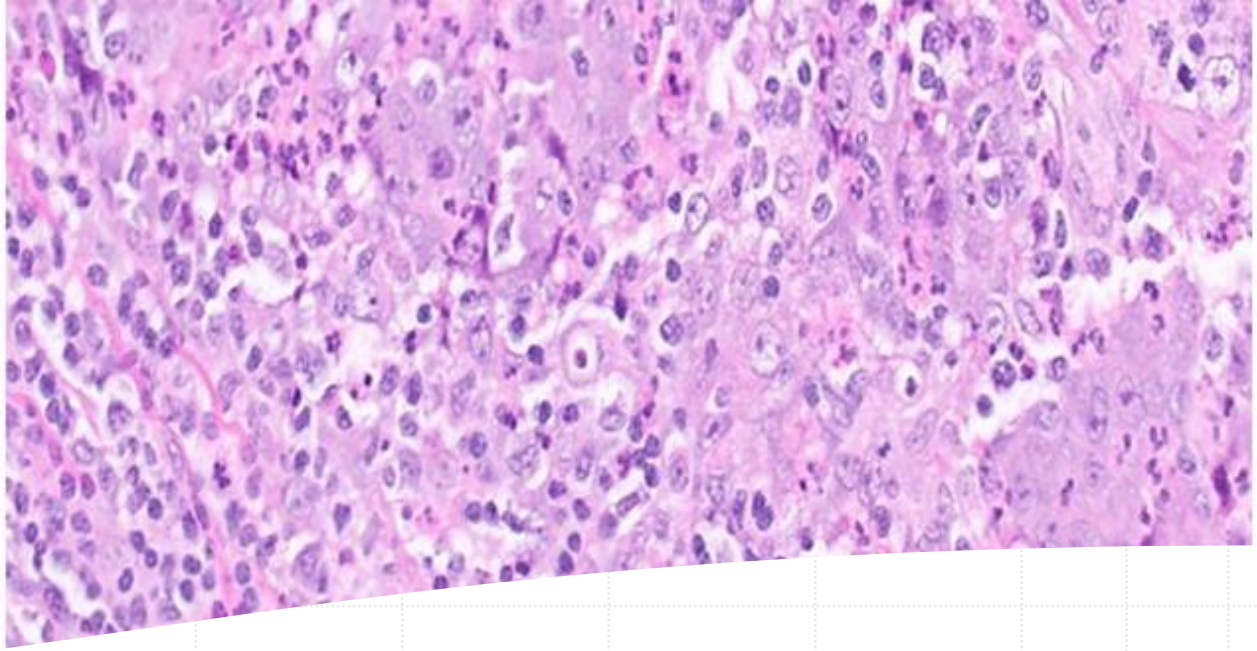
Win AK et al. Cancer Epidemiol Biomarkers Prev. 2017 Mar;26(3):404-412. Epub 2016 Oct 31.

Cumulative Cancer Risk in Lynch Syndrome up to Age 75 Years Stratified by MMR Gene Mutation and Sex

Mismatch Repair Gene	Sex	Cumulative Risk of All Cancers (%)	Cumulative Risk of Colorectal Carcinoma (%)	Mean Age of Diagnosis Of CRC (Years)	Cumulative Risk of Endometrial Carcinoma	Cumulative Risk of Ovarian Carcinoma
MLH1	Male	71.4	57.1	43		
	Female	81.0	48.3	43	35.2	11.0
MSH2	Male	75.2	51.4	44		
	Female	84.3	46.6	44	48.9	17.4
MSH6	Male	41.7	18.2	55		
	Female	61.8	20.3	57	41.1	10.8
PMS2	Male	34.1	10.4	59		
	Female				12.8	3.0

From Dominguez-Valentin M, Sampson JR, Seppala TT, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med*. 2020;22(1):15-25; Jang E, Chung DC. Hereditary colon cancer: Lynch syndrome. *Gut Liver*. 2010;4(2):151-160.

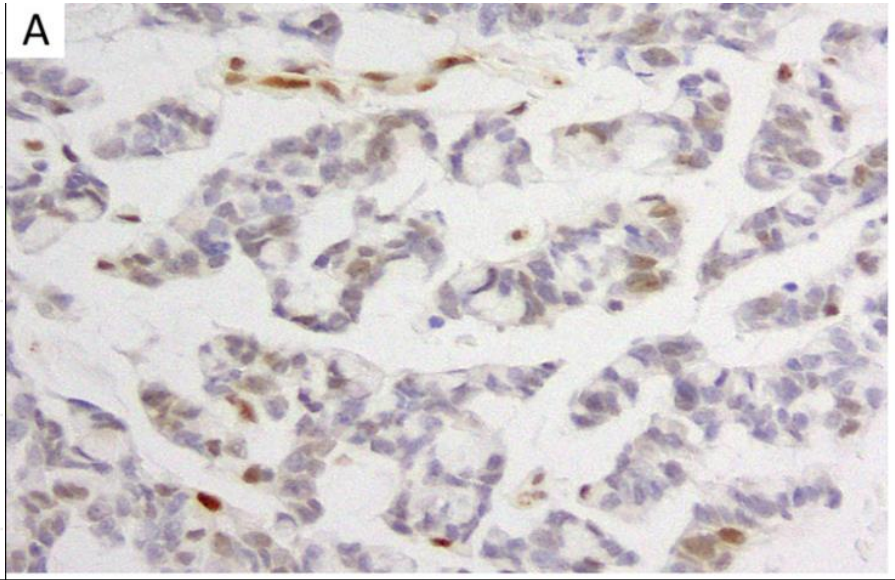
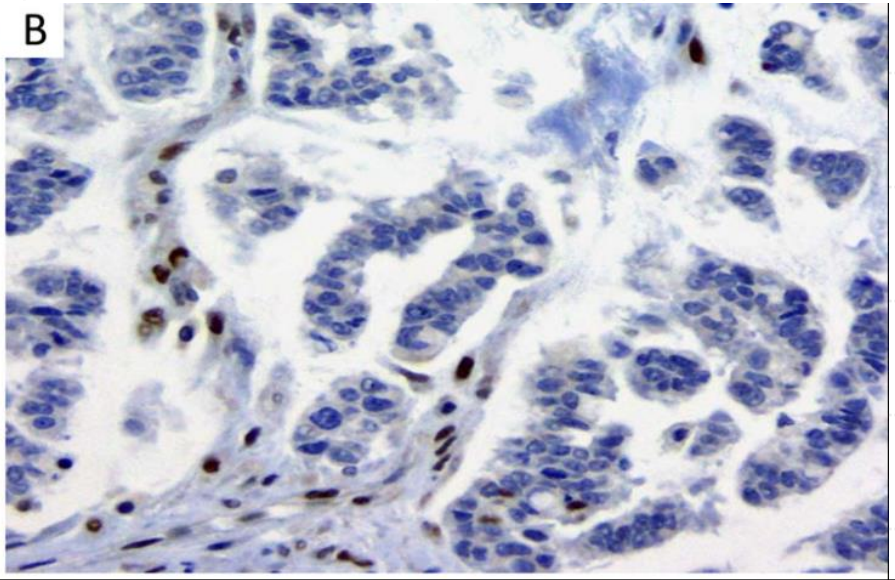
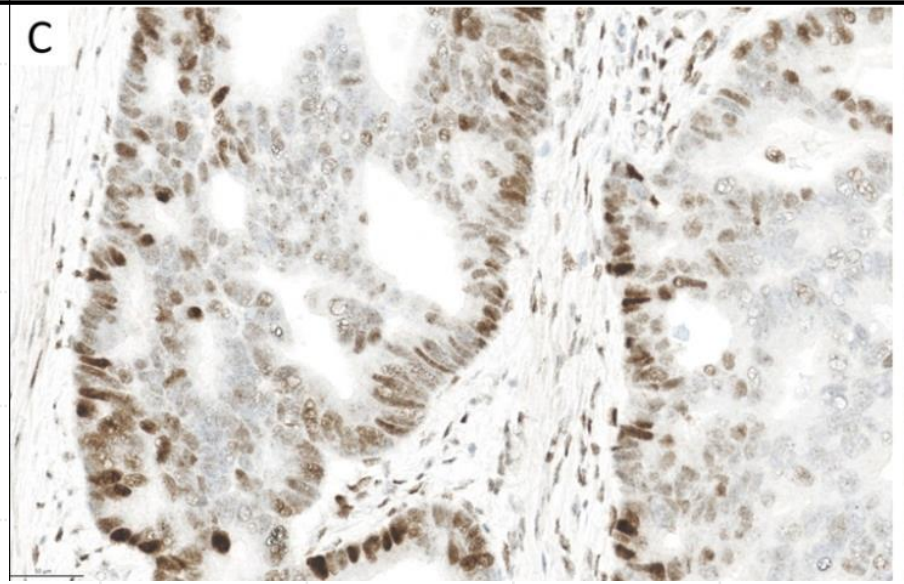
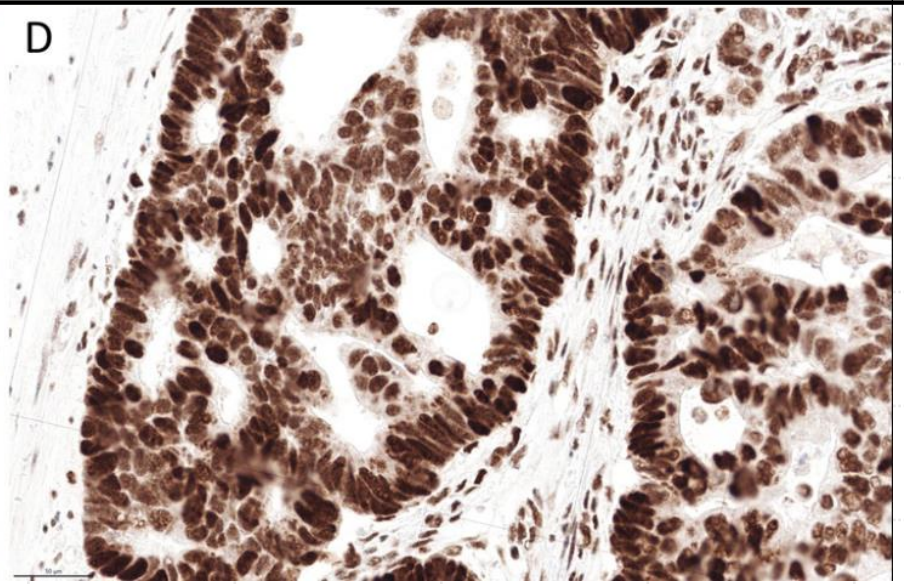




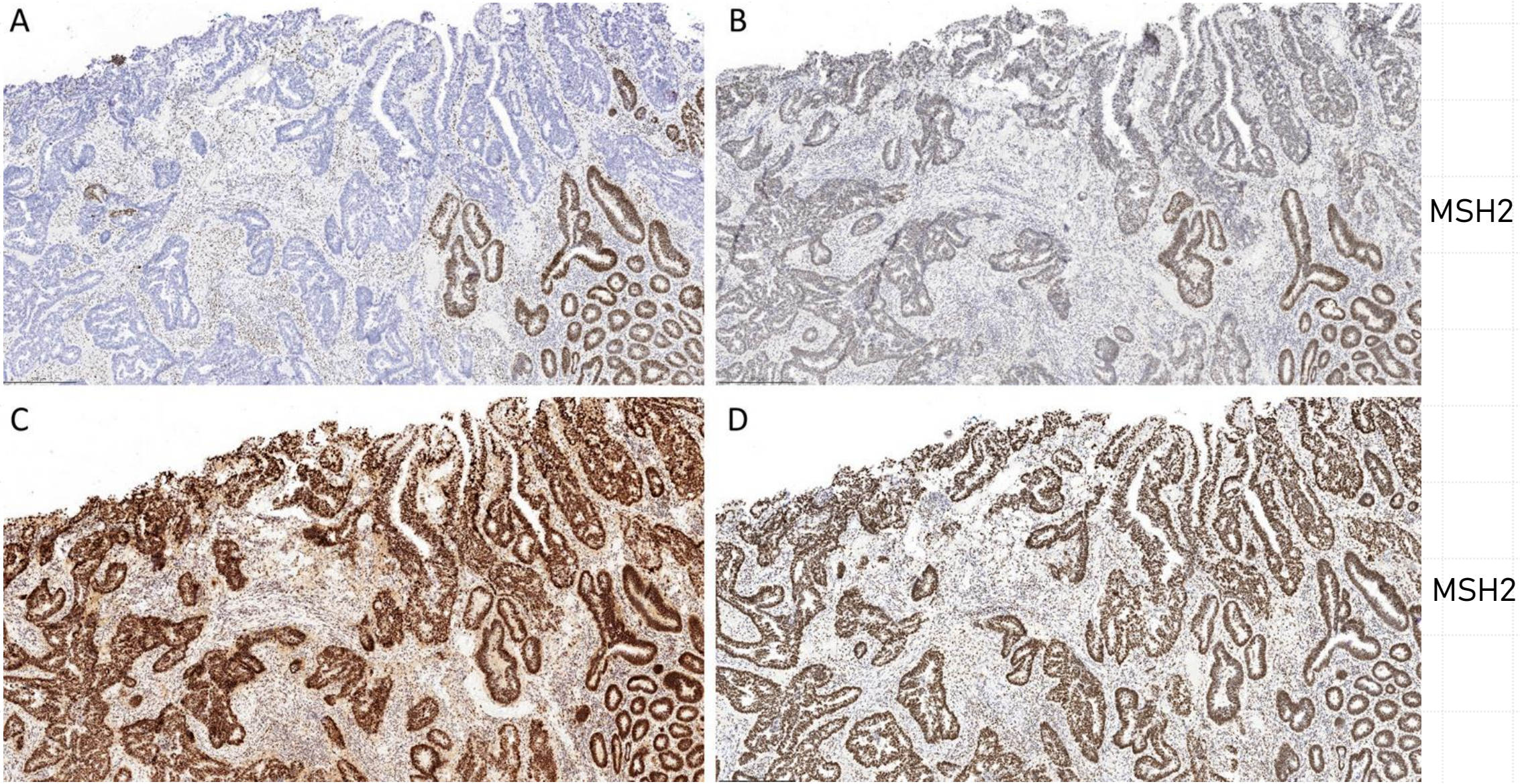
Morphology of MMRd/MSI-H CRC

- More often poorly differentiated, mucinous, and signet-cell ring cell types. (MLH1 hypermethylation/sporadic MMRd).
- Crohn's-like reaction, tumor-infiltrating lymphocytes and tumor budding within the tumor. (Lynch).
- CAP no longer recommends reporting on these morphologic features in synoptic templates.

