MAYO CLINIC

BIOMARKERS AND ACTIONABLE ALTERATIONS IN GASTROINTESTINAL CANCER

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Dr. Matkowskyj attended the University of Illinois in Chicago for her graduate and medical school degrees and then completed her Anatomic Pathology Residency and GI/Liver Pathology Fellowship at Northwestern University/McGaw Medical Center in Chicago. She is a board-certified anatomic pathologist, who practiced at the University of Wisconsin-Madison for 12 years. She recently relocated and is a Professor in the Department of Laboratory Medicine at Mayo Clinic in Rochester, Minnesota. She serves on several NIH review panels, and is a member of the CAP Cancer Committee and Gastric HER2 Guidelines Panel, She previously served as a member of the NCCN Guideline panels for Esophageal and Gastric Cancers. Dr. Matkowskyj's research focuses on novel biomarkers and experimental therapeutics in gastrointestinal and pancreaticobiliary malignancies.

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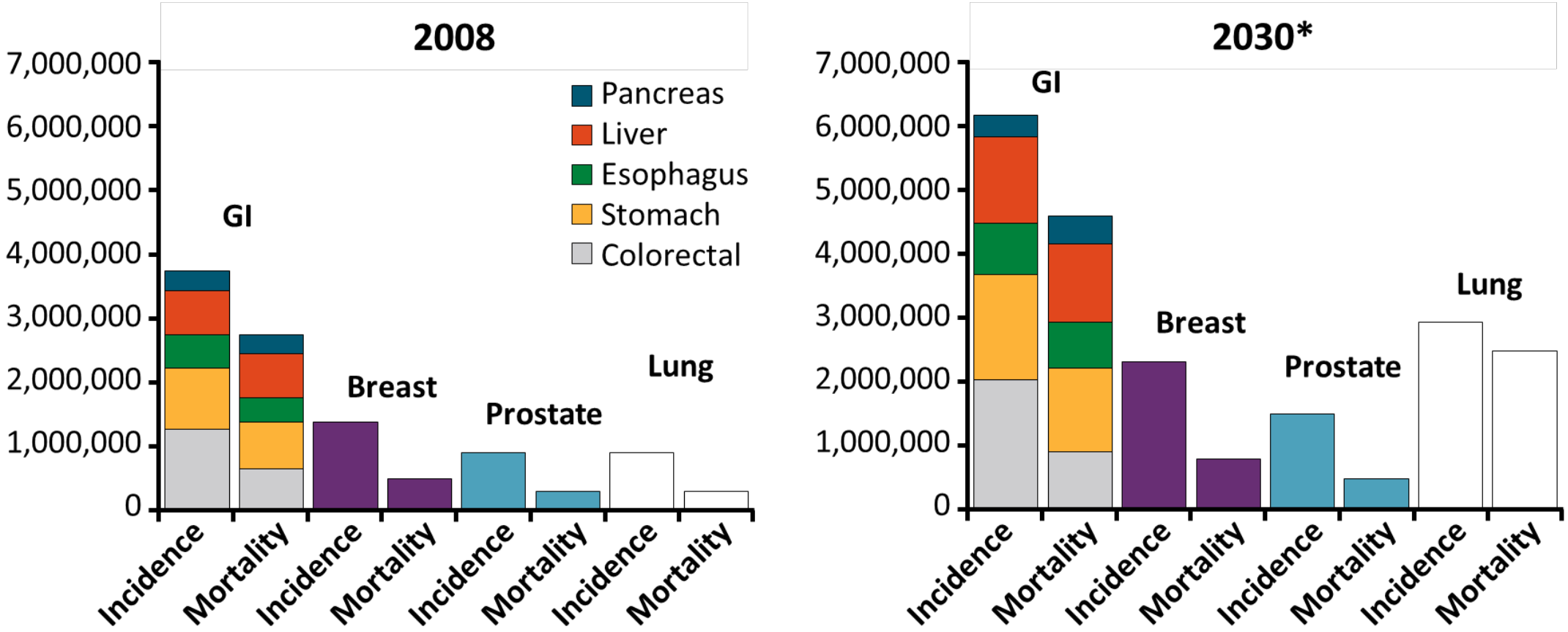
LEARNING OBJECTIVE

- Financial Disclosures
- Role and Repertoire of Current Biomarkers
- Actionable Mutations/Alterations
- Real World Cases
- Recently Approved & Emerging Biomarkers
- Summary

FINANCIAL DISCLOSURES

- AstraZeneca
- Amgen, Inc.
- Astellas Pharma US, Inc.
- Daiichi Sankyo, Inc.
- Elephas Bio, Corp.
- Merck

WORLDWIDE CANCER STATISTICS

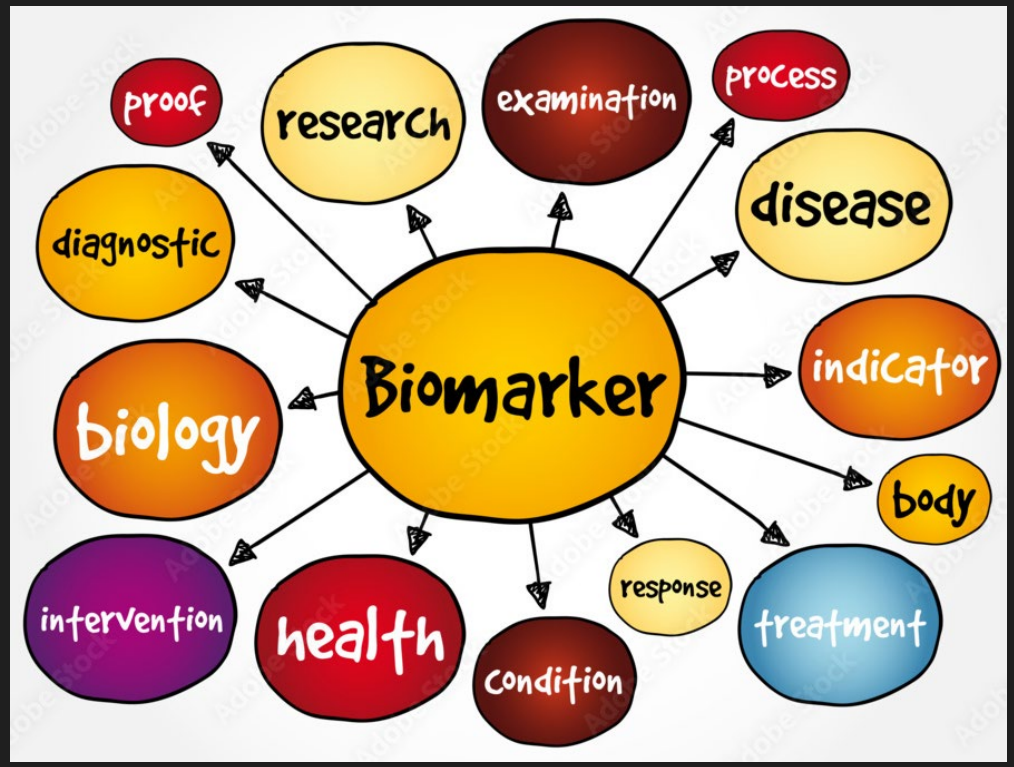


*2030 values estimated using projected incidence and mortality rates from 2008 to 2030 and weighting for prevalence in developed vs developing countries.

Bray. Lancet Oncol. 2012;13:790. Globocan 2018. International Agency for Research on Cancer.

Slide credit: clinicaloptions.com

Role & Repertoire of Current GI Biomarkers



WHAT ARE BIOMARKERS?

- Biomarkers are helpful for disease diagnosis, monitoring disease progression, predicting disease recurrence, and treatment monitoring.
- Clinical cancer biomarker utilization is progressively increasing with early cancer detection and therapeutics enhancement at the core for cancer management.
- Over the last 10 years, several biomarkers have been established and are considered in “regular” use.
- Biomarkers in clinical use need to be ***sensitive, specific, easily interpretable*** and ***clinically convenient*** for ease of implementation.
- Nonetheless, discovery of new cancer biomarkers remains important, especially for tumors with a dismal prognosis.

Silent Polling Question

How would you rank your familiarity with clinical practice guidelines for gastrointestinal cancer recommendations for testing?

A. Not familiar

B. Slightly familiar

C. Moderately familiar

D. Very familiar

BIOMARKERS SPECIFIC TO GI CANCERS

- The identification of clinically actionable biomarkers has enabled their incorporation into clinical practice paradigms/management guidelines.
- Based on NCCN review for gastrointestinal/pancreaticobiliary cancers, common biomarkers include:
  - IHC: MMR, HER2, PD-L1, BRAF
  - Molecular: MSI, TMB, BRAF, RAS, HER2
  - Fusions: NTRK, RET, FGFR
- Despite the availability of these clinically actionable biomarkers, a deeper understanding of the roles they play in tumor biology, optimization of biomarker testing/scoring, the development of additional biomarkers are still needed to improve patient outcomes.

Established Biomarkers in Gastrointestinal Cancers

HER2

A biomarker associated with activation of downstream signalling that leads to uncontrolled **cell-cycle progression**, **cell division/proliferation**, **motility**, **invasion,** and **adhesion**

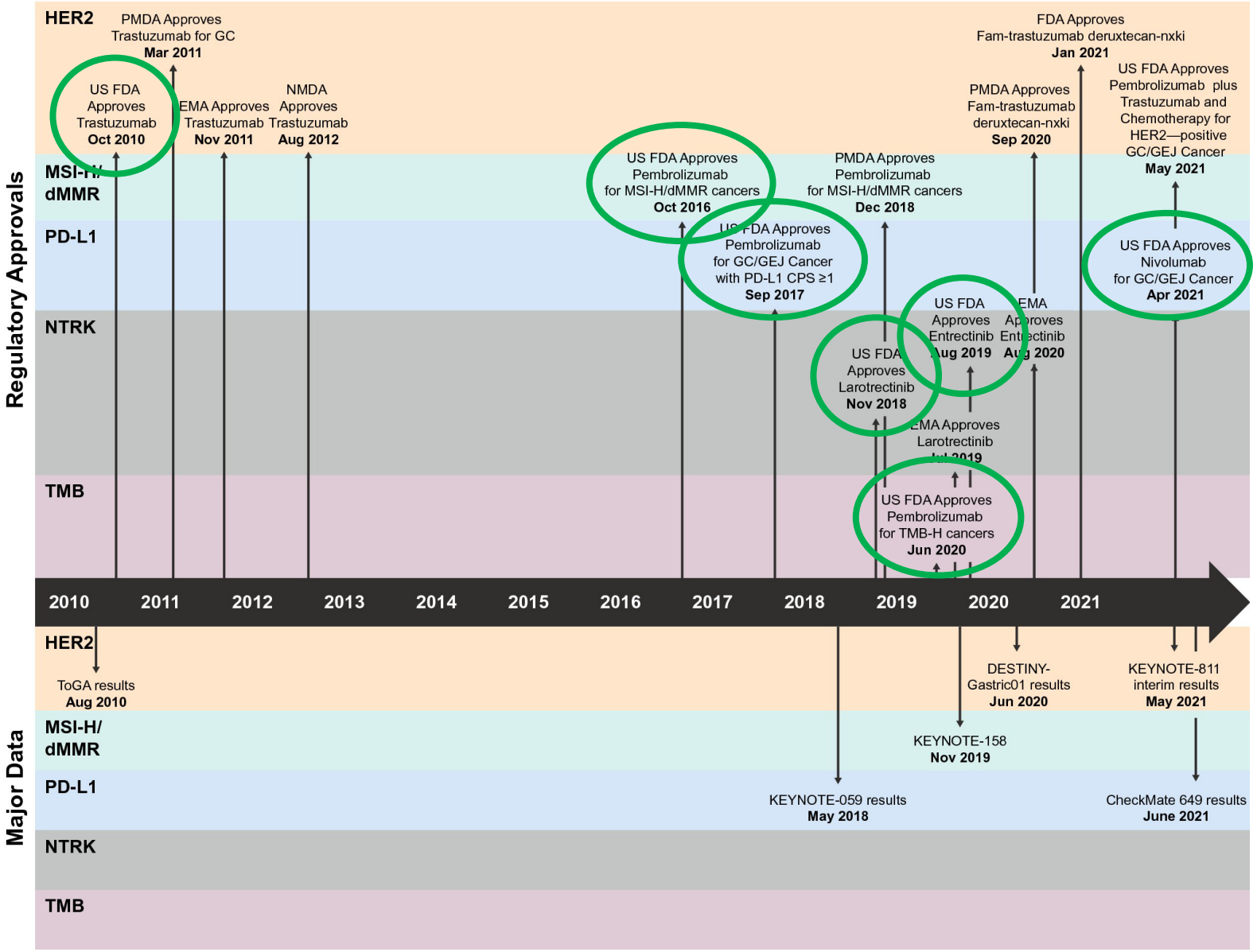
MMR/MSI

A biomarker associated with a **deficiency in the DNA MMR system** resulting in an increased rate of somatic mutations within the cells that can also be identified through **mutations in microsatellite sequences**

PD-L1

A biomarker that can bind to the immune checkpoint receptor PD-1, which allows **tumors to escape immune surveillance**