Tumor staining	Internal control	Interpretation	Technical/biological explanation
Equivocal throughout	Weak or none	Staining not working, repeat test on same or different block	Typically due to poor fixation
Focally weak or lost	Also weak or none in these foci	Regard these foci as non-interpretable, rely on the remaining interpretable regions for results (Fig. 4A, B)	Typically due to regional poor fixation, tissue degeneration, or poor exposure to antibody/reagents during staining
Weaker than internal control	Present and optimal	Correlate with staining of its partner protein as follows:	
		MLH1 weak/PMS2 normal (Fig. 1C, D): - Report both as normal	
		MLH1 weak/PMS2 abnormal (Fig. 1A, B): - Report both as abnormal	
		MLH1 normal/PMS2 weak (unlikely scenario): - Report PMS2 as equivocal	
		MLH1 abnormal/PMS2 weak (unlikely scenario): - Report both as abnormal	
		MSH2 weak (or lost)/MSH6 normal (unlikely scenario): - Report MSH2 as equivocal	Have been observed in *POLE*-mutated cases, mechanism unclear
		MSH2 weak/MSH6 abnormal (Fig. 2): - Report both as abnormal	
		MSH2 normal/MSH6 weak: - Report MSH6 as abnormal	
		MSH2 abnormal/MSH6 weak (Fig. 4D, E): - Report both as abnormal	
Distinct clonal loss	Present and optimal	Report as abnormal:	
		Clonal loss of MLH1 and PMS2 (Fig. 3D–F)	Typically associated with clonal *MLH1* methylation (maybe mutation as well, see below)
		Clonal loss of MSH6 in MLH1/PMS2-deficient tumors	Typically associated with secondary mutation of coding microsatellites in *MSH6* in the tumor
		Clonal loss of MLH1/PMS2, MSH2/MSH6 (Fig. 3A–C), PMS2 alone, or MSH6 alone	Could potentially be associated with germline mutation, suggest genetic workup
Cytoplasmic staining		- Mostly aberrant, regard as non-interpretable; rely on nuclear staining status for result interpretation - When occurring with MSH2, and accompanied by loss of nuclear staining, it could reflect EPCAM/MSH2 abnormality	In some EPCAM-Lynch syndrome cases, cytoplasmic localization of EPCAM-MSH2 fusion proteins can result in cytoplasmic MSH2 staining[21,22]

	MLH1	PMS2	
Tumor 1			Germline MLH1
Tumor 2			Likely technical or other clinically inconsequential etiology
	MLH1	PMS2	

Detecting mismatch repair deficiency in solid neoplasms: immunohistochemistry, microsatellite instability, or both? Wang, Chiyun et al. Modern Pathology, Volume 35, Issue 11, 1515 - 1528



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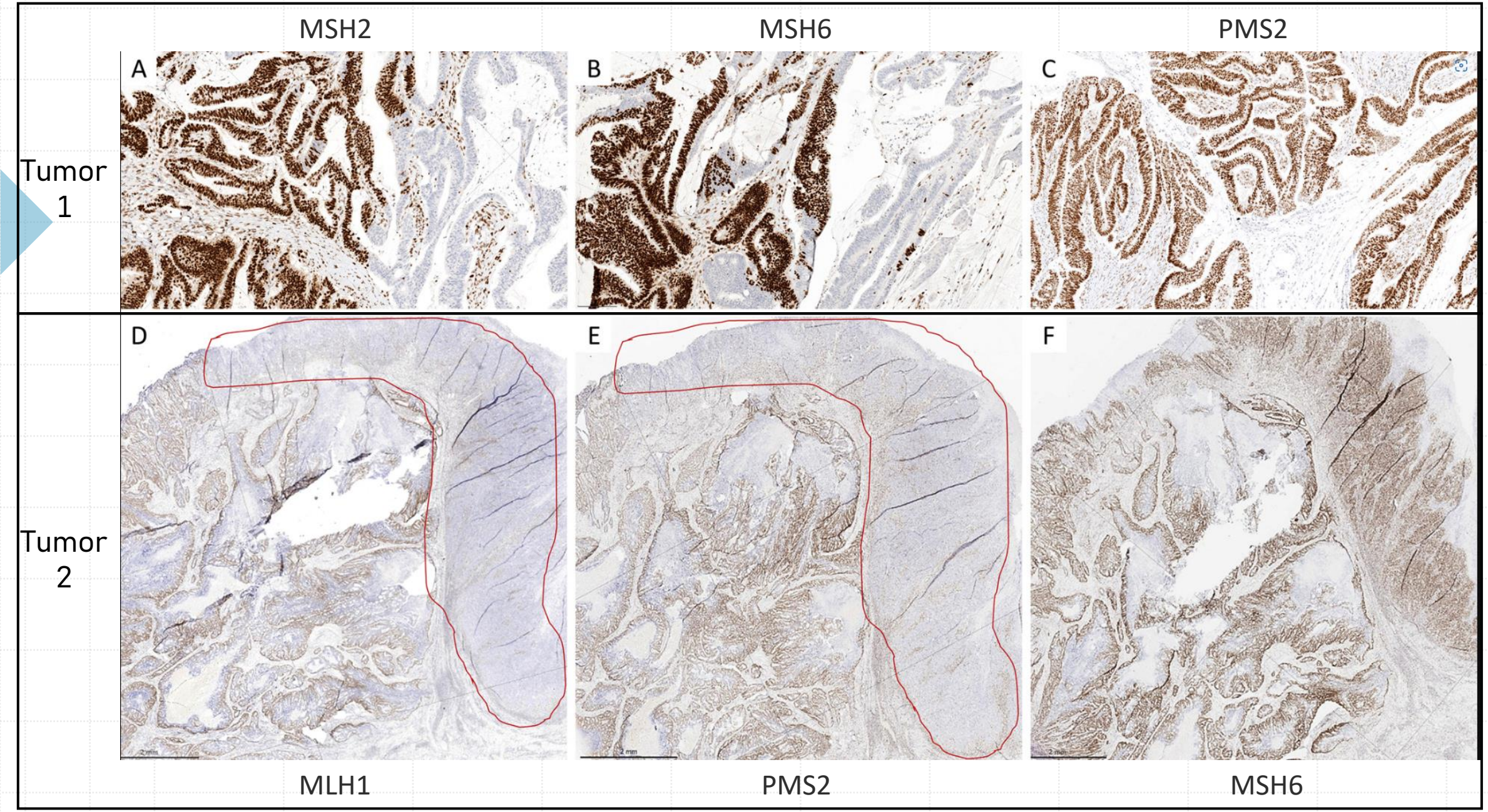
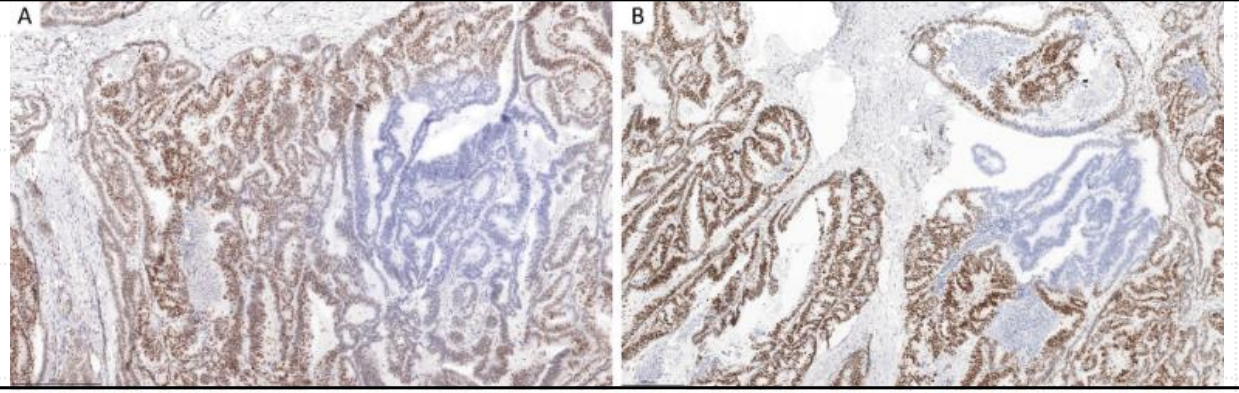


Detecting mismatch repair deficiency in solid neoplasms: immunohistochemistry, microsatellite instability, or both? Wang, Chiyun et al. Modern Pathology, Volume 35, Issue 11, 1515 - 1528

Tumor 1
Loss of staining in all MMR proteins (MSI stable)

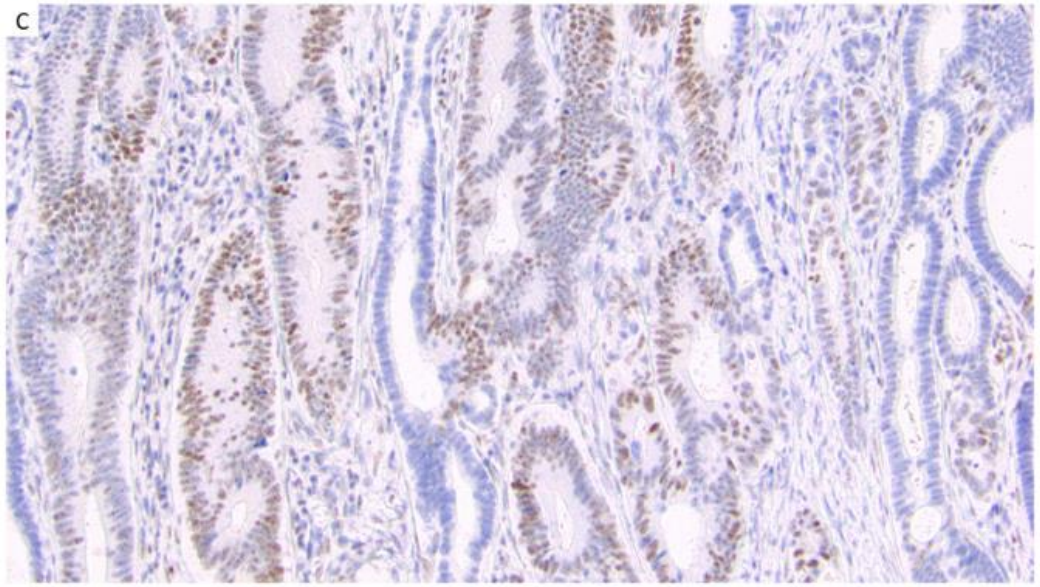
MSH6



PMS2

Tumor 2
MSI stable
No germline alteration

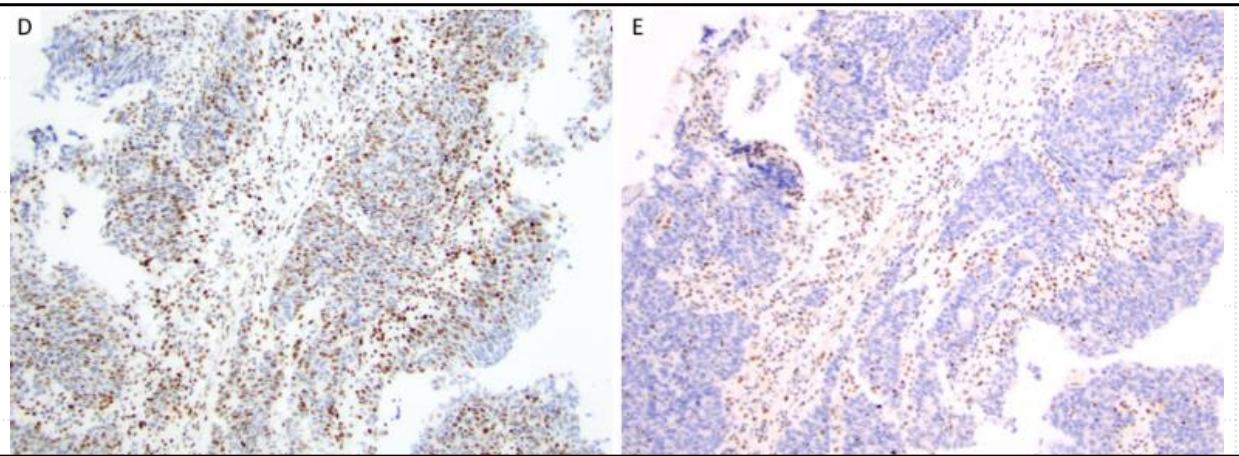
MLH1



Tumor 3

MSH2 lost
Heterogenous MSH6

MSH6



MSH6

Wang, Chiyun et al. Modern Pathology, Volume 35, Issue 11, 1515 - 1528

Take away for IHC assessment of MMRd

- Consider pre-analytic variable(i.e. fixation, edge artifact) for weak staining in tumor than internal control.
- Staining may not follow “all or none” pattern.
  - Areas of tumor with weak staining relative to internal control or areas of tumor with distinct subclonal loss should be further investigated for MSI (PCR or NGS).

Traditional definition	MMRd = Complete loss of nuclear staining in the tumor **All absent = abnormal** **(implying: Partially present = normal)**
Alternative definition	MMRd = Either **complete loss** or **distinct clonal loss** of nuclear staining in the tumor **All absent or partially absent** = abnormal (implying: **All present** = **normal**)

Sporadic dMMR colorectal cancer

- 10-15% sporadic colorectal cancer
- Acquired hypermethylation of MLH1 promoter
- IHC: MLH1/PMS2 loss (same as Lynch syndrome due to germline MLH1 mutation)
- BRAF V600E mutation in about 50%
  - Not common in extra-colonic tumors

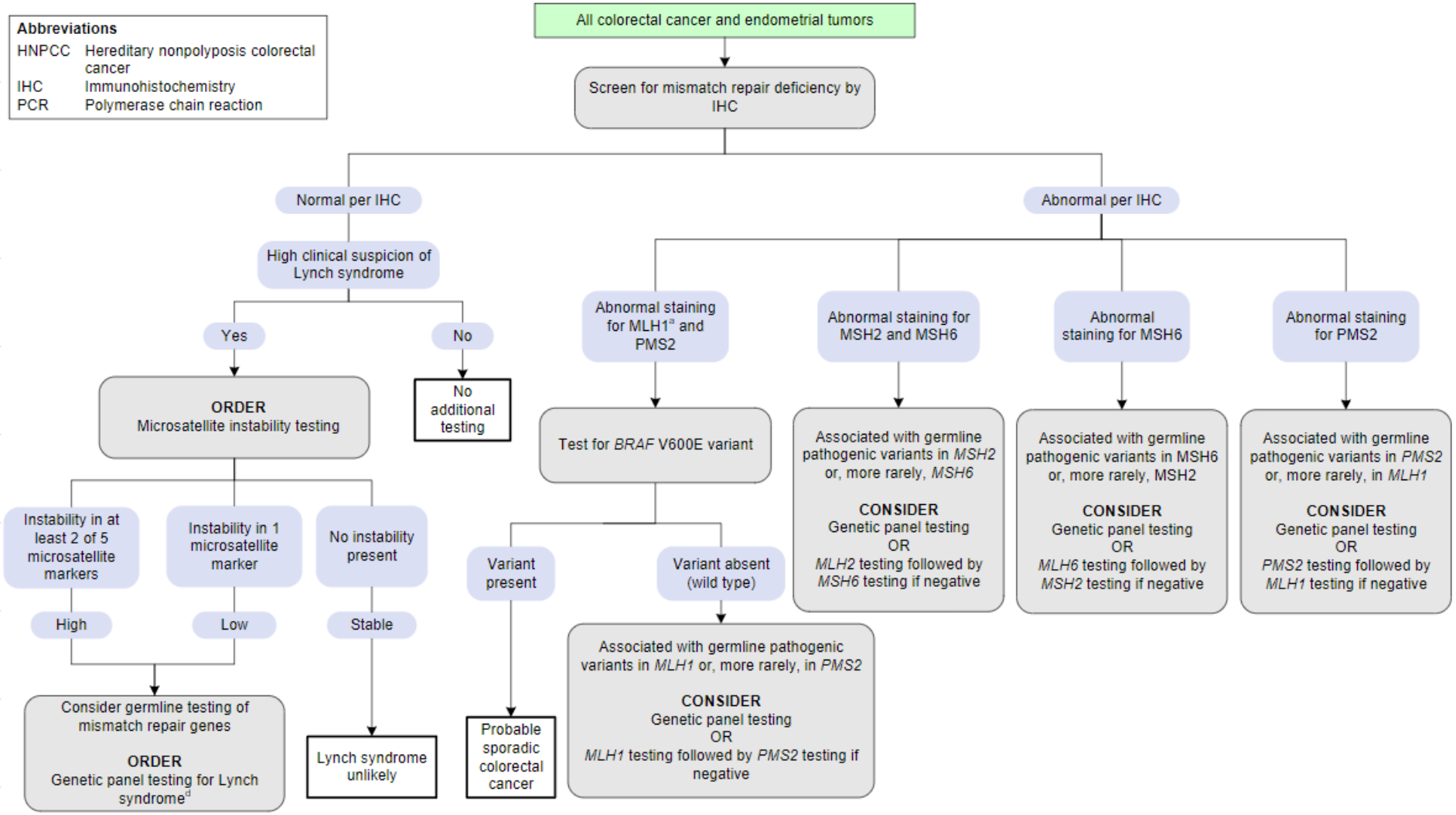
ARUP Consult

Lynch Syndrome (HNPCC) Testing

Click here for topics associated with this algorithm

Abbreviations

HNPCC	Hereditary nonpolyposis colorectal cancer
IHC	Immunohistochemistry
PCR	Polymerase chain reaction



[a] Loss of *MLH1* may be due to either acquired hypermethylation (in sporadic tumors) or a germline mutation (in Lynch syndrome).

[b] Panel (reflex) tests are available (Mismatch Repair by Immunohistochemistry with Reflex to *BRAF* Codon 600 Mutation and *MLH1* Promoter Methylation; Mismatch Repair by Immunohistochemistry with Reflex to *MLH1* Promoter Methylation).

[c] Not applicable to endometrial cancers; order only *MLH1* Promoter Methylation.

[d] Consider targeted testing if a specific variant has been previously identified in a family member.

Private Information

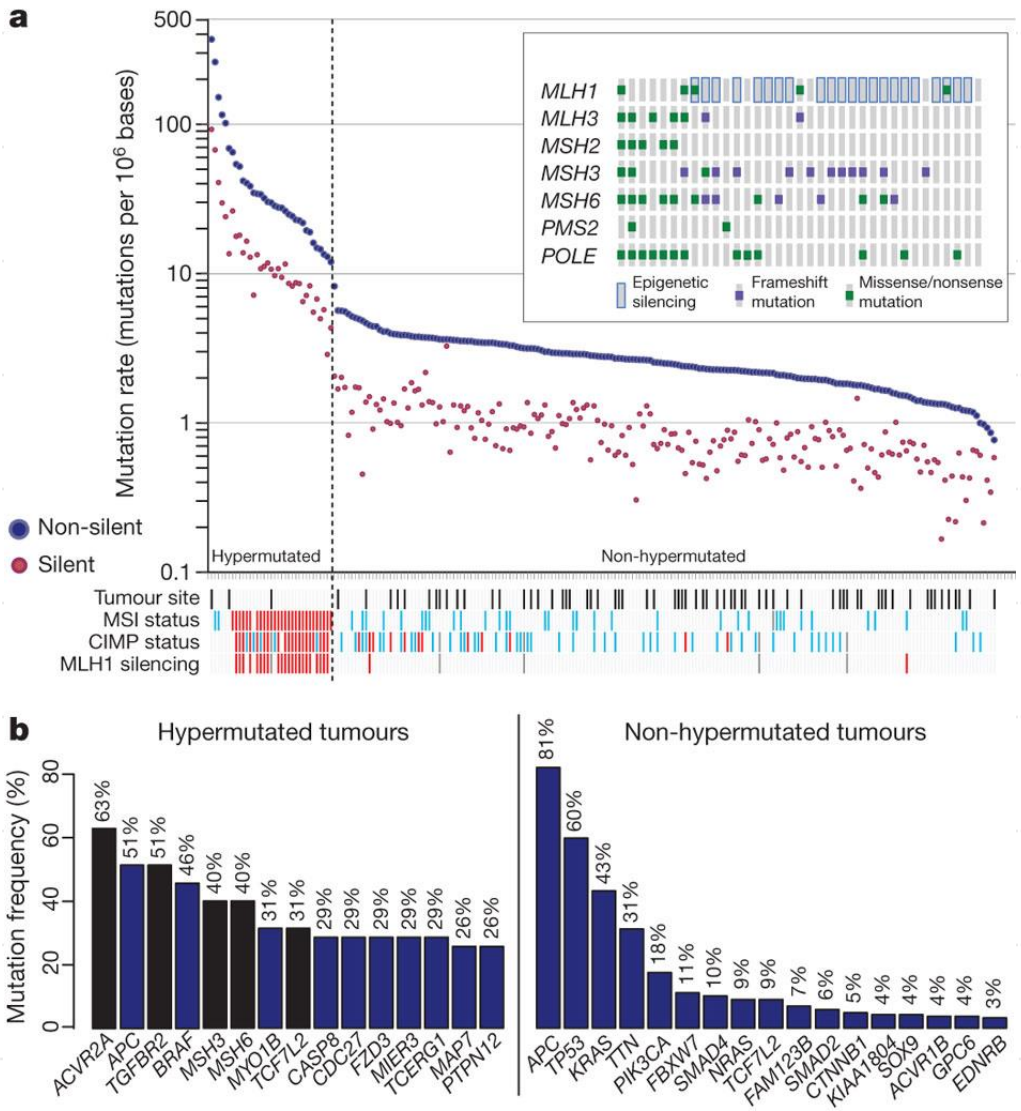
Content reviewed: November 2022 Last updated: January 2024

MMRd/MSI and Prognosis/Response 5-FU

- Ribic et al. NEJM (2003). Pooled analysis of stage II and III CRC from several RCTs. **MMRd/MSI-H tumors (especially stage II) did not respond to 5-FU chemotherapy**.
- QUASAR Trial (2007) – Efficacy of 5-FU monotherapy in stage II CRC, stratified by MMRd/MSI status. **No benefit of 5-FU chemotherapy.**
- Sargent et al. J Clin Oncology (2010). Pooled analysis form 5 RCTs for stage II and III CRC. **No survival benefit for 5-FU monotherapy for MMRd/MSI-H CRC**.

The Cancer Genome Atlas Network, Colon and Rectum

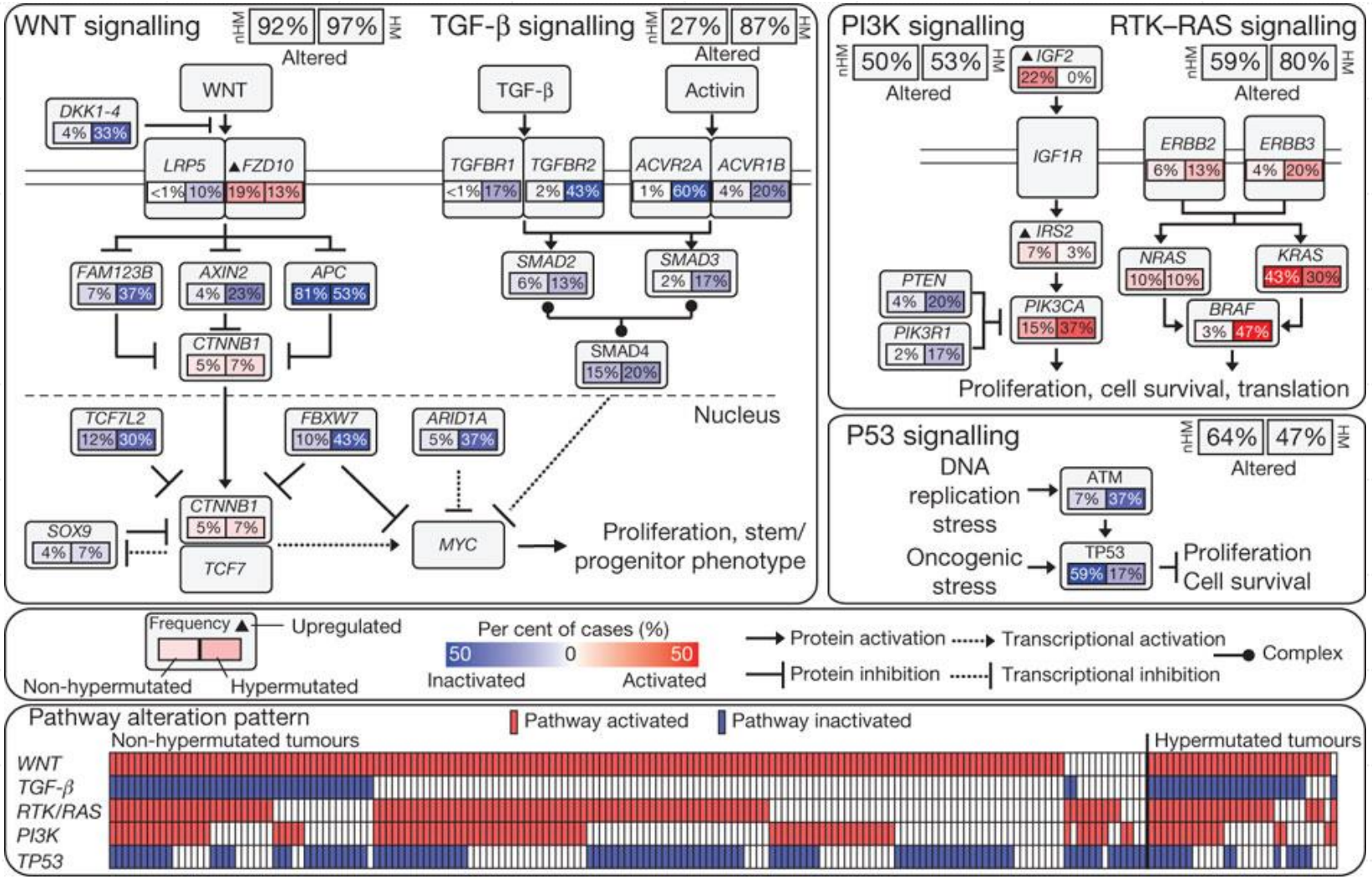
Mutation frequencies in human CRC.



The Cancer Genome Atlas Network Nature **487**, 330-337 (2012) doi:10.1038/nature11252, figure 1.

The Cancer Genome Atlas Network, Colon and Rectum

Diversity and frequency of genetic changes leading to deregulation of signalling pathways in CRC.



The Cancer Genome Atlas Network Nature **487**, 330-337 (2012) doi:10.1038/nature11252, figure 4

Figure 5. Proposed taxonomy of colorectal cancer reflecting significant biological differences in the gene expression-based molecular subtypes.

CMS1 MSI Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGFβ activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

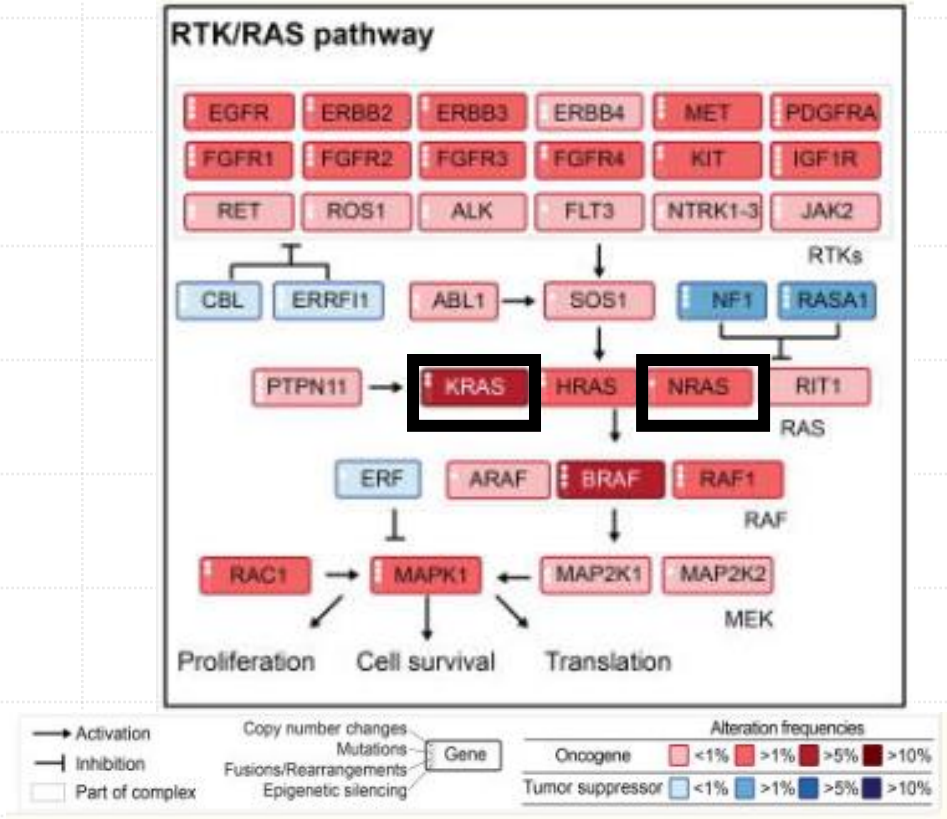
Guinney J et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015 Nov;21(11):1350-6. doi: 10.1038/nm.3967. Epub 2015 Oct 12. PMID: 26457759.