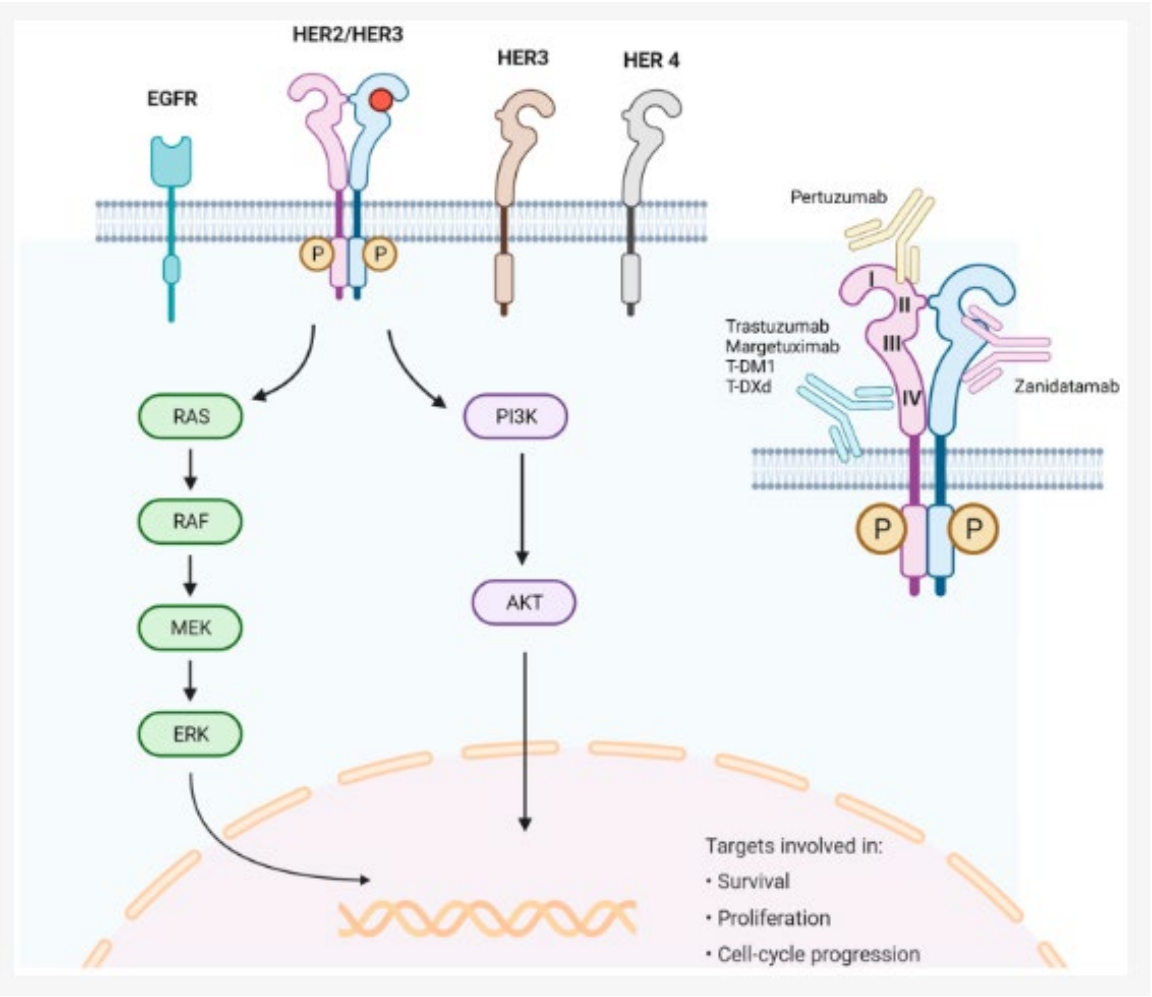
EXAMPLE OF BIOMARKER APPROVAL TIMELINE



Journal of Cancer Research and Clinical Oncology (2022) 148: 2023–2043.

HER2/ERBB2

- The HER2/ERBB2 protein is a receptor on the surface of cells and is part of the epidermal growth factor (EGF) receptor family.
- It binds as a heterodimer, which stabilizes ligand binding and activates downstream signaling pathways.
- Trastuzumab is a monoclonal antibody against human epidermal growth factor receptor 2 (HER2) that is used in combination with chemotherapy in cancer.
- Trastuzumab binds to an extracellular domain of this receptor and inhibits HER2 homodimerization, thereby preventing HER2-mediated signaling.
- It is also thought to facilitate antibody-dependent cellular cytotoxicity, leading to the death of cells that express HER2.
- Traztuzumab and biosimilar compounds like trastuzumab deruxtecan are also used for other HER2 positive cancers.



Bull Cancer 2023; 110: 552–559

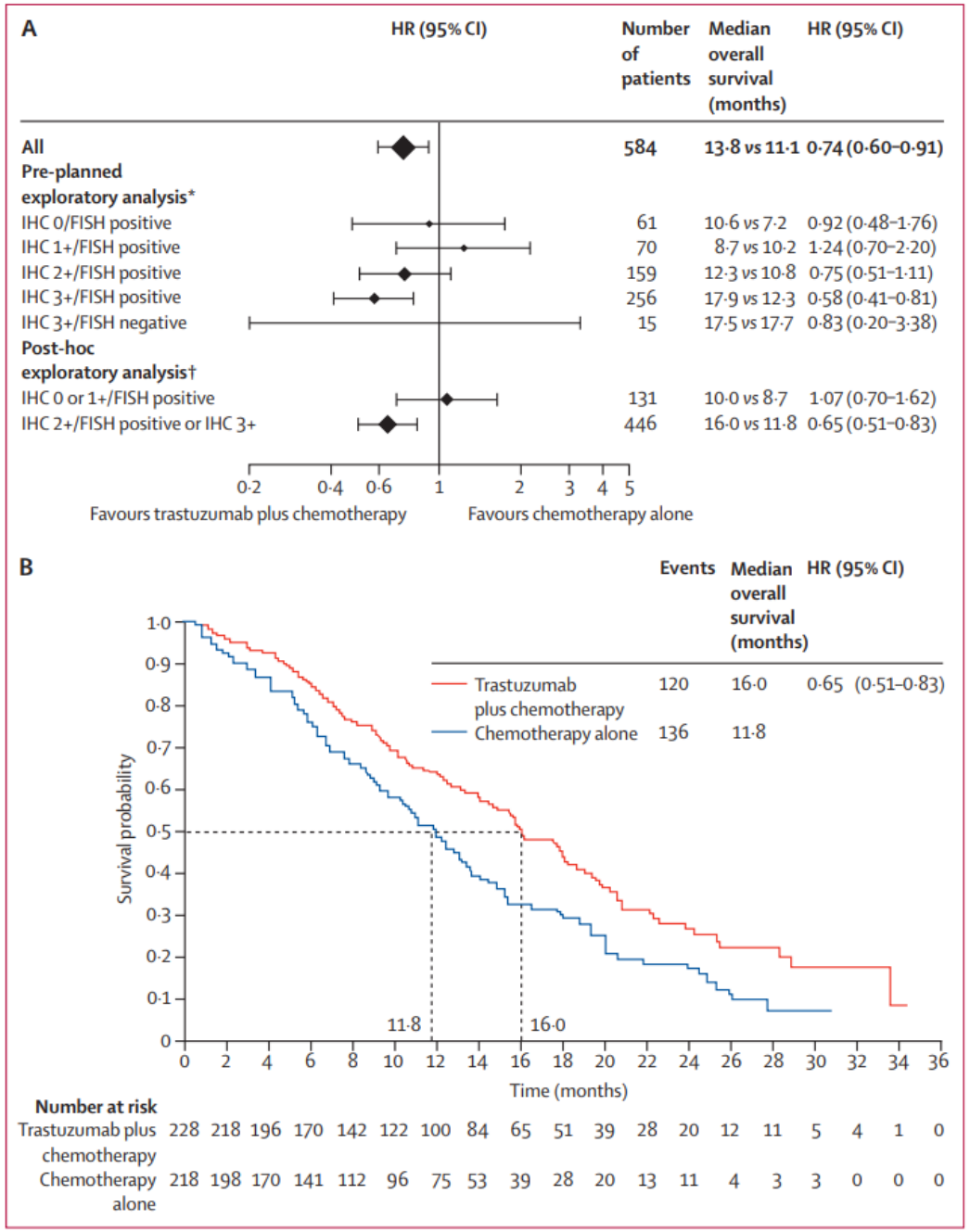
Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial

Yung-Jue Bang, Eric Van Cutsem,* Andrea Feyereislova, Hyun C Chung, Lin Shen, Akira Sawaki, Florian Lordick, Atsushi Ohtsu, Yasushi Omuro, Taroh Satoh, Giuseppe Aprile, Evgeny Kulikov, Julie Hill, Michaela Lehle, Josef Rüschoff, Yoon-Koo Kang, for the ToGA Trial Investigators†*

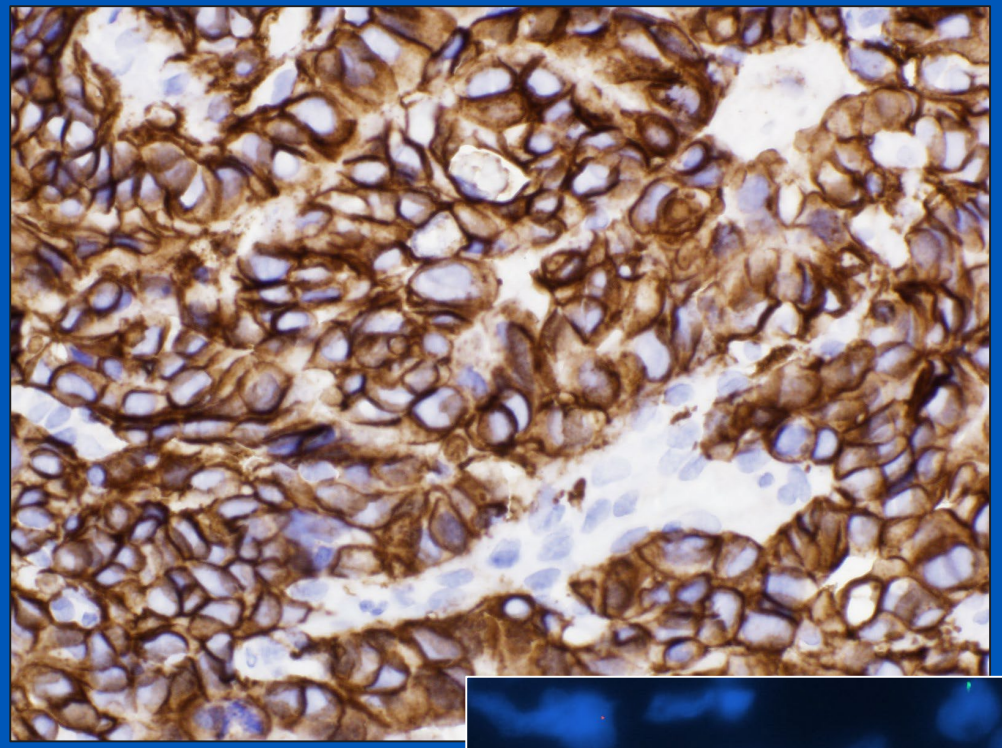
	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

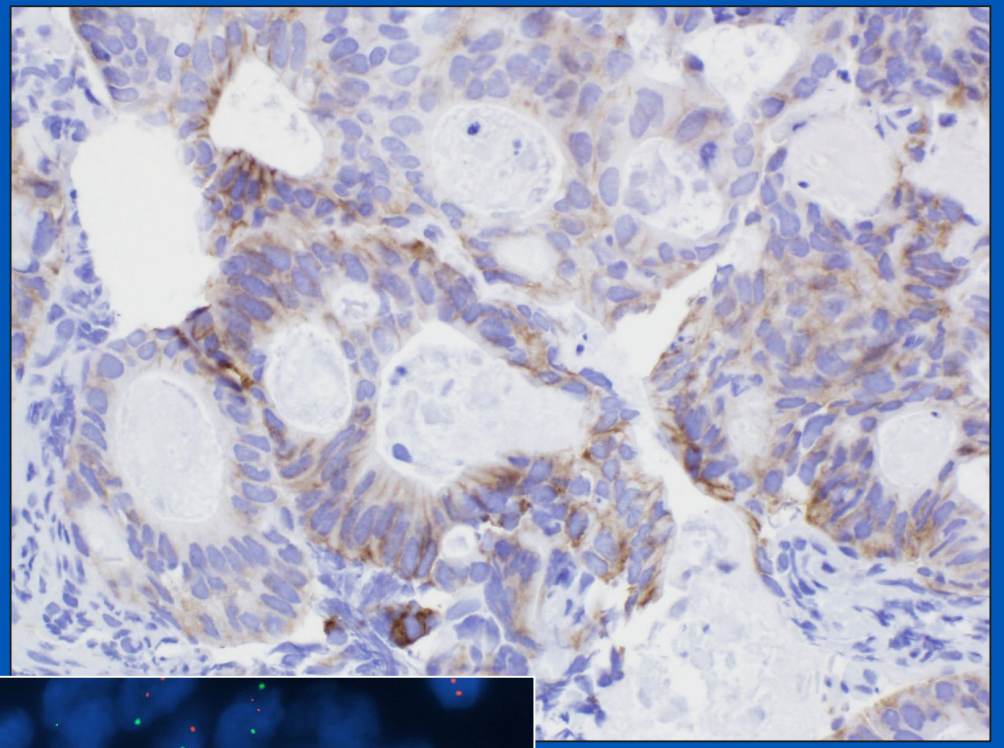
HER2=human epidermal growth factor receptor 2 (also known as ERBB2).

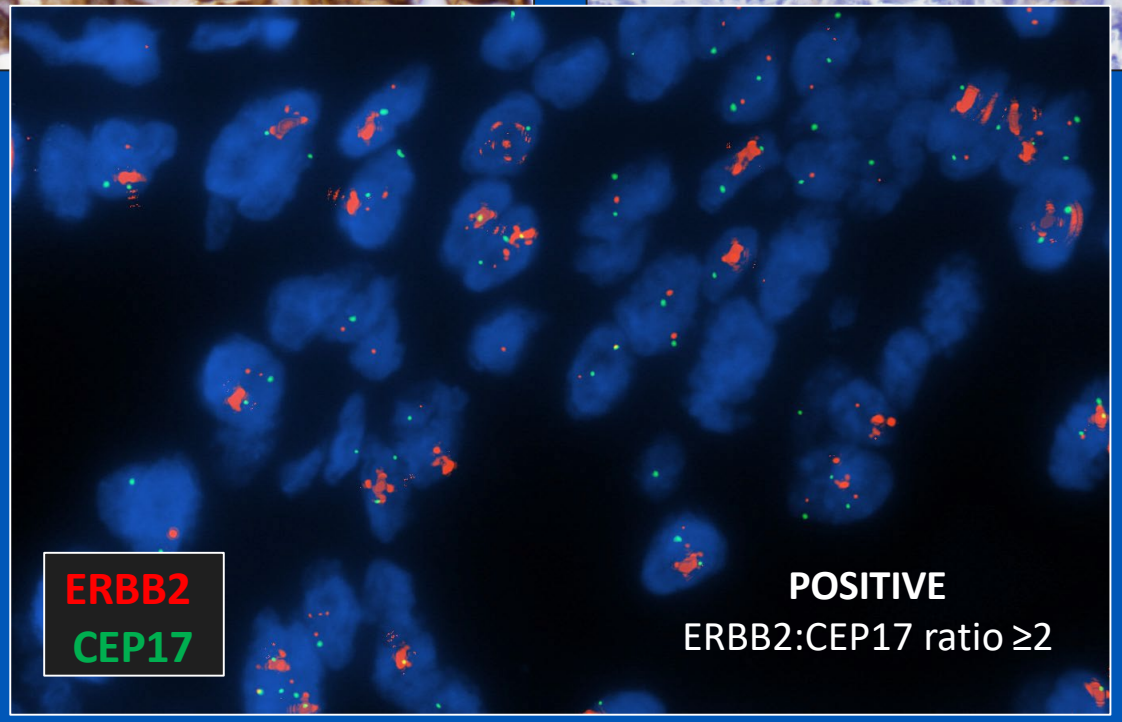
Table 1: **Immunohistochemistry scoring for HER2 in gastric and gastro-oesophageal junction cancer, by type of diagnostic specimen**



Lancet 2010; 376: 687–97

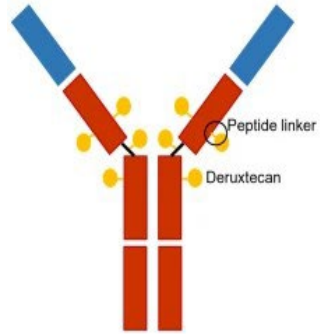




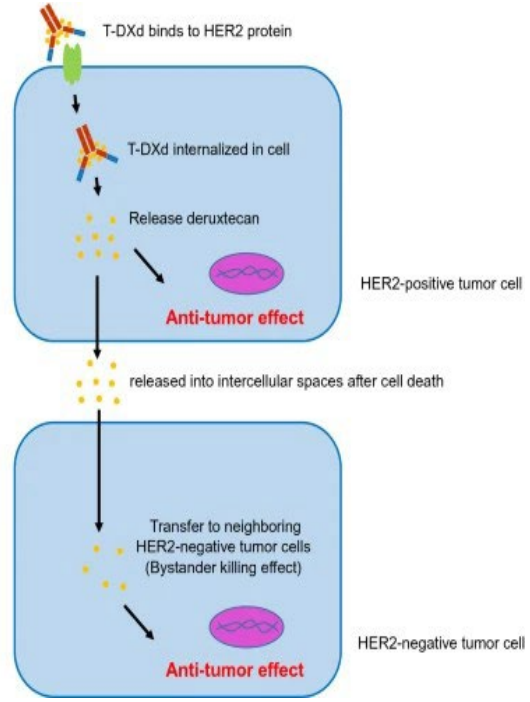


FDA Grants HER2+ Tumor Agnostic Approval

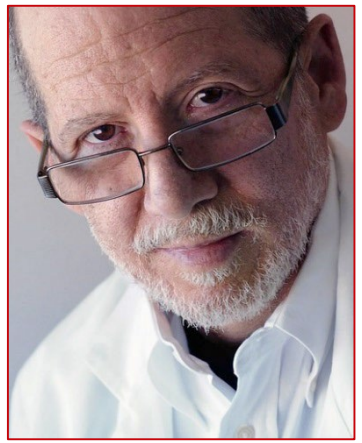
- In April 2024, the FDA granted accelerated approval to trastuzumab deruxtecan for adult patients with unresectable or metastatic HER2-positive (IHC3+) solid tumors.
- Highlights HER2-positive as a tumor-agnostic biomarker.
- Patients may have received prior systemic treatment and have no satisfactory alternative treatment options.
- Approval based on 3 trials showing objective response rate (ORR) of 51.4%, 52.9%, and 46.9%, with median durations of response (DOR) ranging from 5.5 to 19.4 months across the studies :
  - DESTINY-PanTumor02 (NCT04482309)
  - DESTINY-Lung01 (NCT03505710)
  - DESTINY-CRC02 (NCT04744831)

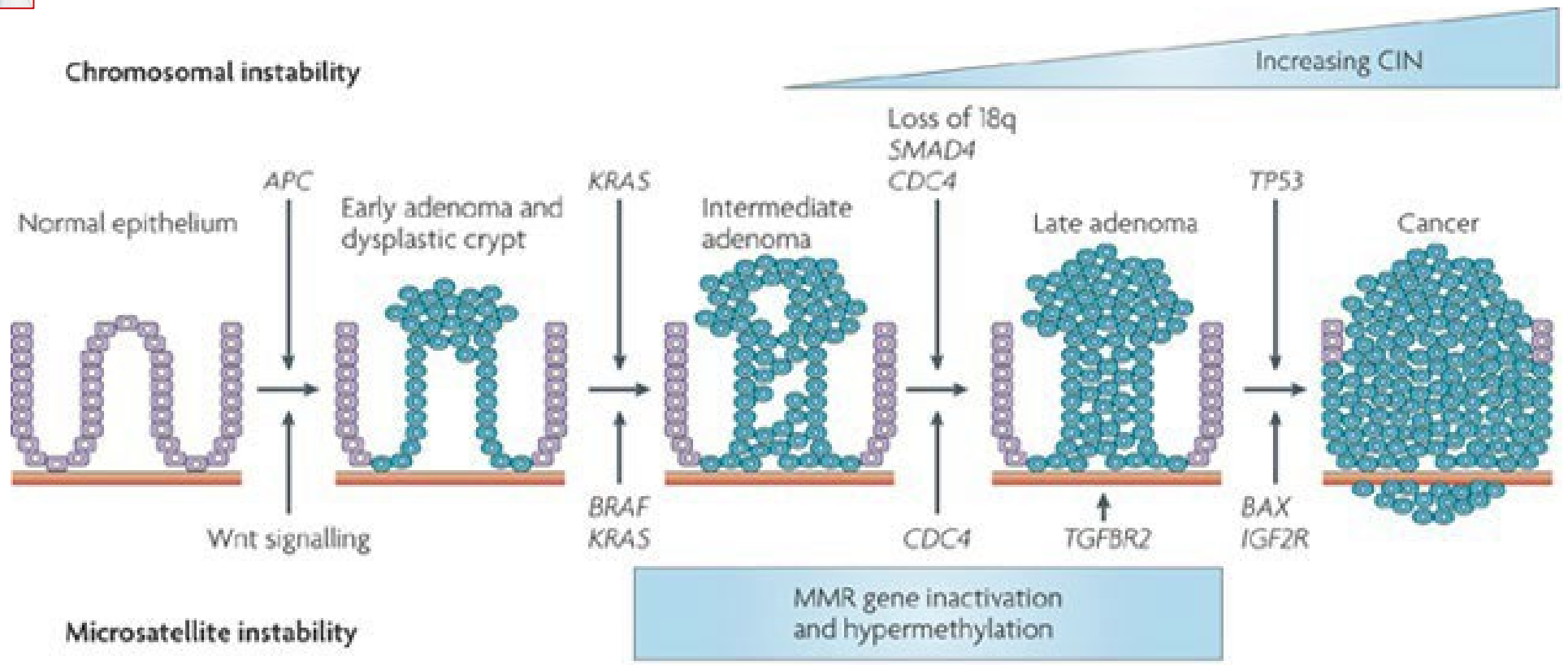


Trastuzumab deruxtecan (T-DXd)

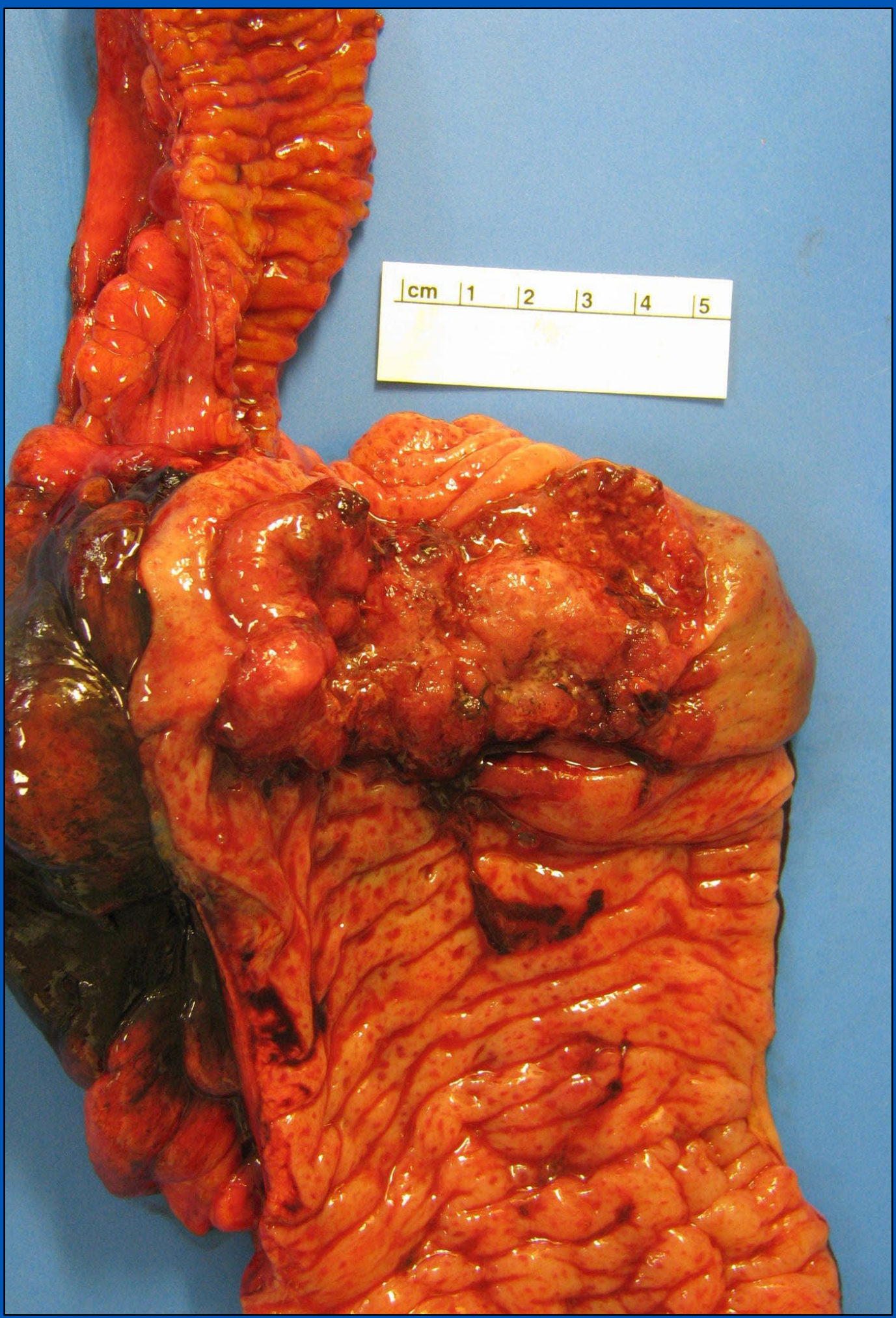


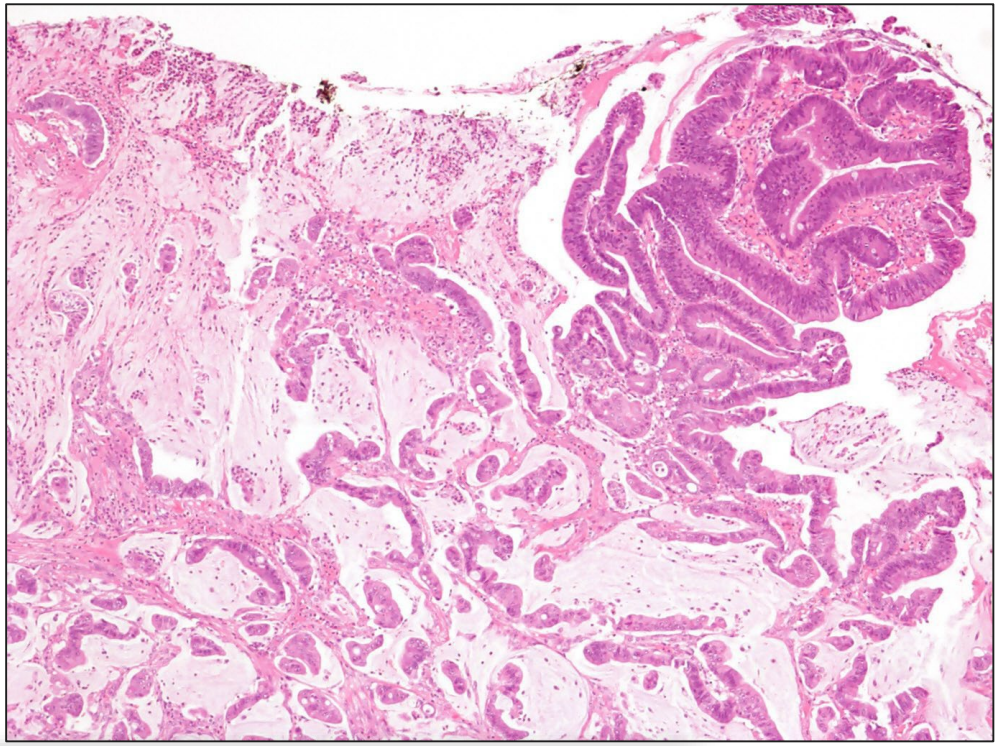
Meric-Bernstam F et al., *J Clin Oncol*. 2024;42(1):47-58.
Smit EF et al., *Lancet Oncol*. 2024;25(4):439-454.
Raghav KPS et al., *J Clin Oncol*. 2023;41(suppl 16).