KRAS and NRAS testing in Colon Cancer

- CRYSTAL study (2008) – Phase III trial FOLFIRI +/- cetuximab in metastatic CRC. KRAS wt pts benefited from anti-EGFR therapy with improved PFS and OS. **KRAS mut pts had no benefit.**
- FIRE-3 study (2014) – Phase III trial FOLFIRI + cetuximab vs. FOLFIRI + bevacizumab. **Pts with Cetuximab and which were KRAS wt had superior OS.**
- 200/201 studies (2014) – Retrospective review of prior trials with NRAS testing. **Patients with NRAS mut did not benefit from anti-EGFR therapies.**
- PRIME study (2019) – Phase III trial FOLFOX +/- panitumumab in metastatic CRC. **KRAS wt pts also benefited with improved PFS and OS.**

KRAS and NRAS testing in Colon Cancer

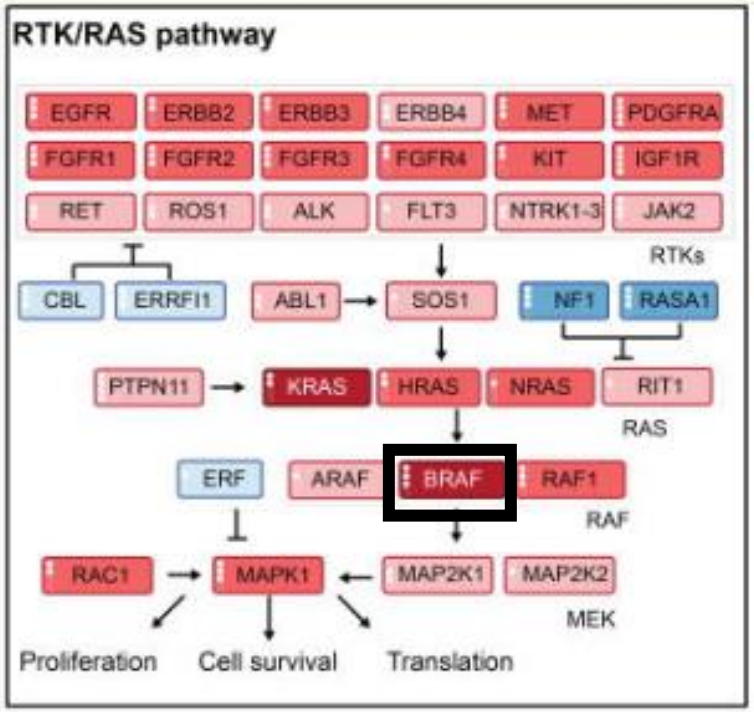
- **Current recommendation (NCCN, ASCO): Extended RAS testing on advanced stage/metastatic CRC.**
- Including KRAS and NRAS testing on common hotspots including exon 2 (codon 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146).
- Methodology: NGS preferred however PCR based panels are also acceptable.



Sanchez-Vega F, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell. 2018 Apr 5;173(2):321-337.e10. PMID: 29625050

BRAF testing in Colon Cancer

- PETACC-3 (2009) – Retrospective analysis of adjuvant therapy in stage III CRC. **BRAF V600E mutations (outside of the setting of MMRd/MSI) were associated with poor OS and poor PFS.**
- TRIBE study (2015) – Phase III trial FOLFOXIRI + bevacizumab vs. FOLFIRI + bevacizumab. **Show improved OS with higher intensity triplet chemotherapy in BRAF mut metastatic CRC.**
- SWOG 1406 Study (2017) – RCT of irinotecan + cetuximab +/- vemurafenib (BRAF inhibitor) vs. irinotecan + cetuximab

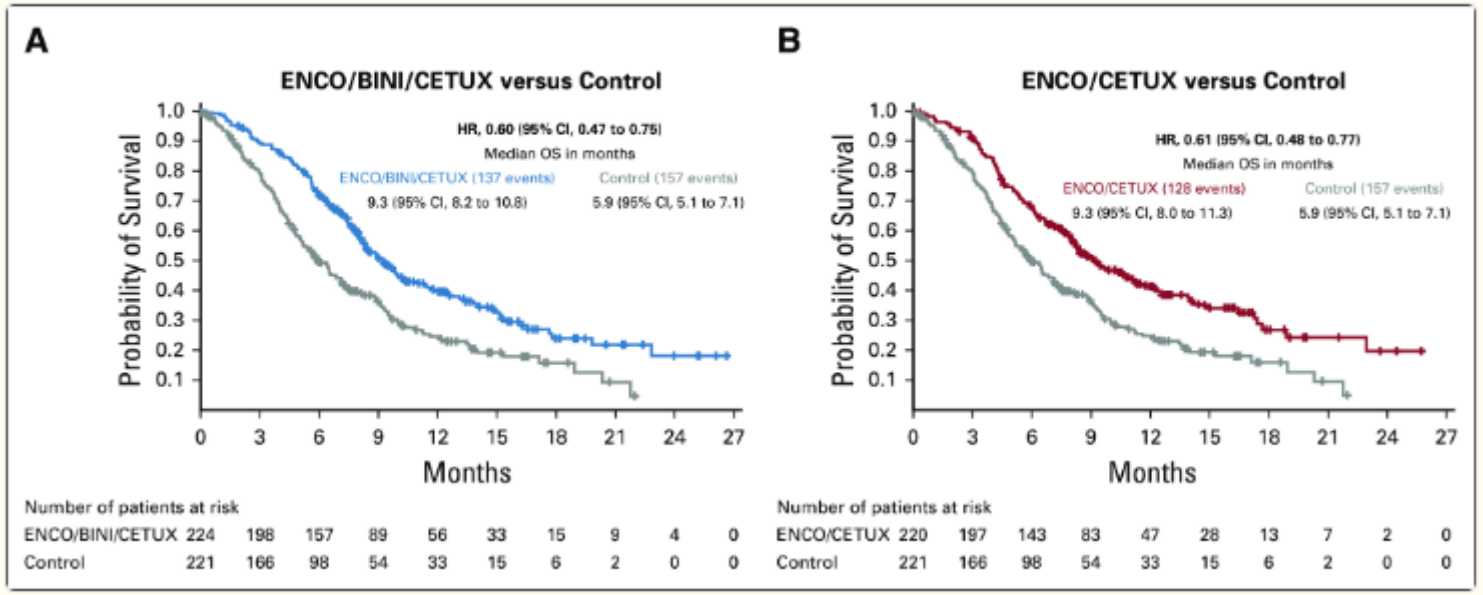


Sanchez-Vega F, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell. 2018 Apr 5;173(2):321-337.e10. PMID: 29625050

BRAF testing in Colon Cancer

- BEACON CRC trial (2019) – Phase III trial Encorafenib + cetuximab +/– binimetinib (MEK inhibitor) in BRAF V600E mut metastatic CRC. **Encorafenib + cetuximab showed improved OS and PFS compared to chemotherapy.**

FIG 1.



Tabernero J et al. Encorafenib Plus Cetuximab as a New Standard of Care for Previously Treated *BRAF* V600E-Mutant Metastatic Colorectal Cancer: Updated Survival Results and Subgroup Analyses from the BEACON Study. J Clin Oncol. 2021 Feb 1;39(4):273-284. PMID: 33503393

- **NCCN recommends BRAF testing V600E while testing for NRAS and KRAS in advanced stage/metastatic CRC.**

HER2 (ERBB2) testing in Colon Cancer

- HERACLES Trial (2016) – Phase II trial of HER2 targeted therapy (trastuzumab + lapatinib) in HER2 positive (3+) , RAS wt metastatic CRC. 30% response rate in HER2 positive patients. **Demonstrated efficacy of dual HER2 blockade.**
- MyPathway Study (2019) – Phase II basket trial including HER2 positive metastatic CRC patients treated with trastuzumab + pertuzumab. **32% response rate in HER2 positive patients.**
- DESTINY-CRC01 Trial (2021) – Phase II study evaluating fam-trastuzumab deruxtecan-nxki in HER2 positive metastatic CRC. **45% response rate. FDA approved for HER2 positive metastatic CRC.**
- Mountaineer Trial (2022) – Phase II trial of trastuzumab + tucatinib in HER2 positive metastatic CRC. **38.1% response rate. FDA approved for HER2 positive metastatic CRC**.

- **All patients with metastatic CRC should be tested for HER2 amplification.**
- Testing methodologies include IHC, FISH, and NGS, confirmatory testing for equivocal results.

Issues with HER2 testing

- **HERACLES Trial**: Amplified HER2 = 3+ staining in ≥ 50% cells, if 3+ in 10-49%, 2+ ≥ 10% of cells then reflexed to FISH (amplified if ERBB2/CEP17 ratio ≥ 2) . 1+ or 2+/3+ staining in <10% of tumor cells considered NEGATIVE.
- **MyPathway study:** No heterogenous 3+ positive category for 10-49% cell staining.
- Expanding spectrum now with HER2 low (IHC 1+ or 2+ staining in ≥ 10% , FISH not amplified) and HER2 ultra low (barely perceptible IHC staining in less than 10% of cells).
  - **DESTINY-CRC01: no response to trastuzumab deruxtecan with IHC 1+ or 2+ staining in ≥ 10% , FISH not amplified**

RESEARCH ARTICLE · Articles in Press, 100654, November 10, 2024

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Evidence for Unified Assessment Criteria of HER2 IHC in Colorectal Carcinoma

Mark G. Evans [1] · Harris B. Krause [1] · Joanne Xiu [1] · ... · David A. Bryant [1] · Matthew J. Oberley [1] · Jaclyn F. Hechtman [1] ... Show more

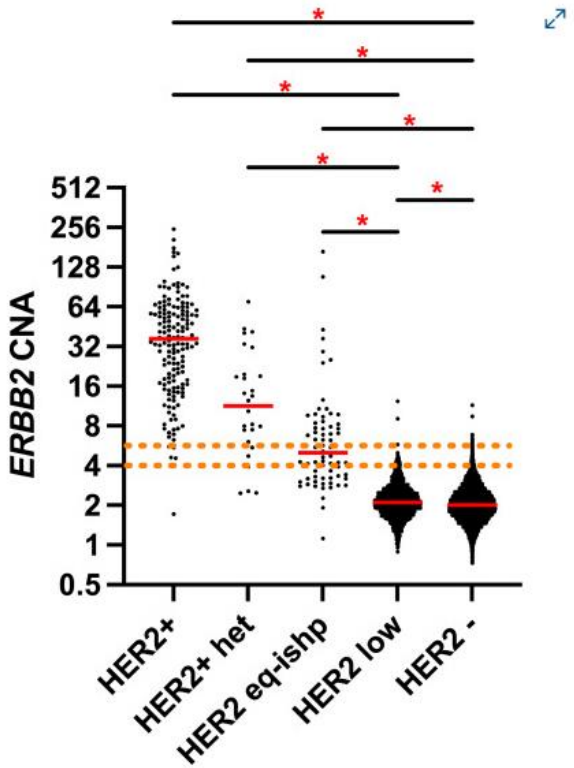
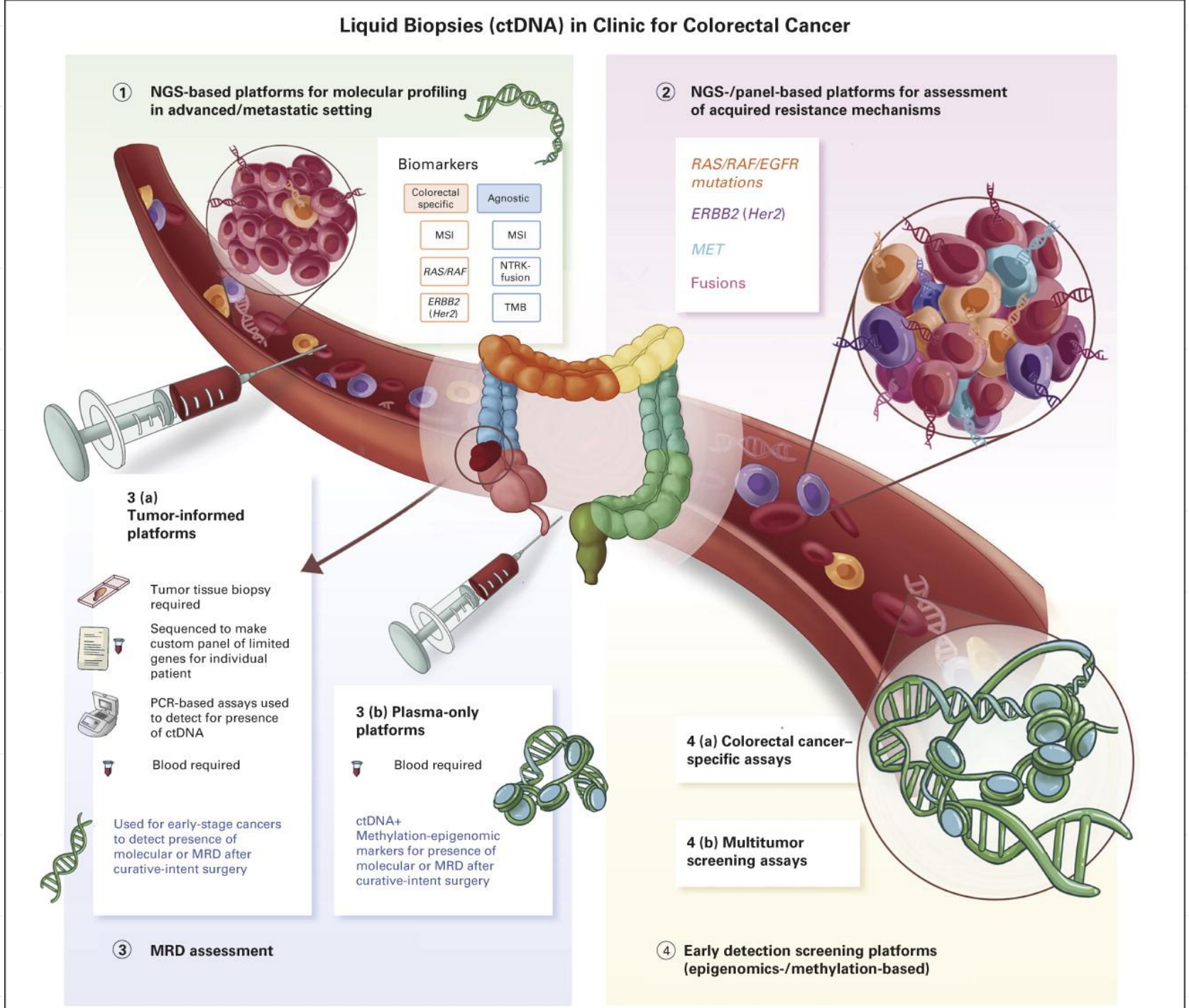