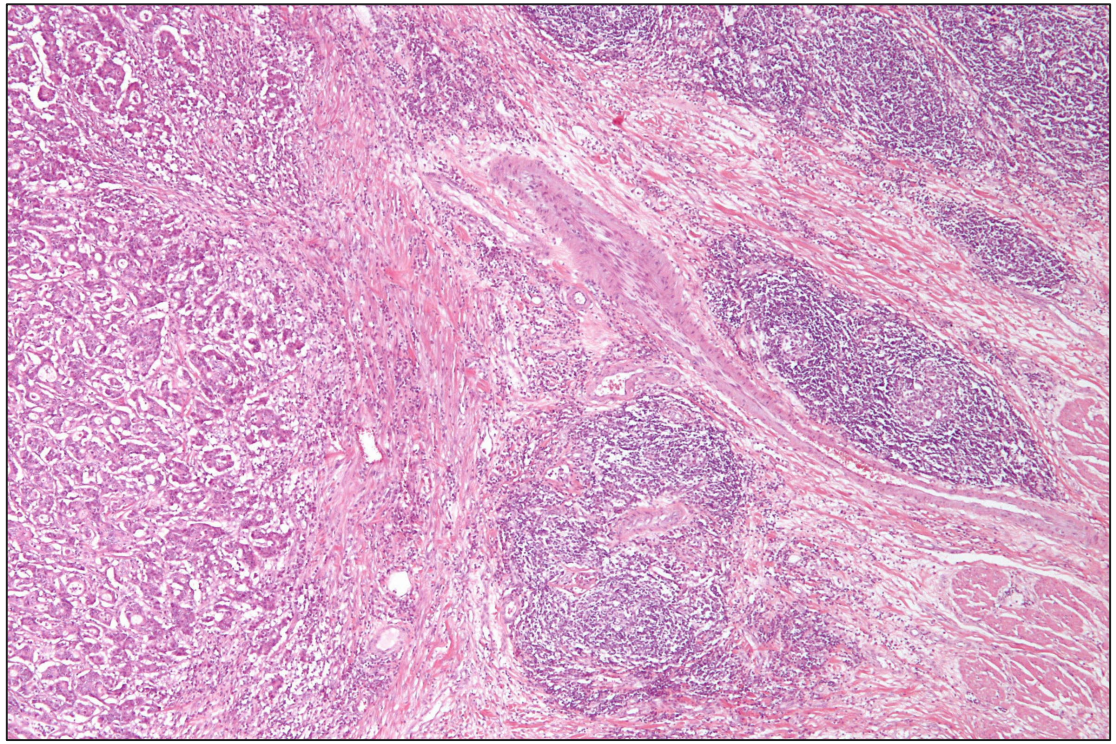


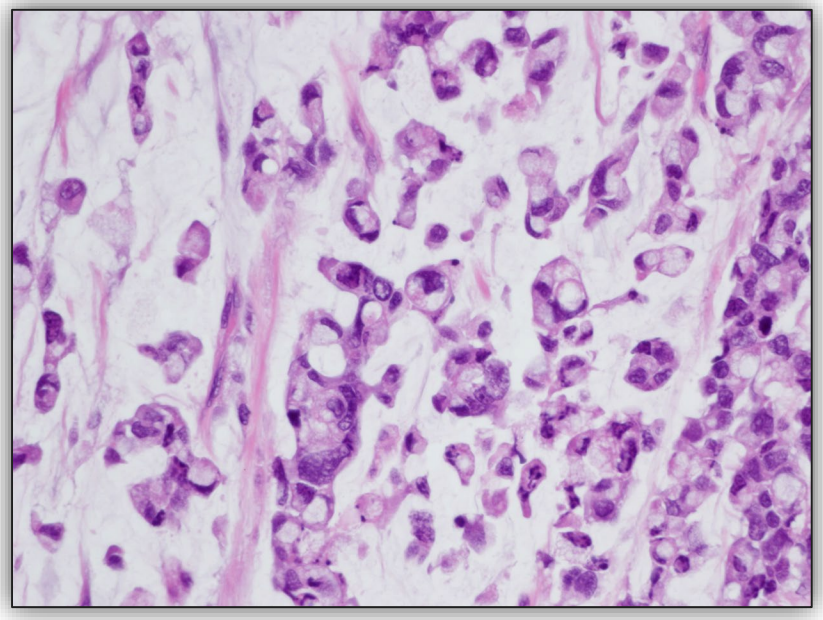


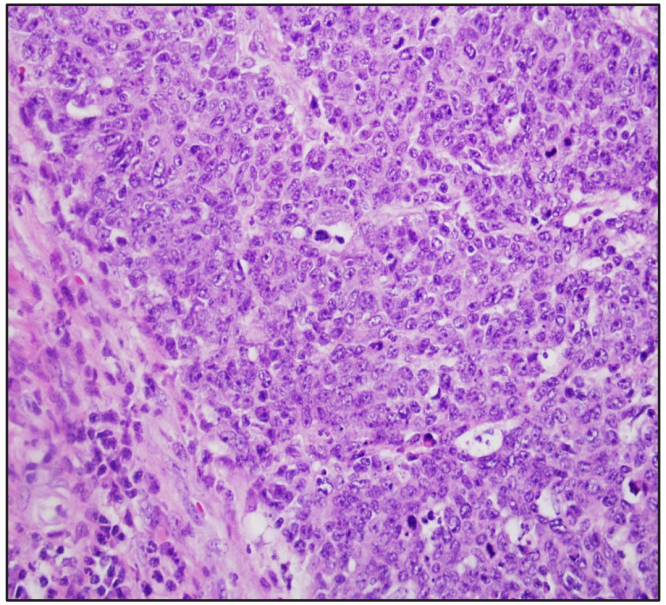
Nature Reviews | Cancer

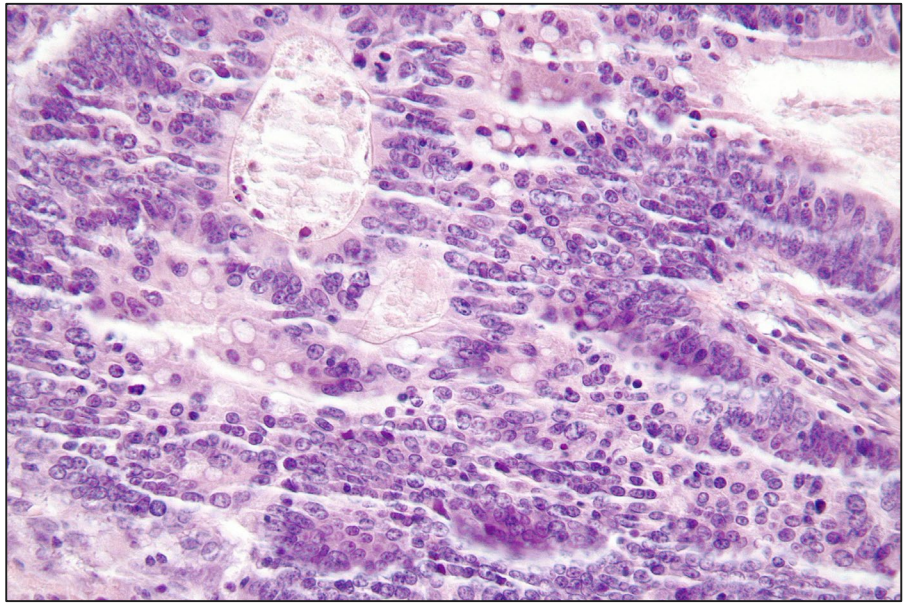


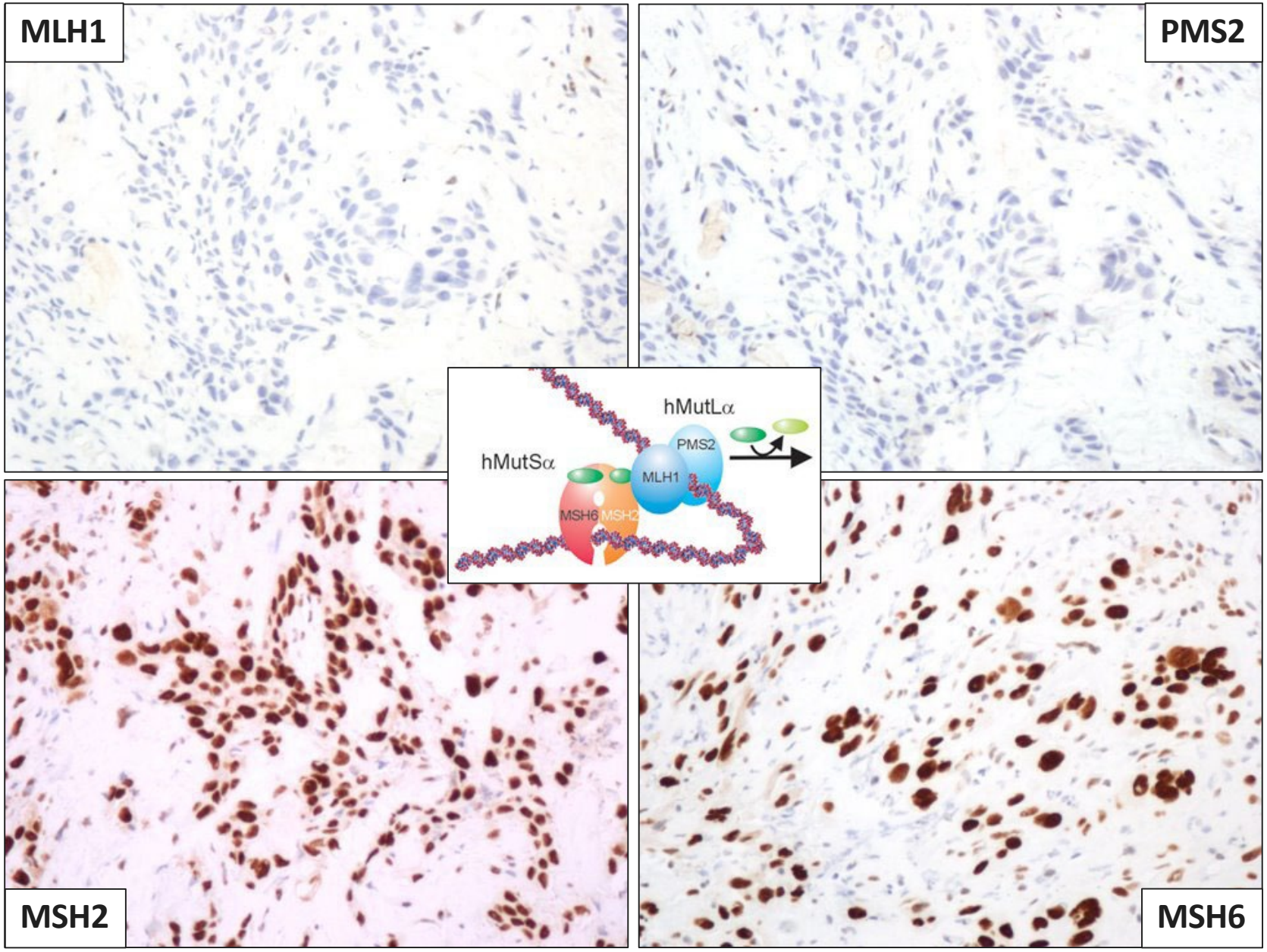












The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

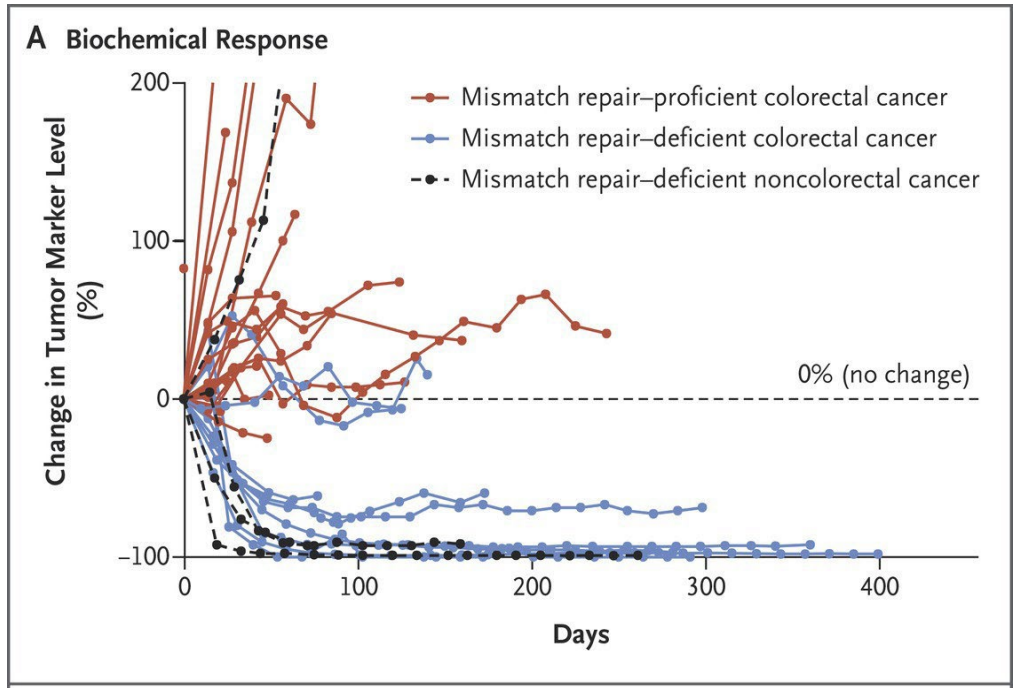
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

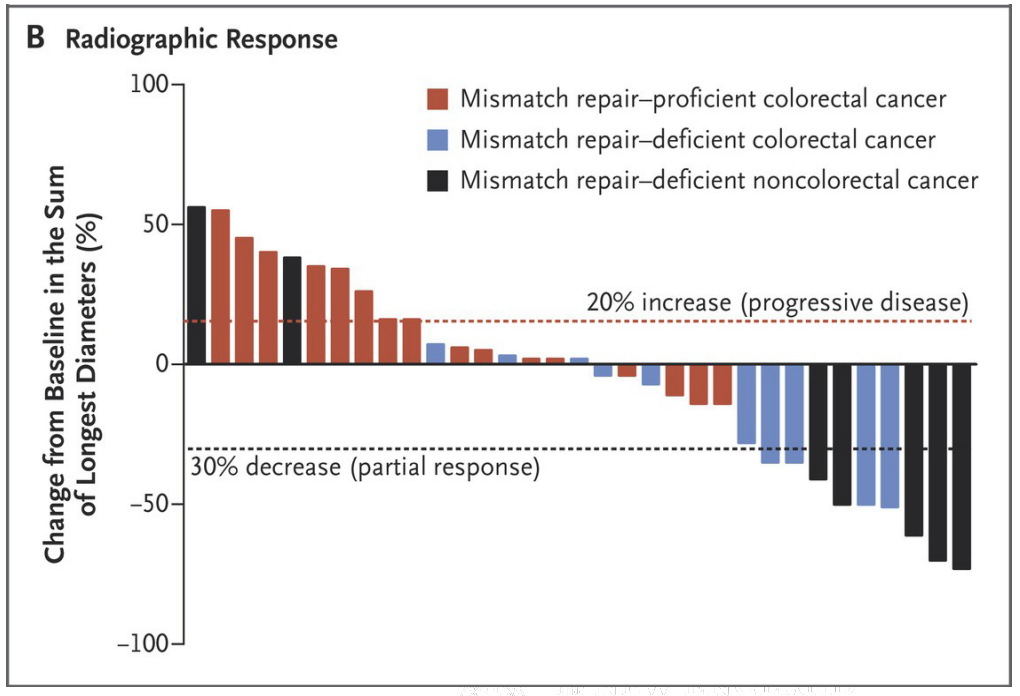
D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, and L.A. Diaz, Jr.

N Engl J Med 2015;372:2509-20.
DOI: 10.1056/NEJMoa1500596

Table 2. Objective Responses According to RECIST Criteria.

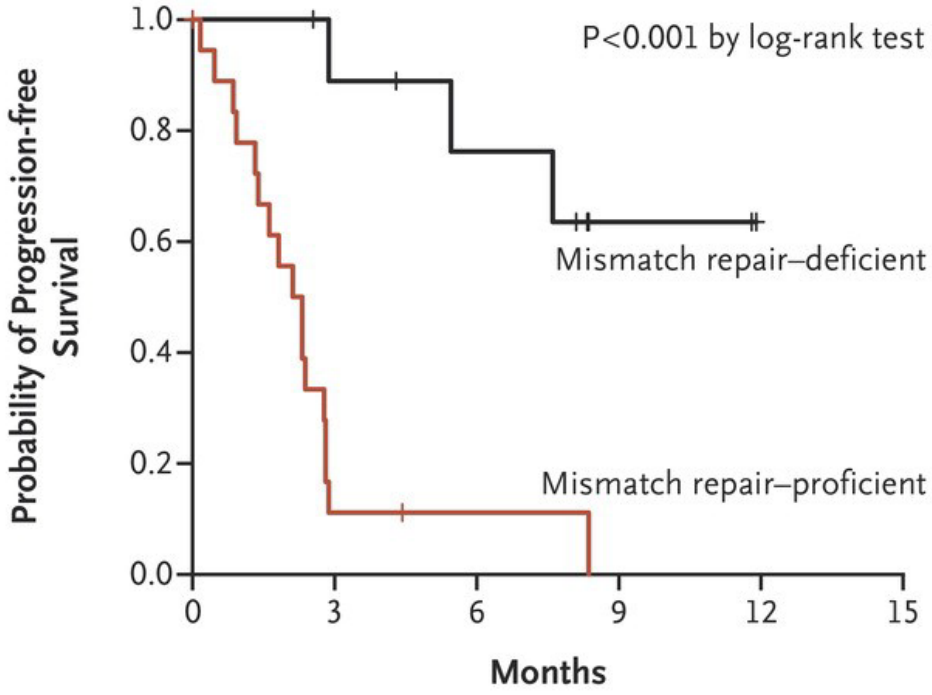
Type of Response	Mismatch Repair–Deficient Colorectal Cancer (N=10)	Mismatch Repair–Proficient Colorectal Cancer (N=18)	Mismatch Repair–Deficient Noncolorectal Cancer (N=7)
Complete response — no. (%)	0	0	1 (14)*
Partial response — no. (%)	4 (40)	0	4 (57)†
Stable disease at week 12 — no. (%)	5 (50)	2 (11)	0
Progressive disease — no. (%)	1 (10)	11 (61)	2 (29)
Could not be evaluated — no. (%)‡	0	5 (28)	0
Objective response rate (95% CI) — %	40 (12–74)	0 (0–19)	71 (29–96)
Disease control rate (95% CI) — %§	90 (55–100)	11 (1–35)	71 (29–96)
Median duration of response — wk	Not reached	NA¶	Not reached
Median time to response (range) — wk	28 (13–35)	NA¶	12 (10–13)





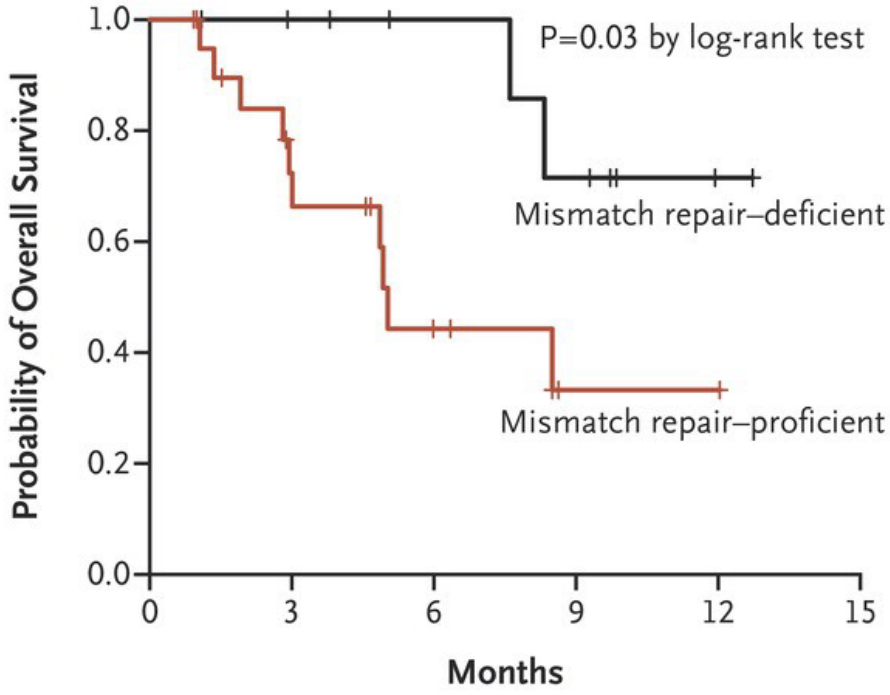
MISMATCH REPAIR STATUS PREDICTED CLINICAL BENEFIT OF IMMUNE CHECKPOINT BLOCKADE WITH PEMBROLIZUMAB

A Progression-free Survival in Cohorts with Colorectal Cancer



No. at Risk						
Mismatch repair–deficient	11	8	6	2	0	0
Mismatch repair–proficient	21	2	1	0	0	0

B Overall Survival in Cohorts with Colorectal Cancer



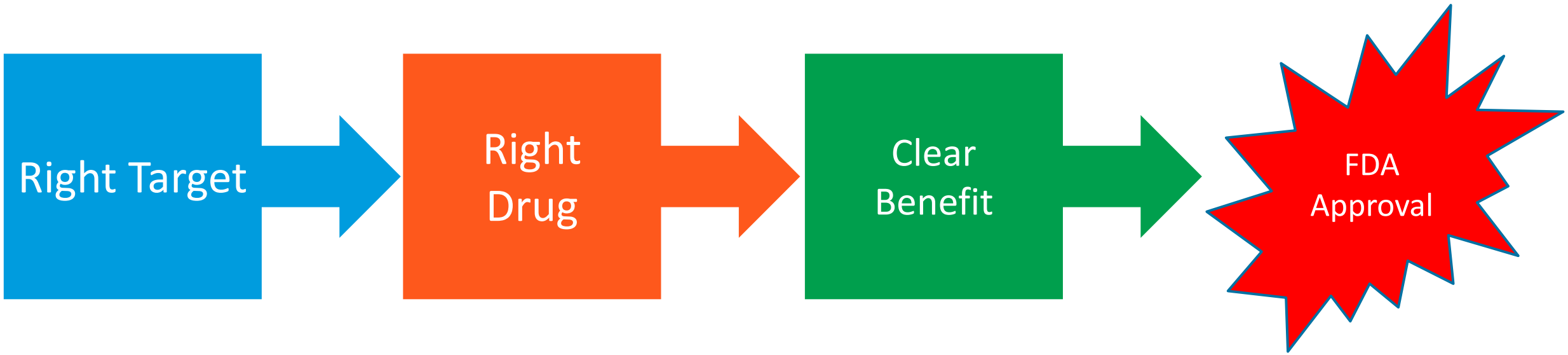
No. at Risk						
Mismatch repair–deficient	11	9	7	5	1	0
Mismatch repair–proficient	21	12	5	1	1	0

The NEW ENGLAND JOURNAL of MEDICINE

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

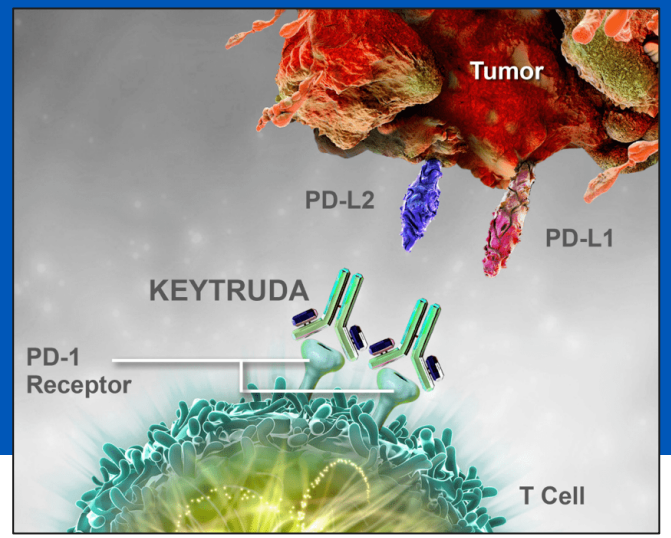
New Order of Clinical Research

- Phase III trials are less necessary
- Drugs are approved for biomarkers, not cancer types
- Guidelines may be just as important as regulatory approval