Figure 2

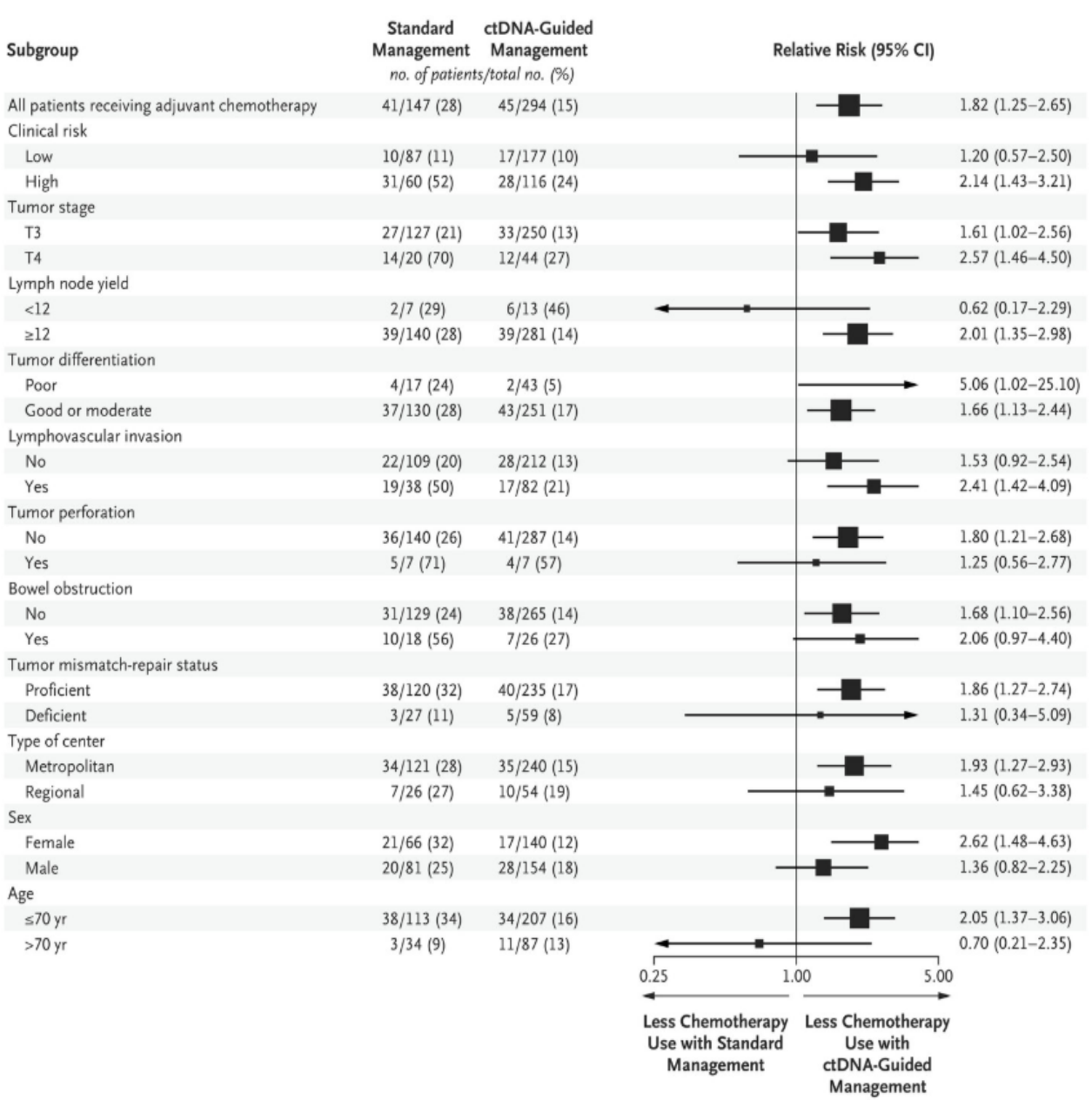
	(HER2 pos)	(HER2 pos het)	(HER2 eq-ishp)	(HER2 low)	(HER2 neg)	Statistic	p-value
IHC result	1.3% (166/13208)	0.2% (28/13208)	0.5% (72/13208)	10.6% (1401/13208)	87.4% (11541/13208)	N/A	N/A
ERBB2 amp	96.2% (153/159)	75% (21/28)	42.0% (29/69)	0.2% (2/1329)	0.1% (7/10832)		
ERBB2 intermediate amp	3.1% (5/159)	10.7% (3/28)	23.2% (16/69)	1.6% (21/1329)	1.2% (125/10832)	Chi squared	<0.001
ERBB2 non-amp	0.6% (1/159)	14.3% (4/28)	34.8% (24/69)	98.3% (1306/1329)	98.8% (10700/10832)		

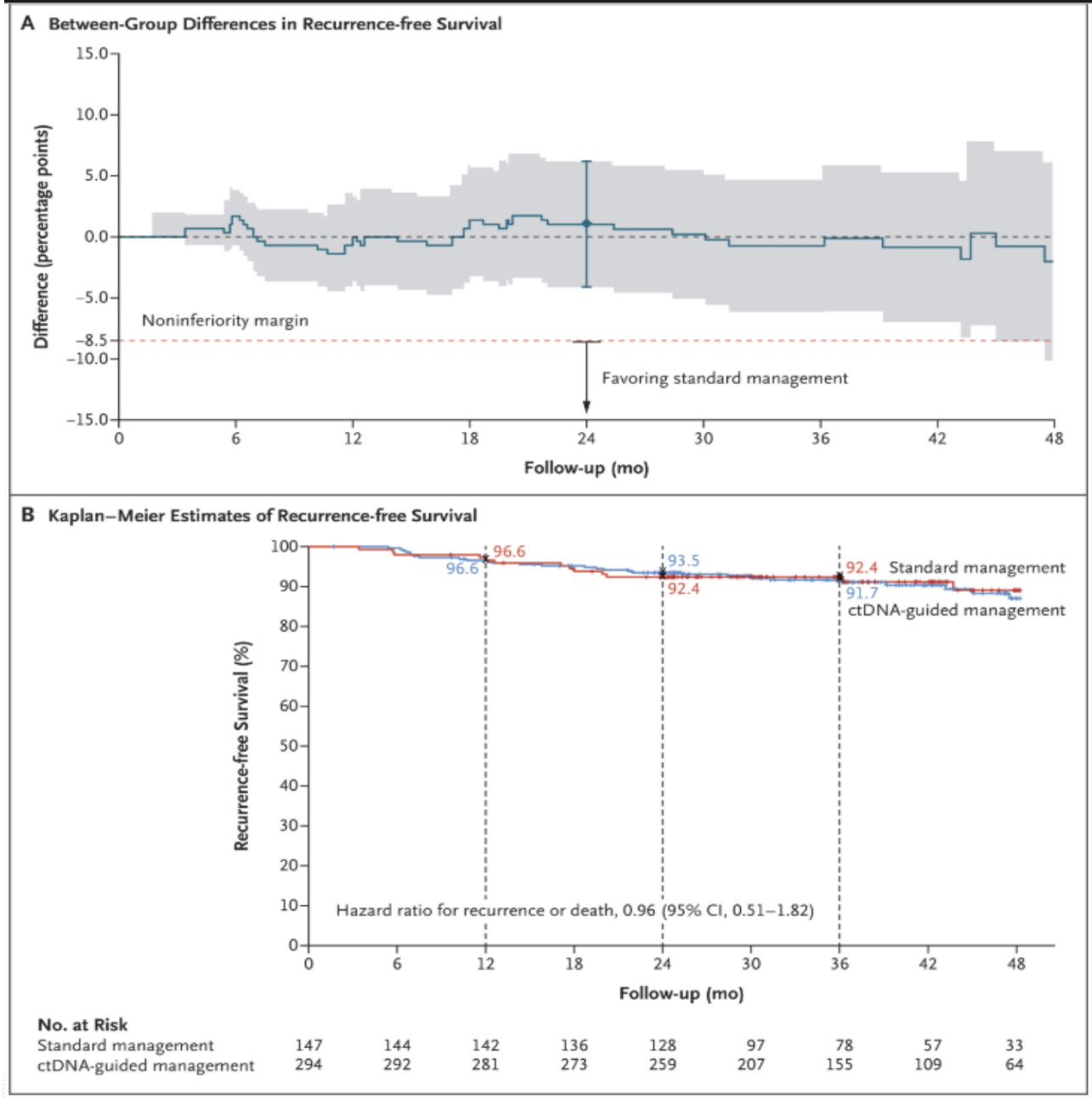
Table 2

100% (22/22) of HER2+ heterogenous (3+ staining in 10-49% of cells were FISH amplified.



•Midhun Malla et al. Using Circulating Tumor DNA in Colorectal Cancer: Current and Evolving Practices. JCO 40, 2846 -2857(2022).





Tie J, et al. DYNAMIC Investigators. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N Engl J Med. 2022 Jun 16;386(24):2261-2272. doi: 10.1056/NEJMoa2200075. Epub 2022 Jun 4. PMID: 35657320;

ctDNA and Colon Cancer

- BESPOKE CRC– RCT 1700 patients with stage 2–3 CRC with multiple ctDNA measured at interval after surgical resection. ctDNA was used to inform adjuvant therapy.
  - **ctDNA-based MRD detection of MRD was prognostic of recurrence.**
  - **ctDNA MRD was predictive: significant benefit adjuvant chemo. MRD+ but not in MRD- pts.**
- GALAXY – 3000 patients with stage 2–4 resectable CRC with pre and post surgery interval ctDNA measurements. ctDNA was used to inform adjuvant therapy.
  - **DFS and OS after 2 years were superior for ctDNA negative patients.**
  - **Patients with positive ctDNA which received adjuvant chemotherapy had better outcomes than those not treated with adjuvant therapy.**

ORIGINAL ARTICLE

A Cell-free DNA Blood-Based Test for Colorectal Cancer Screening

Authors: Daniel C. Chung, M.D., Darrell M. Gray II, M.D., M.P.H., Harminder Singh, M.D., Rachel B. Issaka, M.D., M.A.S., Victoria M. Raymond, M.S., Craig Eagle, M.D., Sylvia Hu, Ph.D., +5, and William M. Grady, M.D. Author Info & Affiliations

Published March 13, 2024 | N Engl J Med 2024;390:973-983 | DOI: 10.1056/NEJMoa2304714 | VOL. 390 NO. 11

- No survival data.
- Poor sensitivity in precancerous lesions.
- Moderately specific.
- Will patients follow up?
- FDA approved as a screening test for average risk adults age 45 and up.

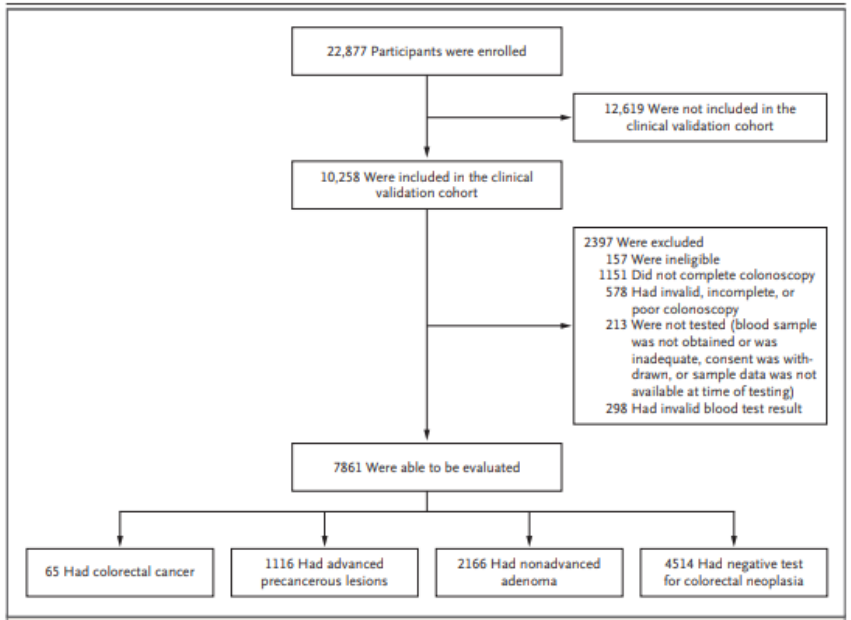


Figure 1. Enrollment and Outcomes.
A total of 22,877 participants were enrolled. Given that the coprimary specificity outcome was sufficiently powered with 7000 participants who were negative for advanced neoplasia on colonoscopy, the population without a diagnosis of colorectal cancer was down-sampled to the target sample of approximately 7000 evaluable participants who were negative for advanced neoplasia on the basis of the expected colonoscopy availability and occurrence of test failure. Sampling was performed with the use of a stratified random approach, such that the age distribution of the selected participants without colorectal cancer followed the 2020 U.S. age distribution. Cohort sampling was completed before sample testing, with age being the only clinical variable considered. Reasons for exclusion are listed in order of priority.

Table 2. Sensitivity and Specificity of the Cell-free DNA (cfDNA) Blood-Based Test for the Most Advanced Findings on Colonoscopy.*

Variable	Most Advanced Finding on Colonoscopy	cfDNA Blood-Based Test	
		Positive Test	Sensitivity (95% CI)
	no.	*no.*	%
Colorectal cancer			
Any	65	54	83.1 (72.2–90.3)
Stage I, II, or III*	48	42	87.5 (75.3–94.1)
Advanced precancerous lesions†	1116	147	13.2 (11.3–15.3)
			Specificity (95% CI)
Nonadvanced adenomas, nonneoplastic findings, and negative colonoscopy	6680	698	89.6 (88.8–90.3)
Nonneoplastic findings and negative colonoscopy	4514	457	89.9 (89.0–90.7)

* Excluded were 10 stage IV and 7 pathologically confirmed, incompletely staged colorectal cancers.
† Advanced precancerous lesions include advanced adenomas and sessile serrated lesions at least 10 mm in the largest dimension.

ORIGINAL ARTICLE

Next-Generation Multitarget Stool DNA Test for Colorectal Cancer Screening

Authors: Thomas F. Imperiale, M.D., Kyle Porter, M.A.S., Julia Zella, Ph.D., Zubin D. Gagrat, B.S., Marilyn C. Olson, Ph.D., Sandi Statz, M.S., Jorge Garces, Ph.D., +6, for the BLUE-C Study Investigators* Author Info & Affiliations

Published March 13, 2024 | N Engl J Med 2024;390:984-993 | DOI: 10.1056/NEJMoa2310336 | VOL. 390 NO. 11

- No survival data.
- 43% sensitivity for advanced precancerous lesions
- Comparison between stool DNA and FIT at one time point.
- FDA approved for average risk adults 45 and older.