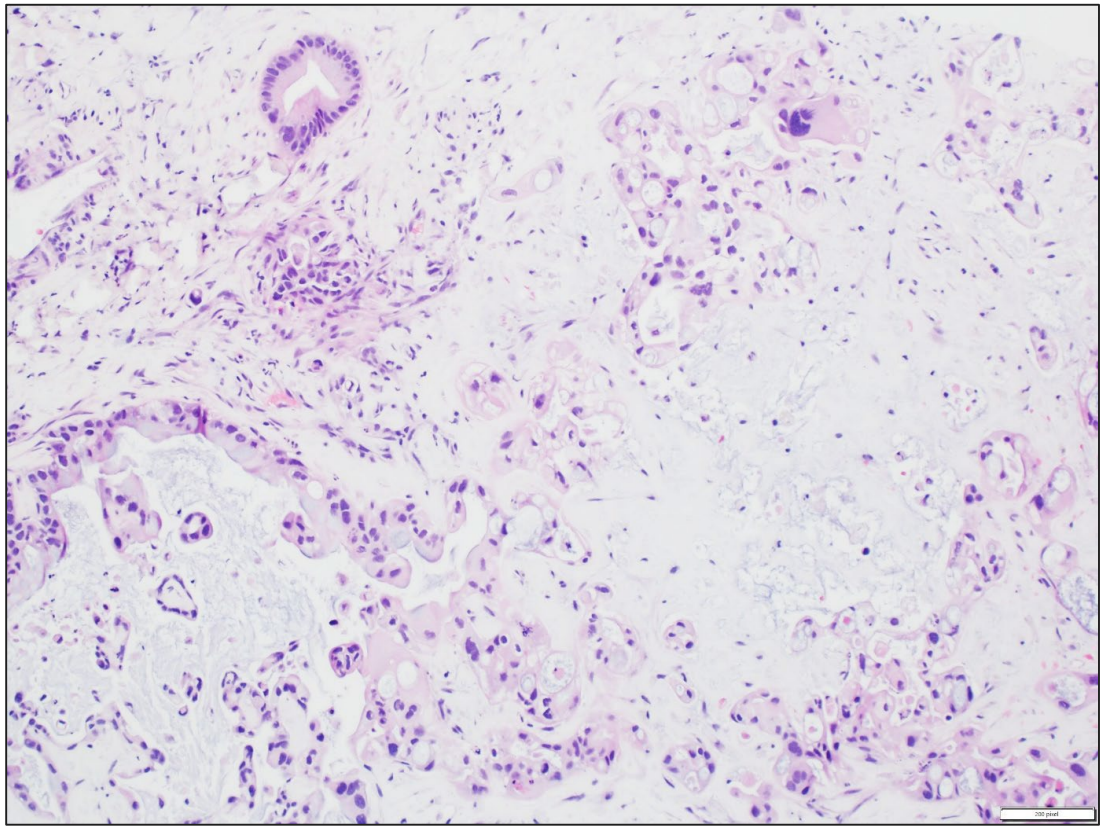


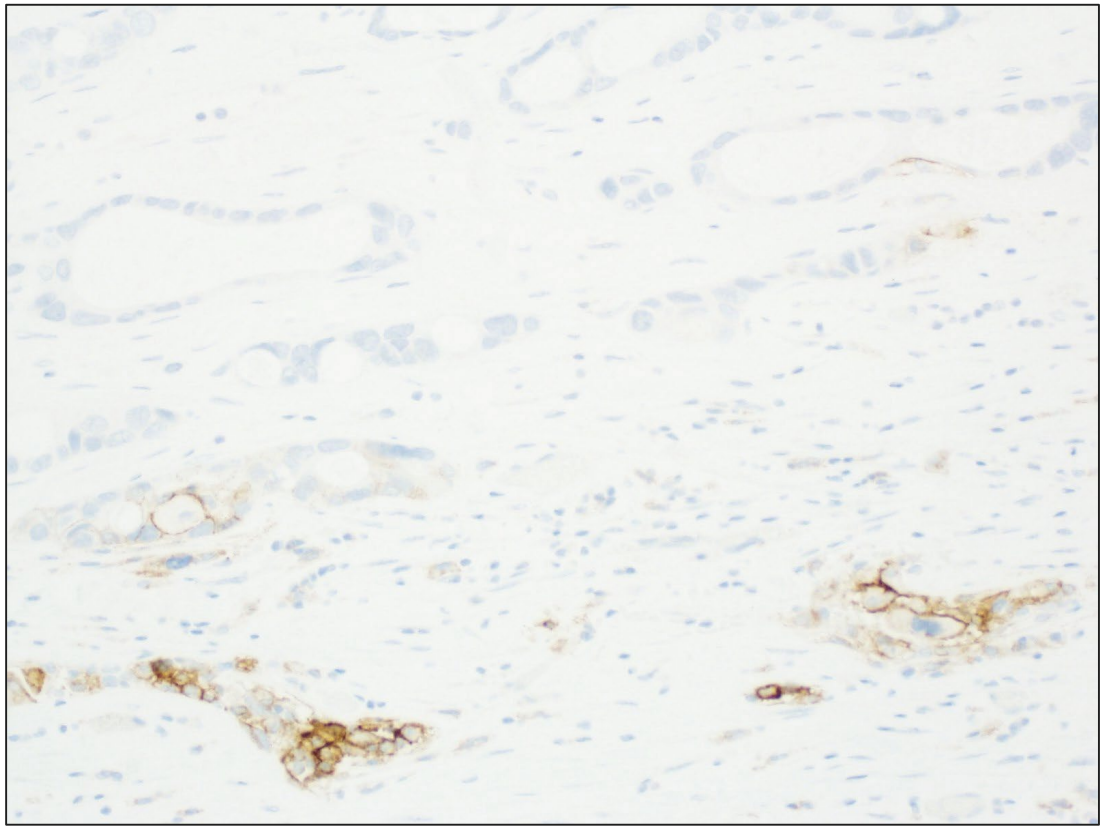
Slide credit: clinicaloptions.com

Immune Checkpoint Inhibitors (ICIs)



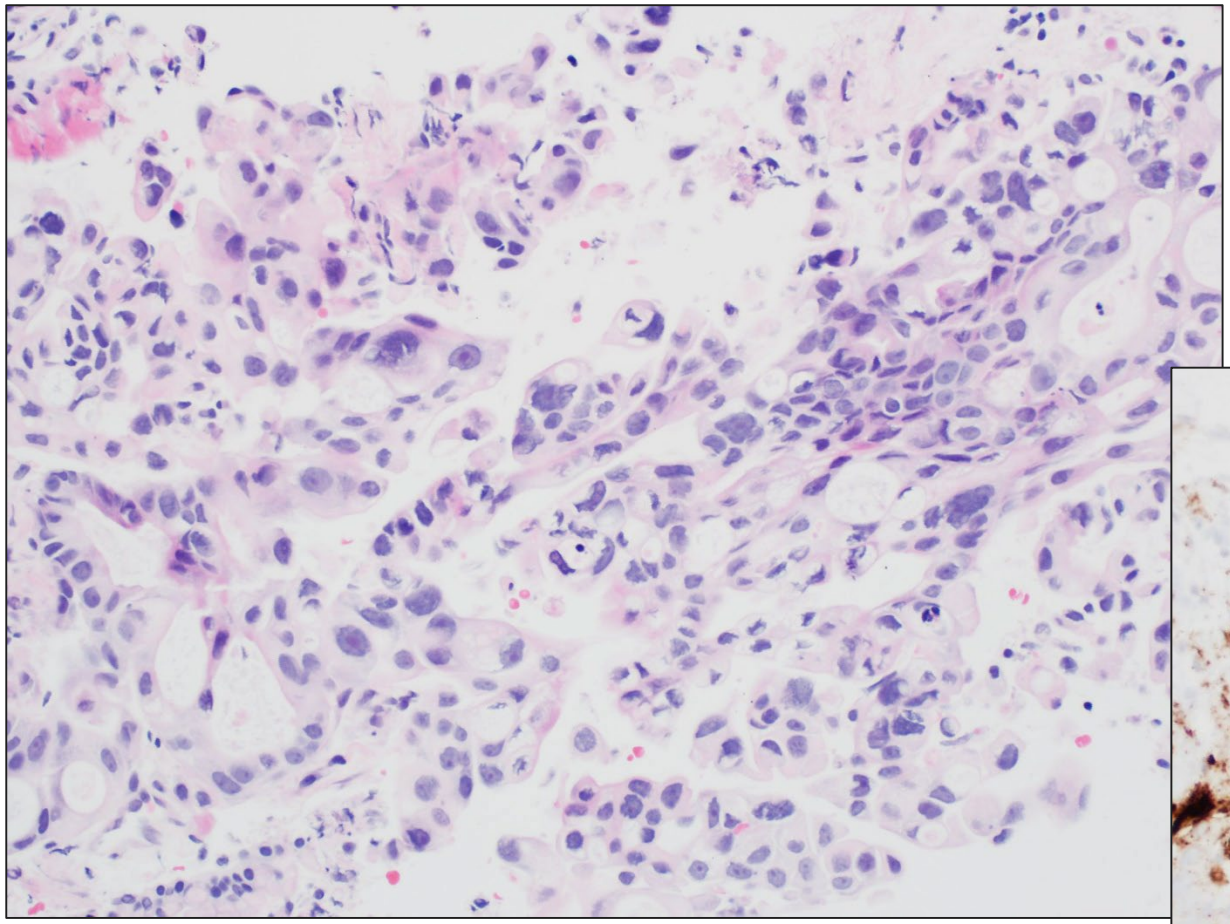
- Immune checkpoints are a normal part of the immune system to prevent destruction of healthy cells.
- Immune checkpoints engage when T cells recognize/bind to partner proteins on other cells; they send an “off” signal to the T cells.
- Immune checkpoint inhibitors block the binding of proteins, allowing T cells to kill cancer cells.
- Original approvals of pembrolizumab and nivolumab were granted agnostic of PD-L1 expression.
  - 2014 Melanoma in 2014
  - 2015 Advanced NSCLC with TPS >1; 2017 metastatic without need for PD-L1
  - 2016 H&N; no PD-L1 needed
  - 2017 for dMMR/MSI-H tumors
  - Original PD-L1 IHC 22C3 pharmDx approved with CPS >1 in Sept 2017
  - Esoph SqCC CPS >10 in July 2019

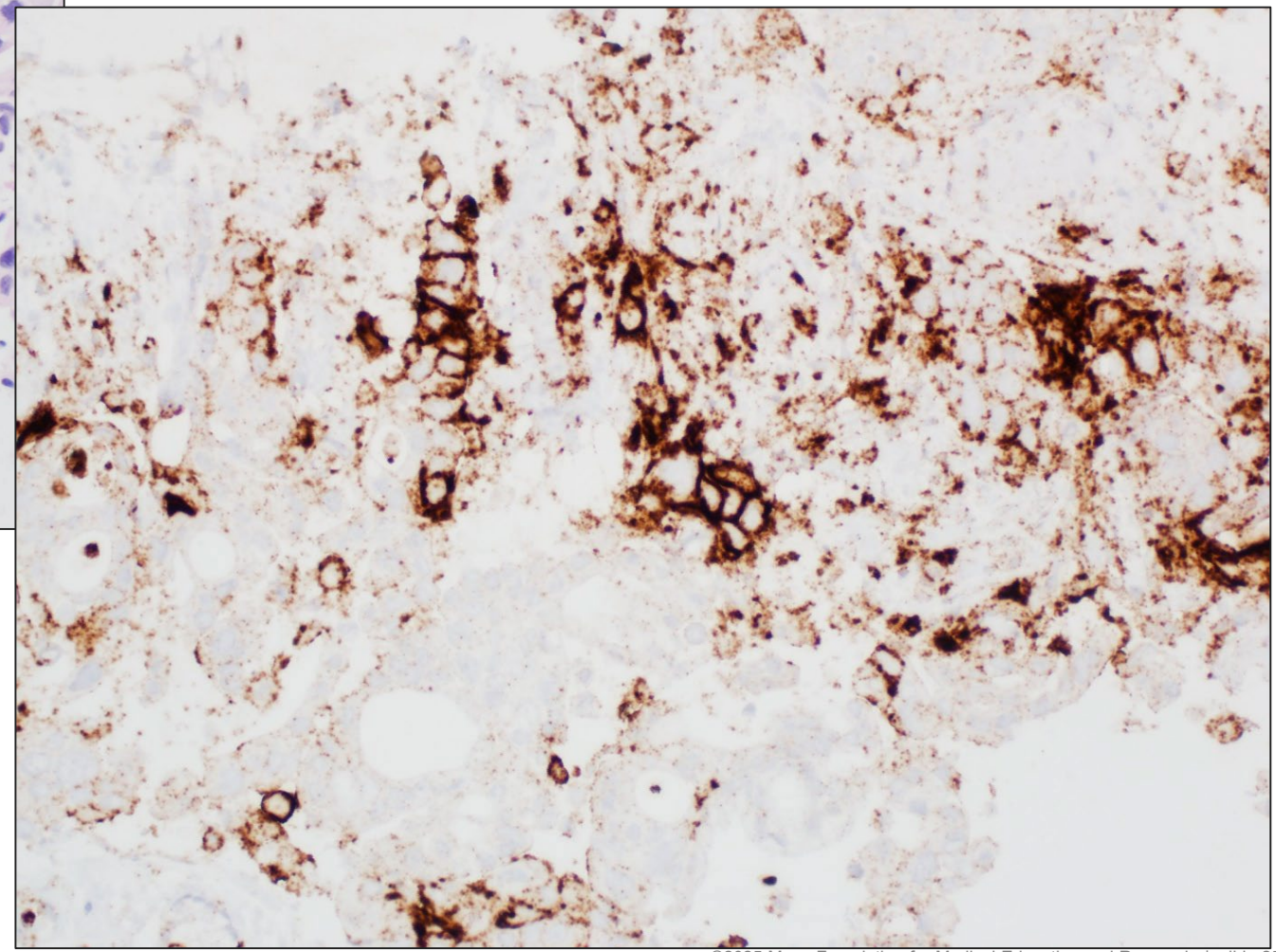




$$\textbf{CPS} = \frac{\text{\# of PD-L1 positive cells (tumor, lymphocytes, macrophages)}}{\text{Total \# of tumor cells}} \times 100$$

- A minimum of 100 tumor cells must be present to be considered adequate.
- A specimen is considered to have PD-L1 expression if the combined positive score (CPS) ≥1.
- Cut-offs for treatment decisions depend on tumor histology and therapy (1st line, 2nd line, etc).