Figure 1

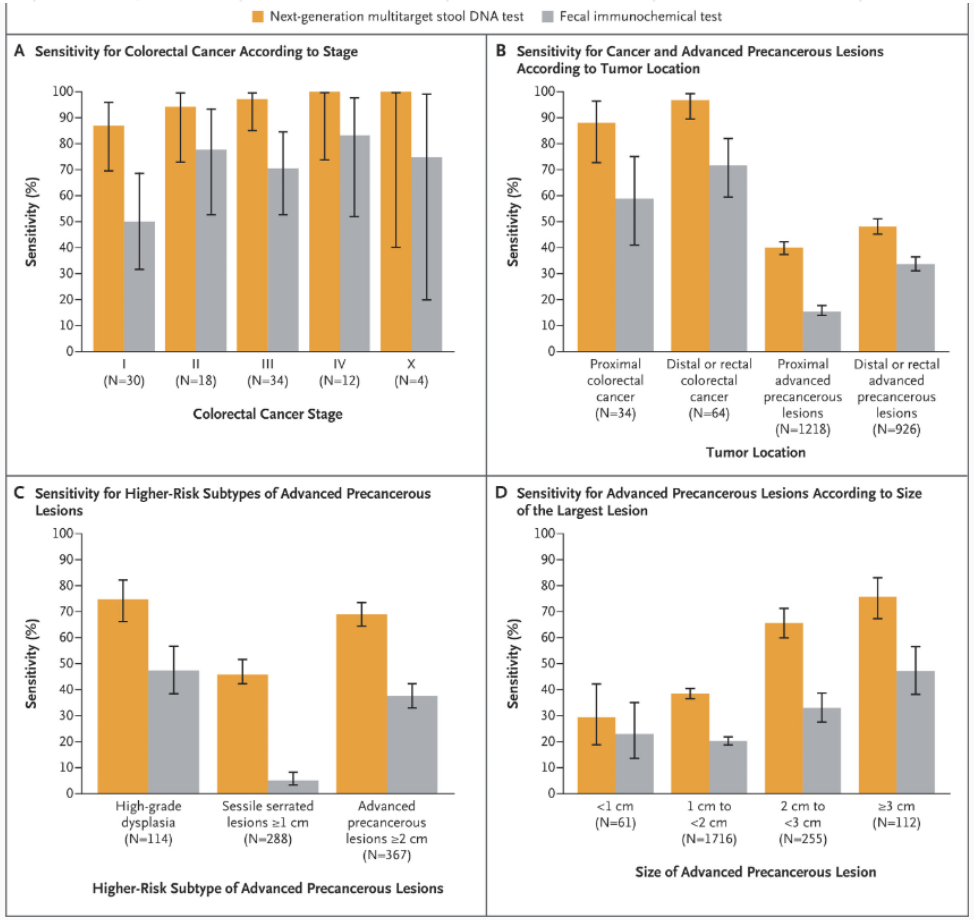


Table 1

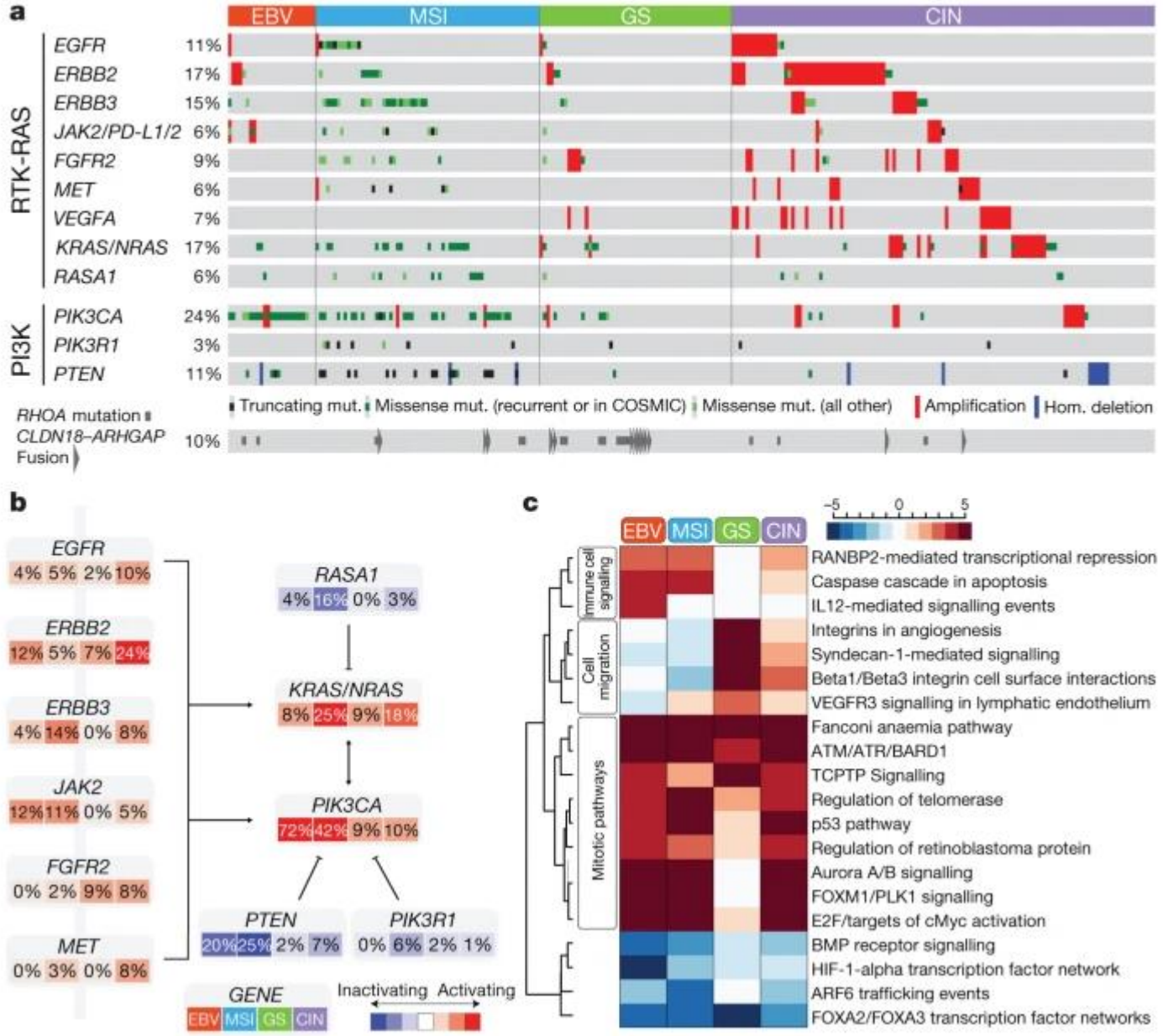
Variable	Colonoscopy (N=20,176)	Next-Generation Multitarget Stool DNA Test (N=20,176)		FIT (N=20,176)	
	No. of Participants	No. of Results	Assessment (95% CI) %	No. of Results	Assessment (95% CI) %
Sensitivity					
Colorectal cancer					
Any	98	92	93.9 (87.1–97.7)†	66	67.3 (57.1–76.5)
Stage I, II, or III‡	82	76	92.7 (84.8–97.3)	53	64.6 (53.3–74.9)
Advanced precancerous lesions	2,144	931	43.4 (41.3–45.6)†	500	23.3 (21.5–25.2)
High-grade dysplasia	114	85	74.6 (65.6–82.3)	54	47.4 (37.9–56.9)
Specificity					
Advanced neoplasia§	17,934	16,245	90.6 (90.1–91.0)	16,997	94.8 (94.4–95.1)¶
Nonneoplastic findings or negative colonoscopy‖	10,961	10,156	92.7 (92.2–93.1)	10,492	95.7 (95.3–96.1)
Negative colonoscopy**	7,510	7,012	93.4 (92.8–93.9)	7,207	96.0 (95.5–96.4)

*
In evaluations of sensitivity, numbers of positive results are shown, and in evaluations of specificity, numbers of negative results are shown. Statistical analyses are presented only for comparisons of the sensitivity for colorectal cancer and advanced precancerous lesions and of the specificity for advanced neoplasia between the next-generation multitarget stool DNA test and the fecal immunochemical test (FIT). CI denotes confidence interval.

†
P<0.001 for the comparison of the next-generation multitarget stool DNA test with FIT.

Gastric and Esophageal Cancers

Figure 5: Integrated molecular description of gastric cancer.



TCGA analysis of gastric cancers

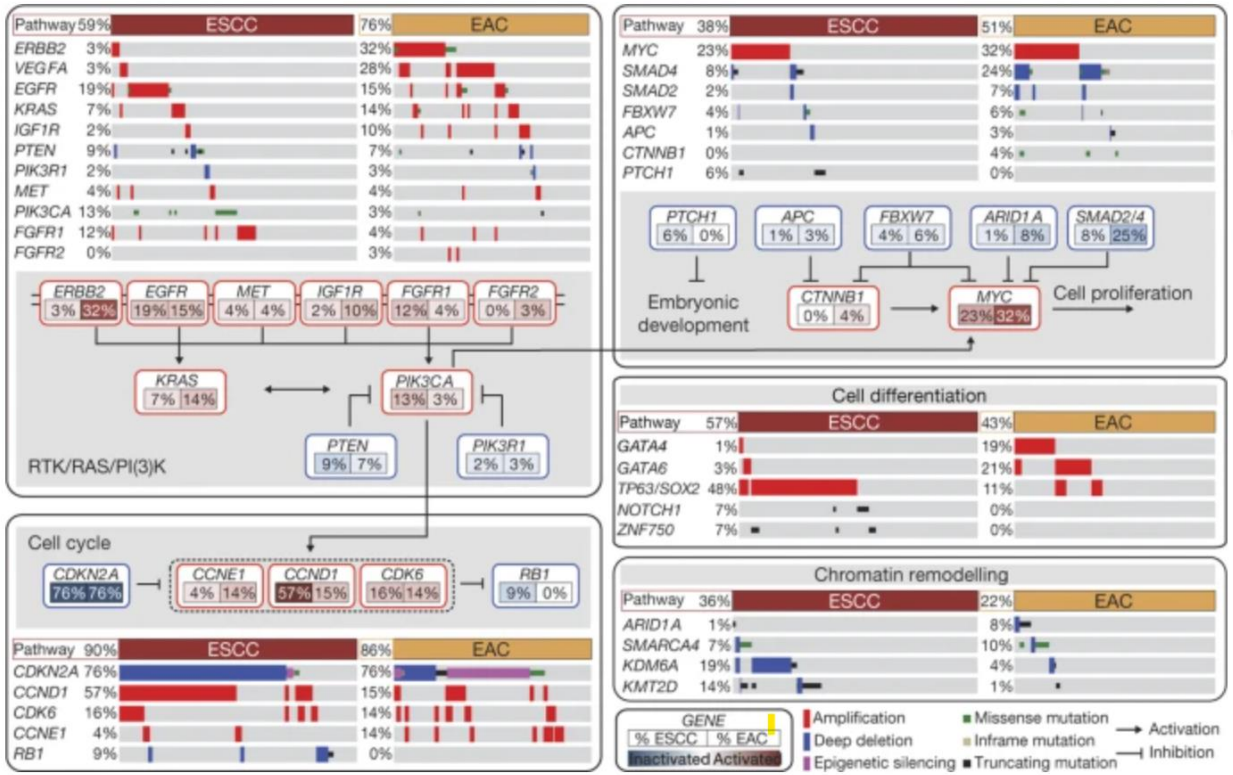
Four subtypes of gastric cancer:

- **Epstein–Barr virus positive tumors**
  - Recurrent PIK3CA mutations
  - DNA hypermethylation
  - Amplification of JAK2, PD-L1, PD-L2
- **Microsatellite unstable tumors**
  - Elevated mutation rate
- **Genomically stable tumors**
  - Frequent RHOA mutations/fusions
- **Chromosomal instability tumors**
  - Aneuploid and amplification of receptor tyrosine kinases

The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* **513**, 202–209 (2014).

TCGA analysis of oesophageal cancers

Figure 2: Integrated molecular comparison of somatic alterations across oesophageal cancer.



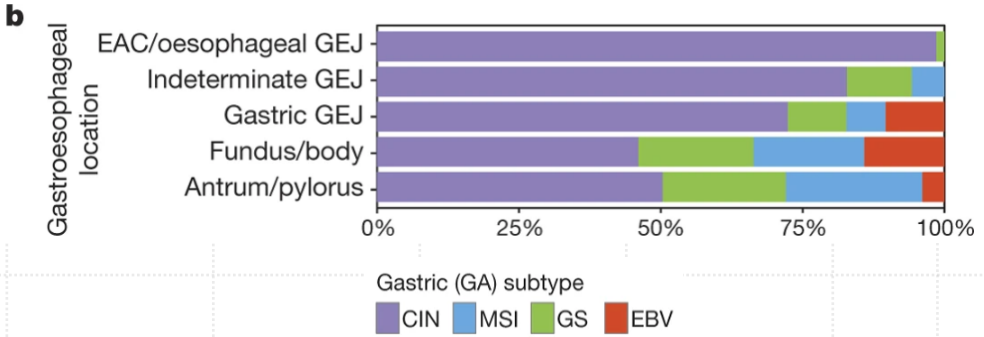
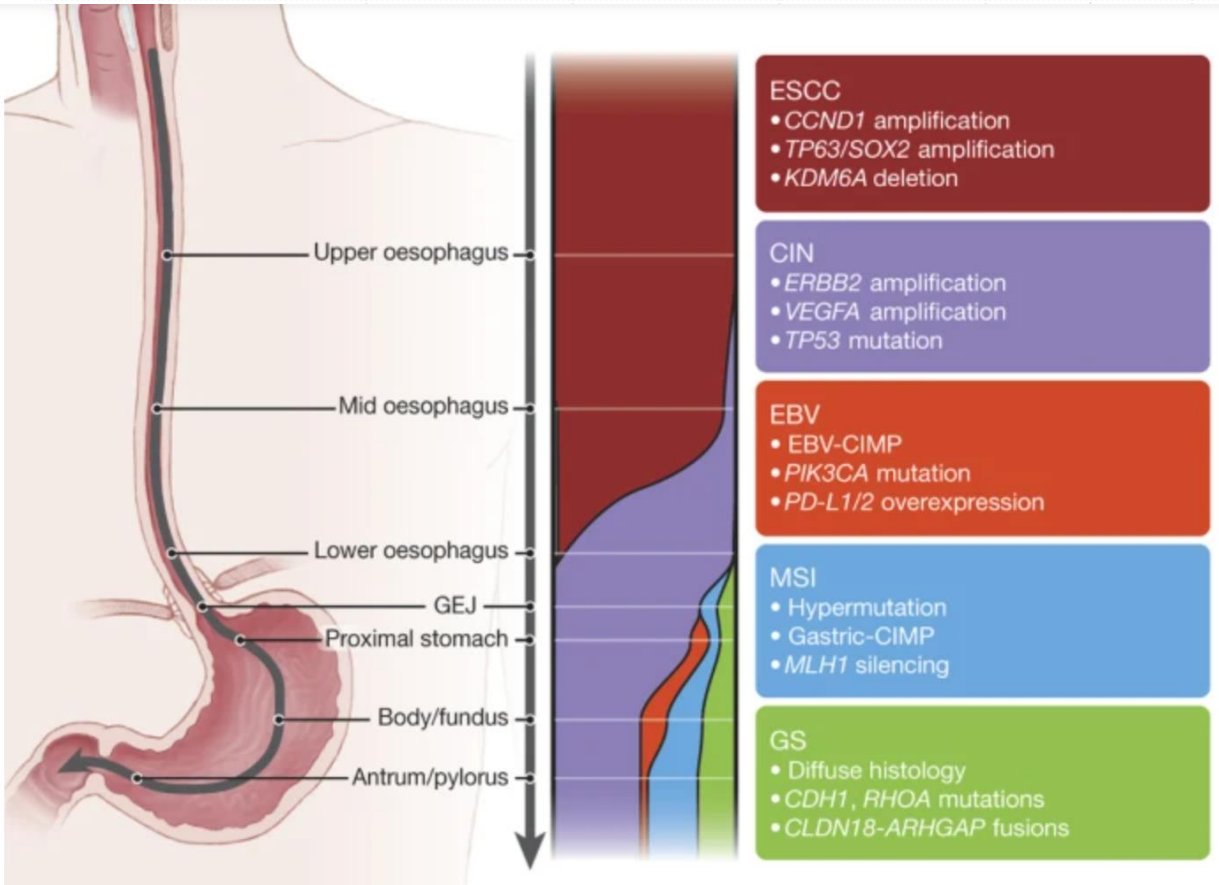