	MERCK	Bristol Myers Squibb	AstraZeneca	Roche	Pfizer MERCK	NOVARTIS
Lead Rx asset	Pembrolizumab KEYTRUDA (anti-PD-1)	Nivolumab OPDIVO (anti-PD-1)	Durvalumab IMFINZI (anti-PD-L1)	Atezolizumab TECENTRIQ (anti-PD-L1)	Avelumab BAVENCIO (anti-PD-L1)	Tislelizumab (anti-PD1)
Diagnostic partner	Dako	Dako	Ventana	Ventana	Dako	Ventana
Clones	22C3	28-8	SP263	SP142/SP263	73-10	SP263
Machines Utilized	Link 48	Link 48	BenchMark series	BenchMark series	Link 48	BenchMark series
Compartment	TM	TM	TM/IC	TC/IC	TC	TM/IC
Scoring	TPS/CPS	TPS/TC/CPS	TC/ICP/IC	TC/IC	TC	TC/TAP

PRINCIPLES OF SYSTEMIC THERAPY

Systemic Therapy for Unresectable Locally Advanced, Recurrent, or Metastatic Disease (where local therapy is not indicated)

First-Line Therapy
- Oxaliplatin is preferred over cisplatin due to lower toxicity.

Preferred Regimens
- HER2 overexpression positive[c]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin and trastuzumab[f]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, trastuzumab[f] and pembrolizumab for PD-L1 CPS ≥1 (category 1)[g,h,17-18]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), cisplatin and trastuzumab (category 1)[f,19]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), cisplatin, trastuzumab[f] and pembrolizumab for PD-L1 CPS ≥1 (category 1)[g,h,17-18]
- HER2 overexpression negative[c]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, and nivolumab (PD-L1 CPS ≥5) (category 1)[g,h,20]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, and pembrolizumab for PD-L1 CPS ≥1[g,h,21] (category 1 for PD-L1 CPS ≥ 10; category 2B for PD-L1 CPS 1 to <10)
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin and zolbetuximab-clzb for CLDN18.2 positive[c] (category 1)[22,23]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine) and oxaliplatin[24-26]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), cisplatin, and pembrolizumab for PD-L1 CPS ≥1[g,h,21] (category 1 for PD-L1 CPS ≥ 10; category 2B for PD-L1 CPS 1 to <10)
  - Fluoropyrimidine (fluorouracil[a] or capecitabine) and cisplatin[24,27-29]
- MSI-H/dMMR tumors (independent of PD-L1 status)[c]
  - Pembrolizumab[g,h,30-32]
  - Dostarlimab-gxly[g,h,33]
  - Nivolumab and ipilimumab[g,h,20]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, and nivolumab[g,h,20]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, and pembrolizumab[g,h,31,32]

Other Recommended Regimens
- Fluorouracil[a,i] and irinotecan[j,34]
- Paclitaxel with or without carboplatin or cisplatin[j,35-39]
- Docetaxel with or without cisplatin[j,40-43]
- Fluoropyrimidine[j,28,44,45] (fluorouracil[a] or capecitabine)
- Docetaxel, cisplatin or oxaliplatin, and fluorouracil[a,j,46,47]

Useful in Certain Circumstances
- HER2 overexpression negative[c]
  - Fluoropyrimidine (fluorouracil[a] or capecitabine), oxaliplatin, and nivolumab (PD-L1 CPS <5) (category 2B)[g,h,20]

PD-L1 IN THE GASTRIC CANCER SPACE

FDA'S CHALLENGES BROAD USE OF PD-1 DRUGS

- PD-L1 expression appears to be a predictive biomarker for HER2-negative gastric/GEJ adenocarcinoma.
- Uncertainty surrounds the efficacy of PD-1 inhibitors in patients with a PD-L1 expression of less than 1, and that the use of immune checkpoint inhibitors exposes patients to potential added toxicities.
- The ***FDA's Oncologic Drugs Advisory Committee (ODAC)*** voted 10 to 2 with 1 abstention against the risk:benefit profile of PD-1 inhibitors in the first-line treatment of patients with advanced HER2-negative, microsatellite stable (MSS) gastric/gastroesophageal junction (GEJ) adenocarcinoma with a PD-L1 expression of less than 1.
- The data for pembrolizumab, nivolumab, and tislelizumab were marginal or not favorable in patients with PD-L1 expression under 1 and moderate in patients with intermediate PD-L1 expression under 10.

Actionable Mutations/Alterations

TUMOR-AGNOSTIC INDICATIONS

Treatment	Indication	Key Supporting Trials
Pembrolizumab	▪ Unresectable/metastatic **MSI-H or dMMR** solid tumors with PD after previous treatment with no satisfactory alternative treatment options	▪ KEYNOTE-016, -164, -012, -028, -158
	▪ Unresectable/metastatic **TMB-H** (≥10 mut/Mb) solid tumors with PD after previous treatment with no satisfactory alternative treatment options	▪ KEYNOTE-158
Dostarlimab	▪ Recurrent/advanced **dMMR** solid tumors with PD after previous treatment with no satisfactory alternative treatment options	▪ GARNET
Entrectinib	▪ Unresectable/metastatic **NTRK gene fusion**–positive solid tumors with PD after previous treatment or with no satisfactory alternative treatment options	▪ ALKA, STARTRK-1, STARTRK-2
Larotrectinib	▪ Unresectable/metastatic **NTRK gene fusion**–positive solid tumors without a known acquired resistance mutation with PD after previous treatment or with no satisfactory alternative treatment options	▪ LOXO-TRK-14001, SCOUT, NAVIGATE
Dabrafenib + trametinib	▪ Unresectable/metastatic solid tumors with **BRAF V600E** with PD after previous treatment with no satisfactory alternative treatment options	▪ BRF117019, NCI-MATCH, CTMT212X2101, COMBI-d, COMBI-v, BRF113928
Selpercatinib	▪ Locally advanced/metastatic solid tumors with a **RET gene fusion** with PD after previous treatment with no satisfactory alternative treatment options	▪ LIBRETTO-001

Slide credit: clinicaloptions.com

Tumor Mutation Burden

- Tumor mutation burden (TMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations occurring in a tumor specimen.
- In the setting of many tumors, TMB-high is defined as ≥10 Muts/Mb.

Most common calculation: $\frac{\text{\# of non-silent mutations}}{\text{genomic area in which mutation are reported}}$

Genomic Alterations Identified†

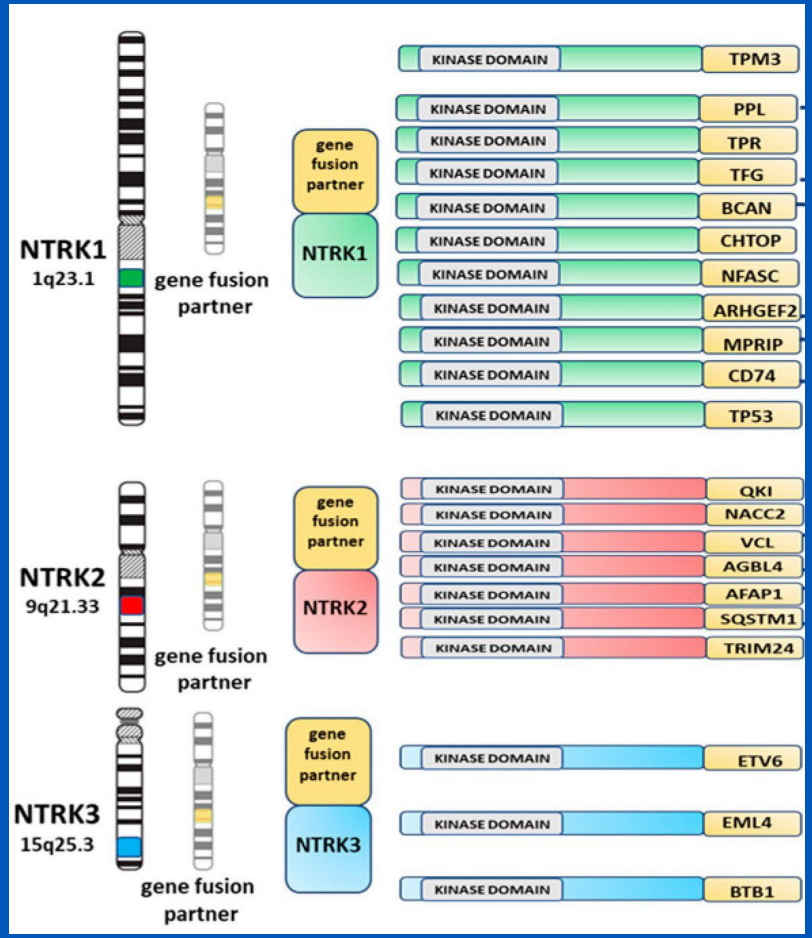
BRCA2 C616*
ATM D2708N
FANCC splice site 1155-2A>G
APC S2197fs*2
ASXL1 G645fs*58
ERBB4 E836K
MLH1 splice site 790+1G>A
MLL2 R5086*
NOTCH2 T1808fs*38
TNFAIP3 A586T
TP53 splice site 993+1G>T

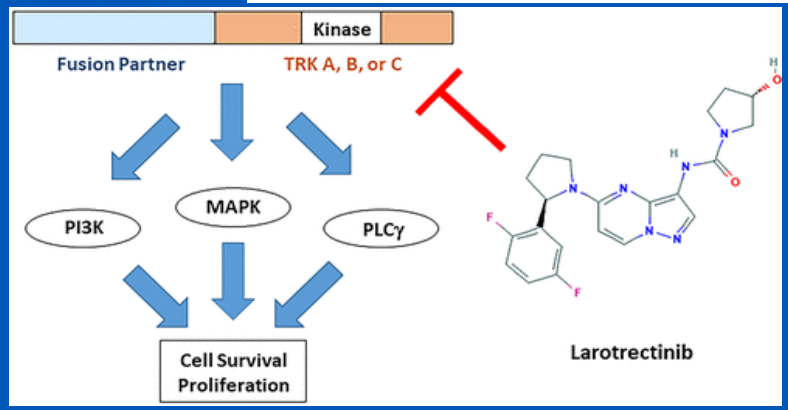
Additional Findings†

Microsatellite status MSI-High
Tumor Mutation Burden TMB-High; 27 Muts/Mb

NTRK Fusions

- Neurotrophic tyrosine receptor kinase (NTRK) gene fusion.
- *NTRK1* was first identified as an oncogene in 1982 during gene transfer assays aimed at identifying genes with transforming capacities (in this case, of a colon cancer).
- FDA granted approval to larotrectinib and entrectinib for adults and adolescents aged 12 or older who have solid tumors that harbor a specific genetic alteration.
- **Fusions** involving *NTRK1, NTRK2*, or *NTRK3* **are** the most common mechanisms of oncogenic TRK activation and are predictive of response.
- ***Mutations DO NOT*** appear to be oncogenic driver events.
- RNA NGS is preferred for fusion detection; can detect additional gene fusions relative to DNA NGS.