Figure 6: Gradations of molecular subclasses of gastroesophageal carcinoma



The Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. *Nature* **541**, 169–175 (2017). https://doi.org/10.1038/nature20805

HER2 (ERBB2) in Gastric Cancer

- ToGA trial (2020) – Phase III RCT comparing trastuzumab + chemotherapy (capecitabine/cisplatin or 5-FU/cisplatin) vs. chemo along in metastatic HER2 positive (3+)/FISH amplified gastric or gastroesophageal junction cancer**. Improved OS and PFS.**
- JACOB trial (2023) – Phase III RCT trastuzumab + pertuzumab + chemo vs. trastuzumab + chemo in HER2 positive metastatic gastric or gastroesophageal junction cancer. **No improvement OS with addition of pertuzumab.**
- KEYNOTE-811 Trial (2023) – Phase III RCT Trastuzumab + chemo + Pembrolizumab vs. without pembro in HER2 positive metastatic gastric or gastroesophageal junction cancer. **Improved response rate and PFS.**
- DESTINY – Gastric 01 Trial – Phase II RCR trastuzumab deruxtecan vs. trastuzumab + chemo in metastatic gastric cancers. **Improved OS and increased response rate.**

PD-L1 testing in Gastric/GEJ Cancers

$$CPS = \frac{\#\ PD\text{-}L1\ staining\ cells\ (tumor\ cells,\ lymphocytes,\ macrophages)}{Total\ \#\ of\ viable\ tumor\ cells} \times 100$$

- "For determination of PD-L1 expression, an objective of **20x magnification** is required assessing tumor cells for **partial or complete linear membrane staining** (at any intensity) that is perceived distinct from cytoplasmic staining."
- "Lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the **tumor nests and/or adjacent supporting stroma** with **convincing membrane and/or cytoplasmic staining (at any intensity).** MICs must be directly associated with the response against the tumor"

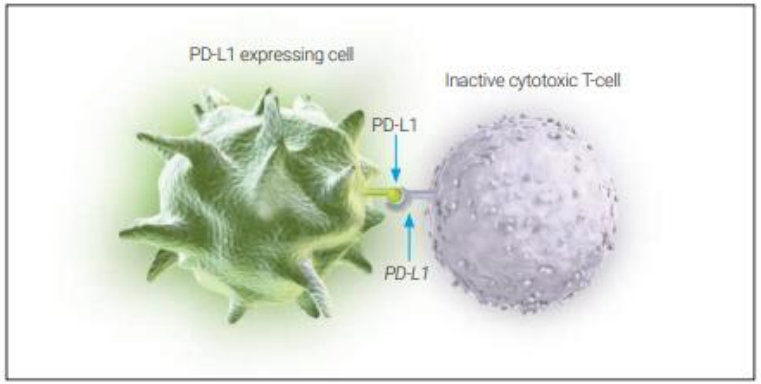


Figure 1: Inactivation of T-cells limits damage to normal tissue.

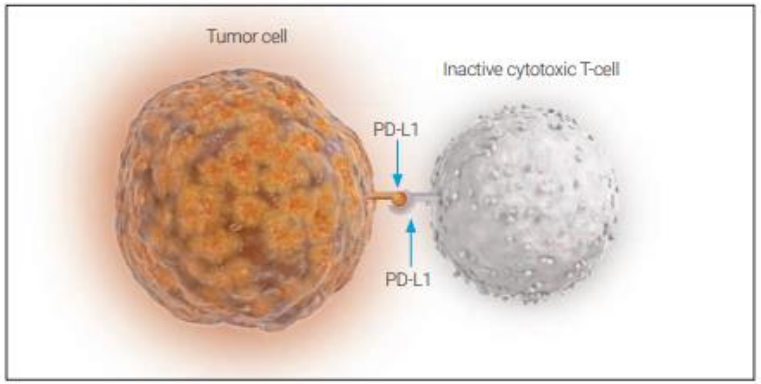


Figure 2: Inactivation of T-cells reduces tumor cell death and elimination.

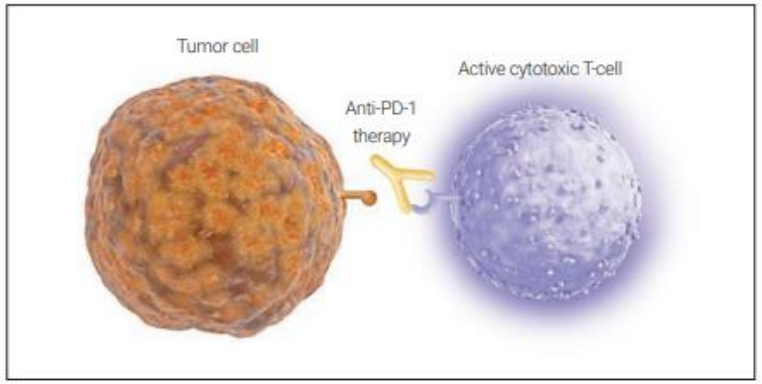


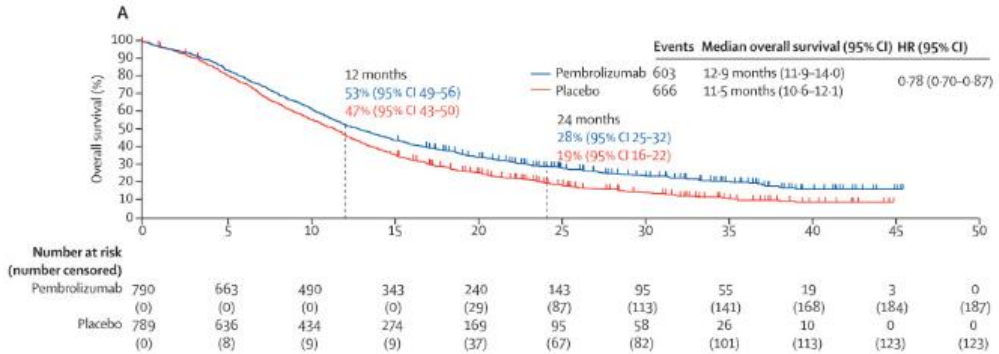
Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T-cells and tumor cell death and elimination.

Image: Agilent PD-L1 22C3 user manual

PD-L1 testing in Gastric/GEJ Cancers (Pembrolizumab)- 22C3

- KEYNOTE 859 –
  - Phase III RCT, unresectable or metastatic HER2 negative gastric or GEJ adenocarcinoma with no prior therapy.
  - Treatment pembrolizumab + investigators choice of chemo. vs. chemo.
  - **Regardless of PD-L1 status, patients with Pembrolizumab + chemo benefited**.
    - Subgroup analysis: Patients with higher CPS benefited from Pembro. more with improved OS and PFS.

	Pembrolizumab group, n/N	Placebo group, n/N	HR (95% CI)
Overall	603/790	666/789	0·78 (0·70–0·87)
PD-L1 CPS at baseline			
<1	139/172	140/172	0·92 (0·73–1·17)
≥1	464/618	526/617	0·73 (0·65–0·83)
1–9	274/337	300/345	0·83 (0·70–0·98)
<10	413/509	440/517	0·86 (0·75–0·98)
≥10	188/279	226/272	0·64 (0·52–0·77)



PD-L1 testing in Gastric/GEJ Cancers (Pembrolizumab)- 22C3

- KEYNOTE 811
  - Phase III RCT, unresectable or metastatic HER2 POSITIVE gastric or GEJ adenocarcinoma with no prior therapy.
  - Treatment pembrolizumab + trastuzumab + investigators choice of chemo. vs. trastuzumab + chemo.
  - OS and PFS still maturing, interim findings: **higher response rate and complete response rates.**

Pembrolizumab group

Placebo group

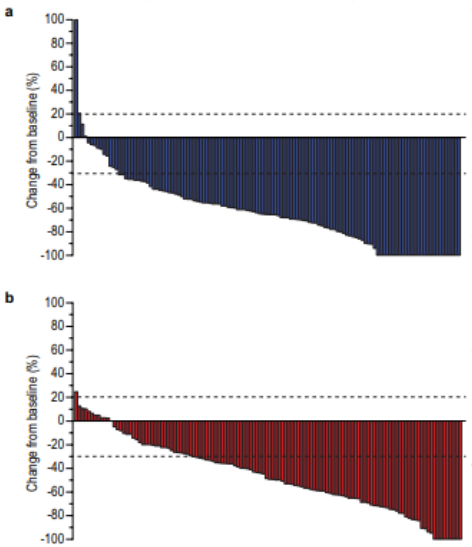


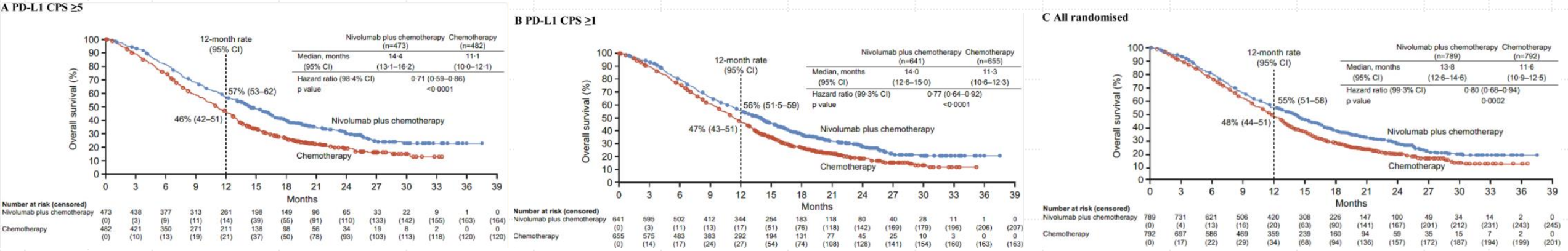
Fig. 1 | Best percentage change from baseline in the size of target lesions among participants in the efficacy population. a, Pembrolizumab group.

Table 1 | Summary of confirmed objective response in the efficacy population

Variable	Pembrolizumab group (*n* = 133)	Placebo group (*n* = 131)
Objective response (% (95% confidence interval))[a]	74.4 (66.2–81.6)	51.9 (43.0–60.7)
Disease control (% (95% confidence interval))[b]	96.2 (91.4–98.8)	89.3 (82.7–94.0)
Best overall response (number (%))		
Complete response	15 (11.3)	4 (3.1)
Partial response	84 (63.2)	64 (48.9)
Stable disease	29 (21.8)	49 (37.4)
Progressive disease	5 (3.8)	7 (5.3)
Not evaluable[c]	0 (0.0)	2 (1.5)
Not assessed[c]	0 (0.0)	5 (3.8)

PD-L1 testing in Gastric/GEJ Cancers (Nivolumab)- 28-8

- CHECKMATE- 649
  - Phase III RCT Nivolumab + chemotherapy (FOLFOX) vs. chemo alone in unresectable gastric/GEJ.
  - **CPS ≥ 5 and CPS ≥ 1 had improved OS and PFS than chemo but more so with CPS ≥ 5.**
- ATTRACTION-2
  - Phase III RCT Nivolumab monotherapy vs. placebo in heavily pretreated gastric/GEJ cancers.
  - **Improved OS regardless of PD-L1 status, although PD-L1 expression did predict durable response.**



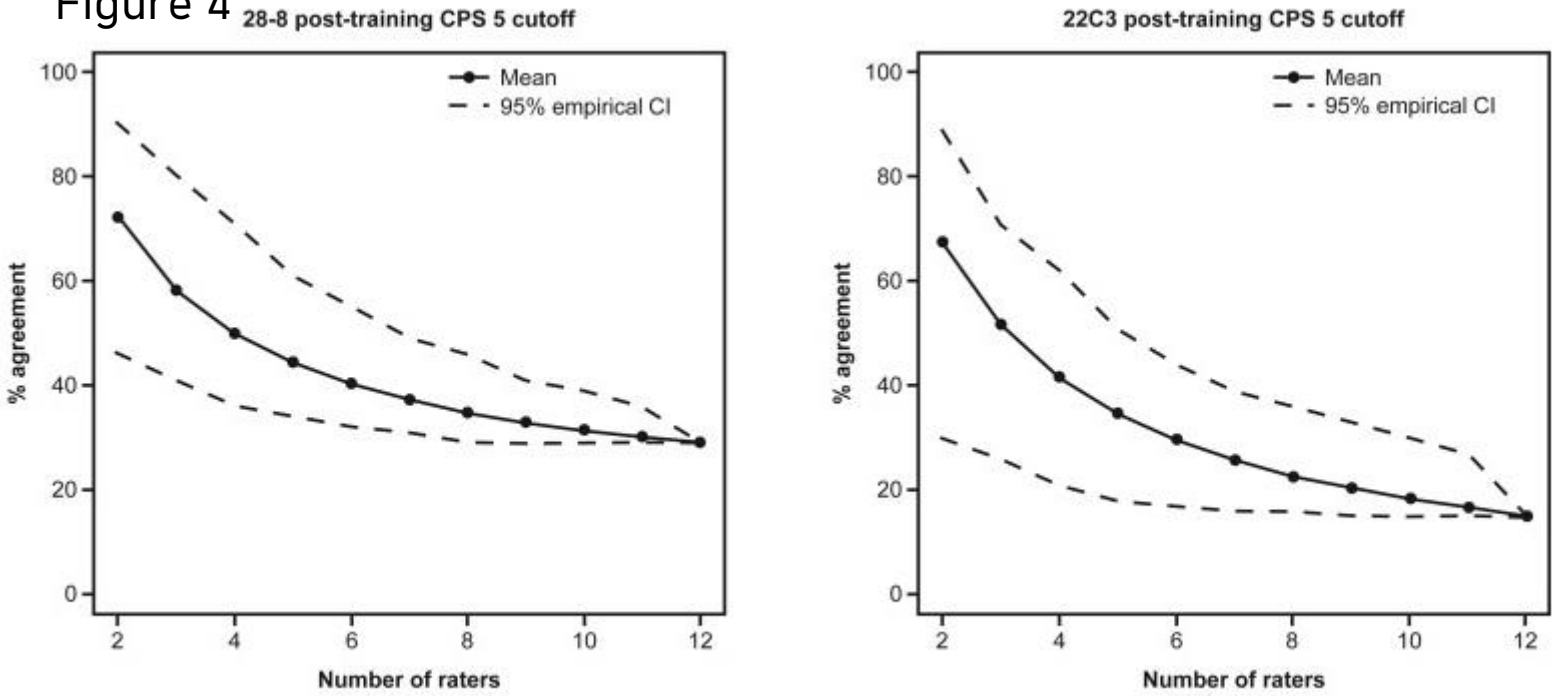
CHECKMATE- 649

CPS cut-offs for Gastric/GEJ

- **CPS ≥ 1: Minimal threshold for calling PD-L1 expression in stomach/GEJ.**
- **CPS ≥ 5: Stronger predictor of benefit with Nivolumab + chemo.**
- **CPS ≥ 10: Identified patients most likely to benefit from Pembrolizumab monotherapy.**

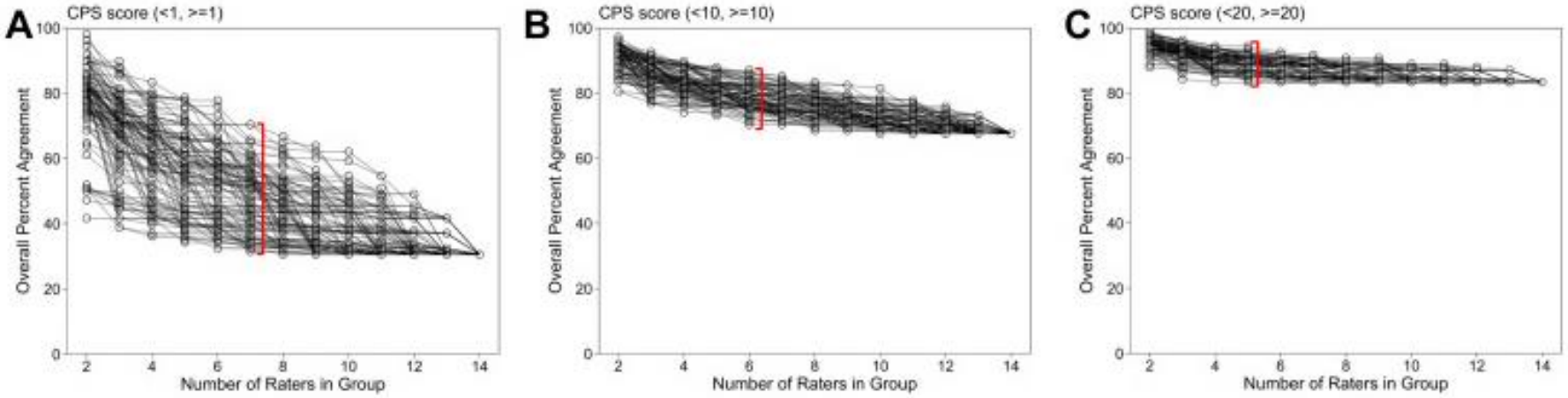
- Testing is recommended at the time of diagnosis for advanced, unresectable, or metastatic gastric/GEJ cancers.

Figure 4



High Interobserver Variability Among Pathologists Using Combined Positive Score to Evaluate PD-L1 Expression in Gastric, Gastroesophageal Junction, and Esophageal Adenocarcinoma
Robert, Marie E. et al. Modern Pathology, Volume 36, Issue 5, 100154

Figure viewer

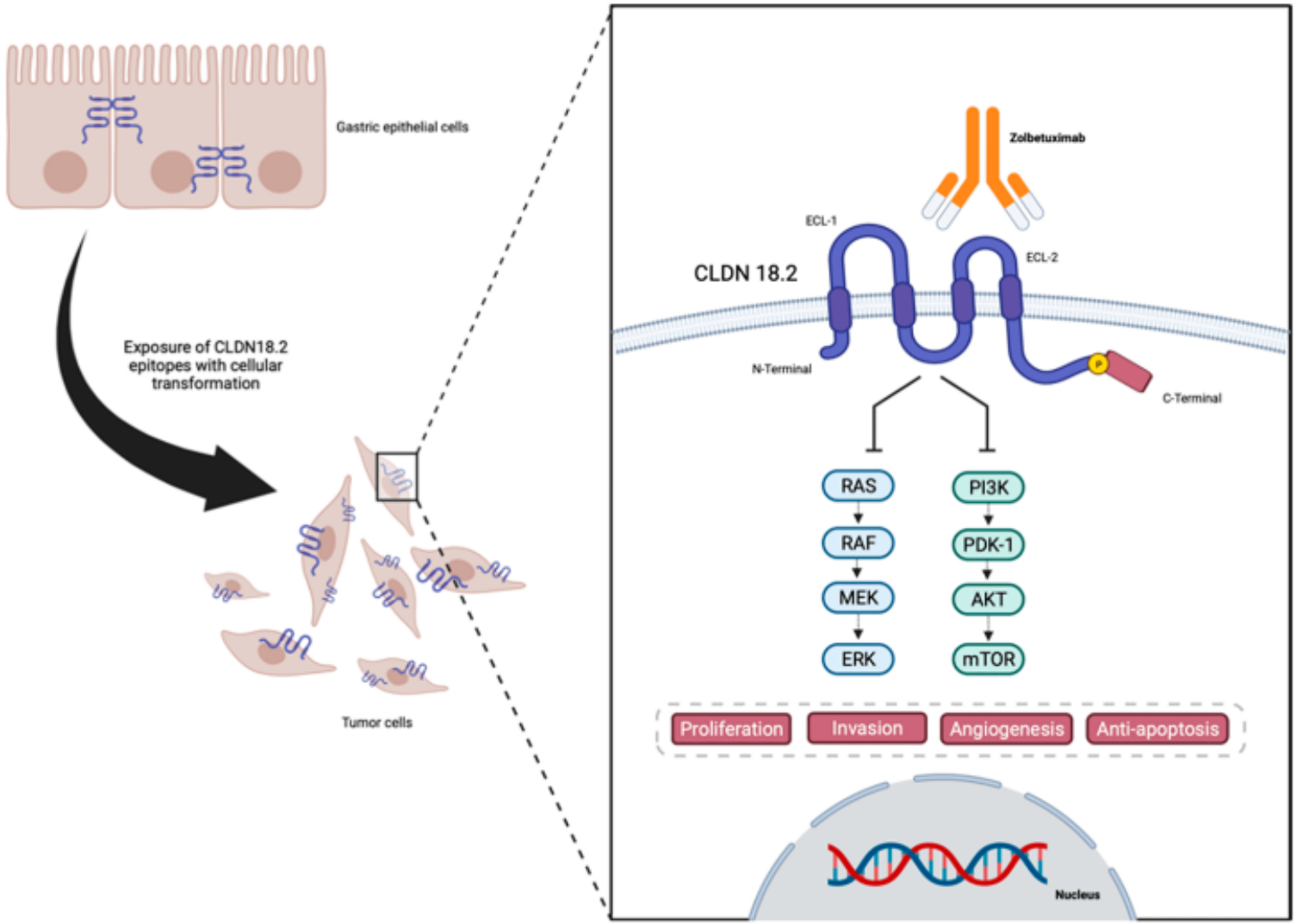


Fernandez AI et al. Multi-Institutional Study of Pathologist Reading of the Programmed Cell Death Ligand-1 Combined Positive Score Immunohistochemistry Assay for Gastric or Gastroesophageal Junction Cancer. Mod Pathol. 2023 May;36(5):100128.

Figure 4

Claudin 18.2

- Claudin 18.2 is an isoform of claudin-18 and a member of a class of transmembrane proteins, which are components of tight junctions between epithelial cells.
- Expressed in 30-40% of gastric cancers, with a slightly greater expression profile in diffuse type gastric cancer.
- During malignant transformation, the loss of cell polarity exposes the Claudin 18.2 epitope, making it more accessible for targeting.
- Claudin 18.2 is expressed almost exclusively in the gastric tissue (or epithelium with gastric type differentiation).



Claudin 18.2

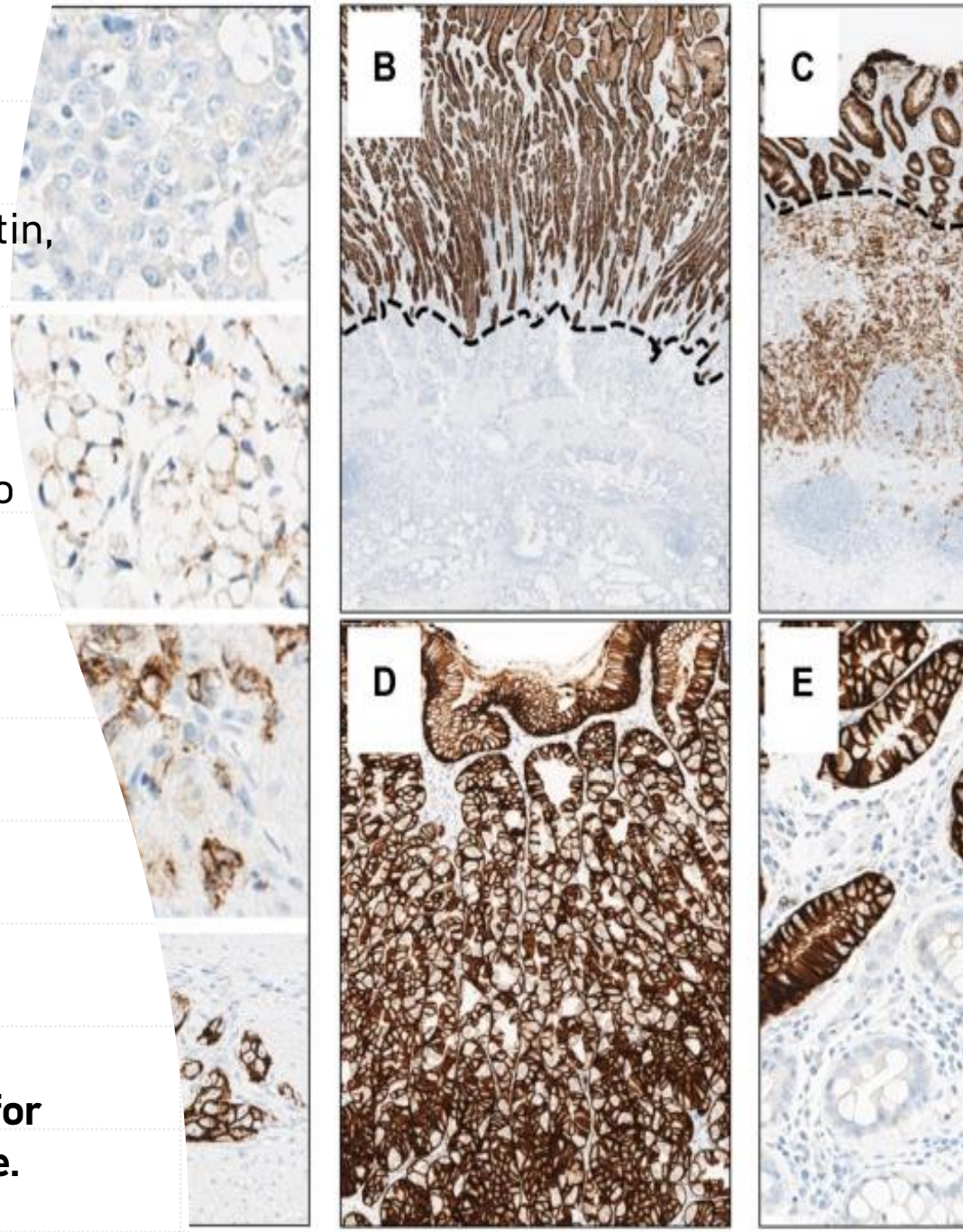
- FAST trial
  - Phase II Zolbetuximab + chemotherapy (epirubicin, oxaliplatin, capecitabin (EOX) vs chemo. alone.
  - **Improved OS and PFS with Zolbetuximab.**

- SPOTLIGHT trial
  - Phase III Zolbetuximab + chemotherapy (FOLFOX) vs. chemo alone in unresectable gastric/GEJ cancers.
  - **Significant PFS and OS benefit in Zolbetuximab arm in first line treatment (preliminary data).**

- GLOW trial
  - Phase III Zolbetuximab + chemotherapy (CAPOX) vs. chemo alone in unresectable gastric/GEJ cancers.
  - **Improved PFS and OS benefit in Zolbetuximab arm in first line treatment (preliminary data).**

NCCN recommendation –

Zolbetuximab with chemotherapy is recommended in the first line for advanced/unresectable gastric/GEJ cancers which are HER2 negative.



Claudin 18.2

- POSITIVE: ≥ 75% viable tumor cells demonstrating moderate to strong membrane CLDN1.8.2 staining (2+ or 3+ intensity)
- NEGATIVE: < 75% viable tumor cells demonstrating moderate to strong membrane CLDN18.2 staining.

Investigational biomarkers for gastric/GEJ cancers

- **FGFR2 inhibitors for FGFR2 amplified tumors (5–10% of gastric or GEJ cancers)**
- **MET inhibitors of MET amplified tumors (5–10% of gastric or GEJ cancers)**

Esophageal squamous cell carcinoma

Esophageal SCC biomarkers

- Pan-cancer markers
  - BRAF
  - MMR
  - TMB
  - NTRK
  - RET
  - HER2
- PD-L1

PD-L1 for ESCC

- KEYNOTE-181 – Phase III trial pembrolizumab vs. investigator choice chemo. for metastatic esophageal cancer (predominantly SCC). **PD-L1 CPS cutoff of 10. Pembrolizumab led to improved OS.**
- KEYNOTE-590 – Phase III trial pembrolizumab + chemo. vs. chemo. alone. for advanced esophageal cancer (SCC and adeno). **Pembrolizumab plus chemo. led to significant OS and PFS for ESCC with CPS ≥ 10.**
- CHECKMATE- 648 – Phase III trial nivolumab + chemo. or Nivolumab + ipilimumab vs. chemo. For advanced esophageal SCC. Evaluated CPS at CPS ≥ 1 and CPS ≥ 10. **Improved OS for nivolumab + chemo for CPS ≥ 1 and CPS ≥ 10.**
- ATTRACTION – 3 – Phase III trial nivolumab vs. chemo. In second line setting for advanced ESCC. Evaluated CPS at CPS ≥ 1 and CPS ≥ 10.

CPS cut-offs for Esophagus

- **CPS ≥ 1: Minimal threshold for calling PD-L1 expression in esophagus.**
- **CPS ≥ 5: Stronger predictor of benefit with Nivolumab + chemo.**
- **CPS ≥ 10: Identified ESCC patients most likely to benefit from Pembrolizumab.**

} Esophageal adenocarcinoma

- Testing is recommended at the time of diagnosis for advanced, unresectable, or metastatic gastric/GEJ cancers.

Conclusion

- Pan-cancer biomarkers
  - MMR
  - TMB
  - NTRK
  - RET
  - HER2
  - BRAF
- Colon Cancer
  - Extended RAS/RAF testing
  - ctDNA and stool DNA
- Gastric and Esophageal Cancer
  - PDL1
  - Claudin 18.2