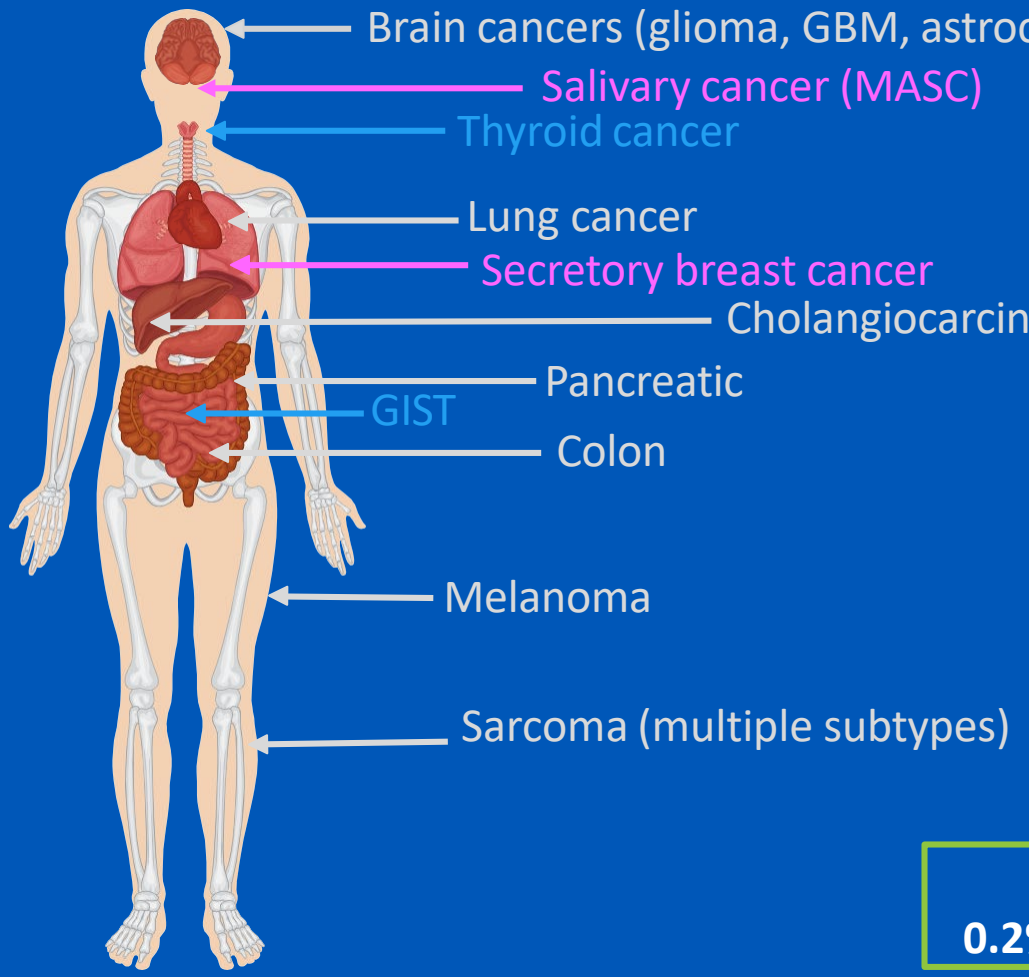


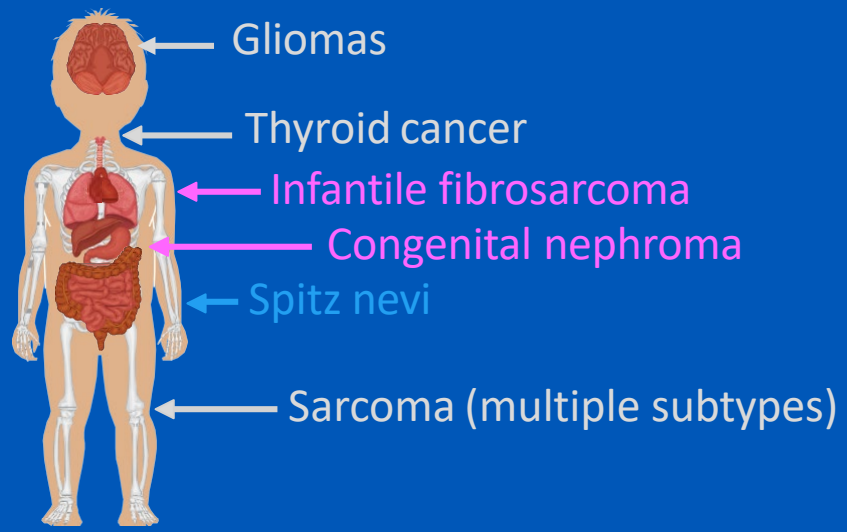


Zito Marino, et. al. *Int. J. Mol. Sci.* **2020**, *21*, 3718.
Wilson, et. al. *Biochemistry* **2019,** 58, 12, 1555–1557.

TRK Fusions Are Found Across Diverse Cancer Types in Adults and Children

Cancers with <5% TRK fusion
Common cancer 5-25% TRK fusions
Rare cancer with high incidence of TRK fusions (>90%)





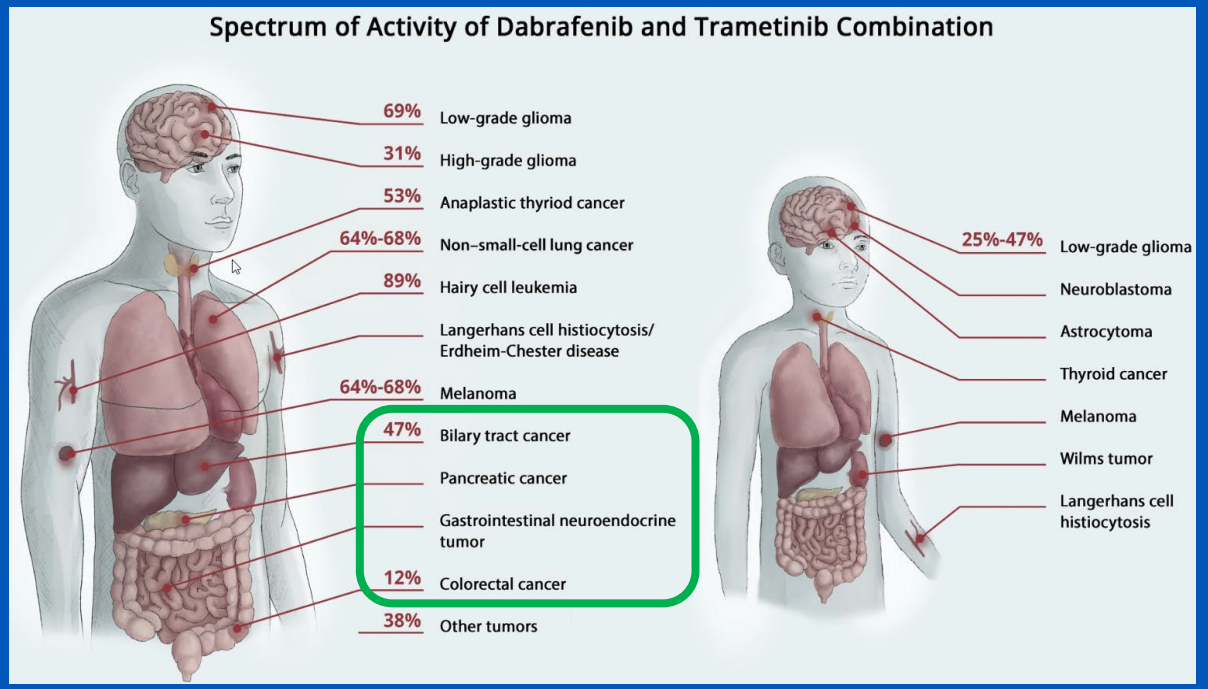
***NTRK* and TRK fusions are rare events:**
0.2% found in screening >11,000 patients with tumors of all types

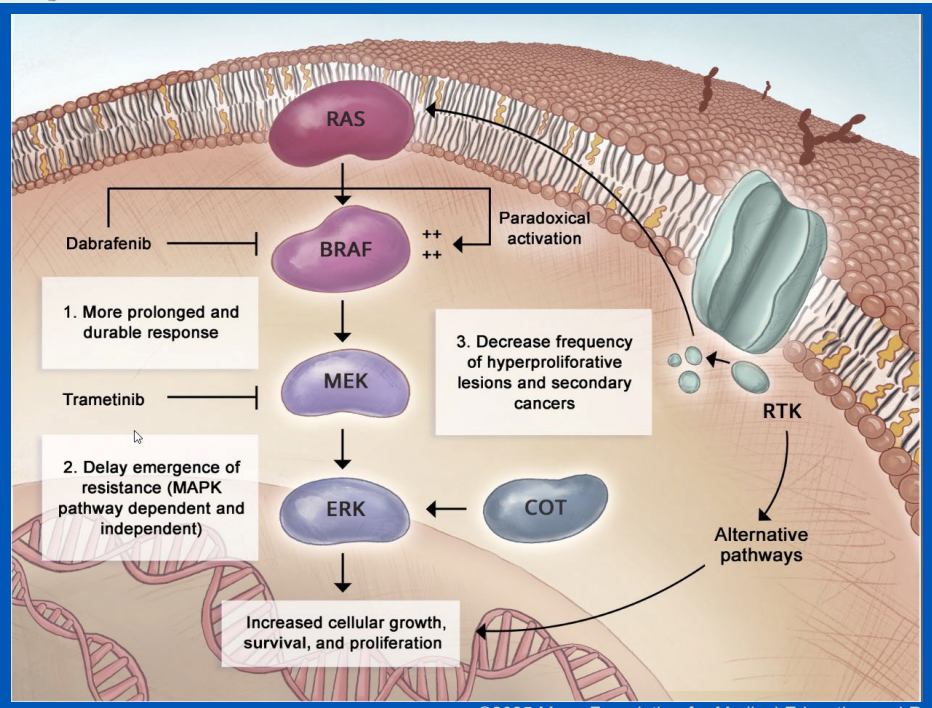
Cocco. Nat Rev Clin Oncol. 2018;15:731.
Urano. Hum Pathol. 2015;46:94.
Watanabe. Cancer Genet Cytogenet. 2002;136:10.
Gatalica. AACR-NCI-EORTC 2017. Abstr A047.
Amatu. ESMO Open. 2016;1:e000023.
Knezevich. Nat Gen. 1998;18:184.
Hyman. ASCO 2017. Abstr LBA2501.

Slide credit: clinicaloptions.com

BRAF V600E

- FDA approved the dabrafenib/trametinib combination as a tissue-agnostic treatment for solid tumors with *BRAF* V600E mutation.
- Data from basket trials have demonstrated consistently good response rates in various tumors, including biliary tract cancer, gliomas, hairy cell leukemia, and multiple other malignancies.
- This has been practice changing in managing *BRAF* V600 mutated cancers.

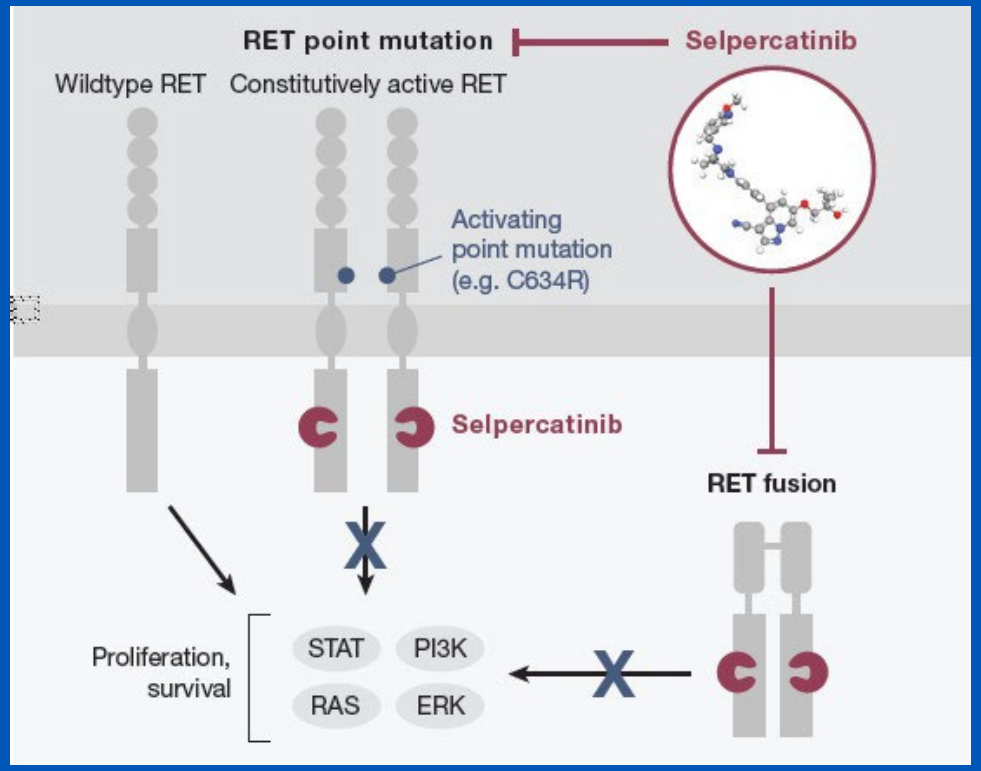


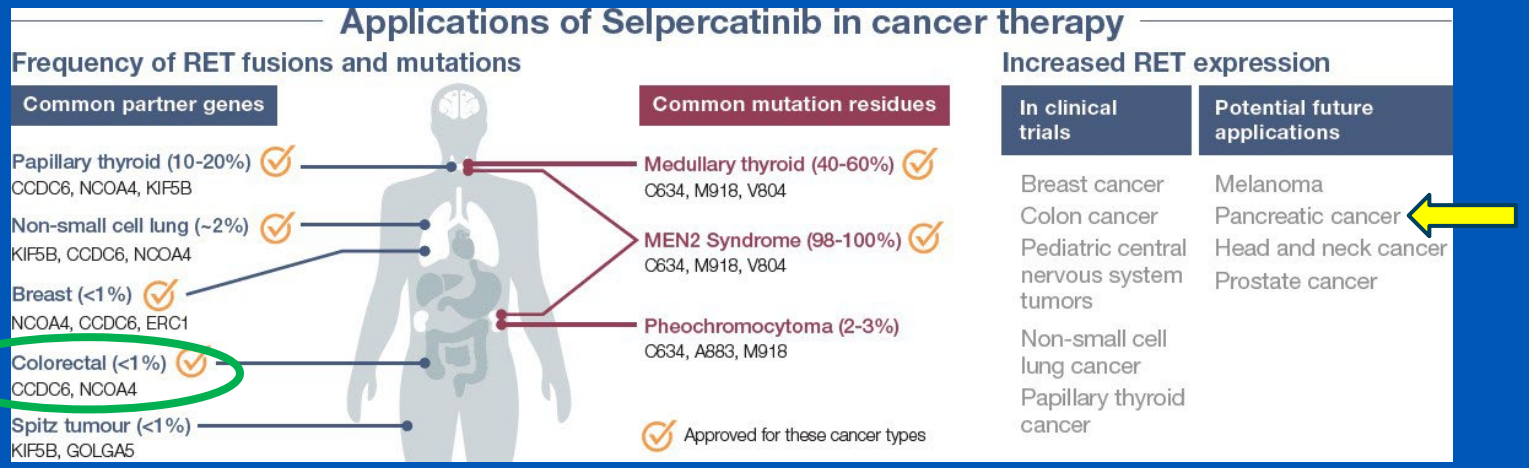


Gouda MA, Subbiah V. American Society of Clinical Oncology Educational Book 2023: 43

RET Fusions

- Approval to selpercatinib for adult patients with locally advanced or metastatic solid tumors with a rearranged during transfection (RET) gene fusion that have progressed on or following prior systemic treatment or who have no satisfactory alternative treatment options.
- Selpercatinib selectively binds to and targets various **RET mutations** and **RET-fusion** products resulting in an inhibition of cell growth of tumors cells.



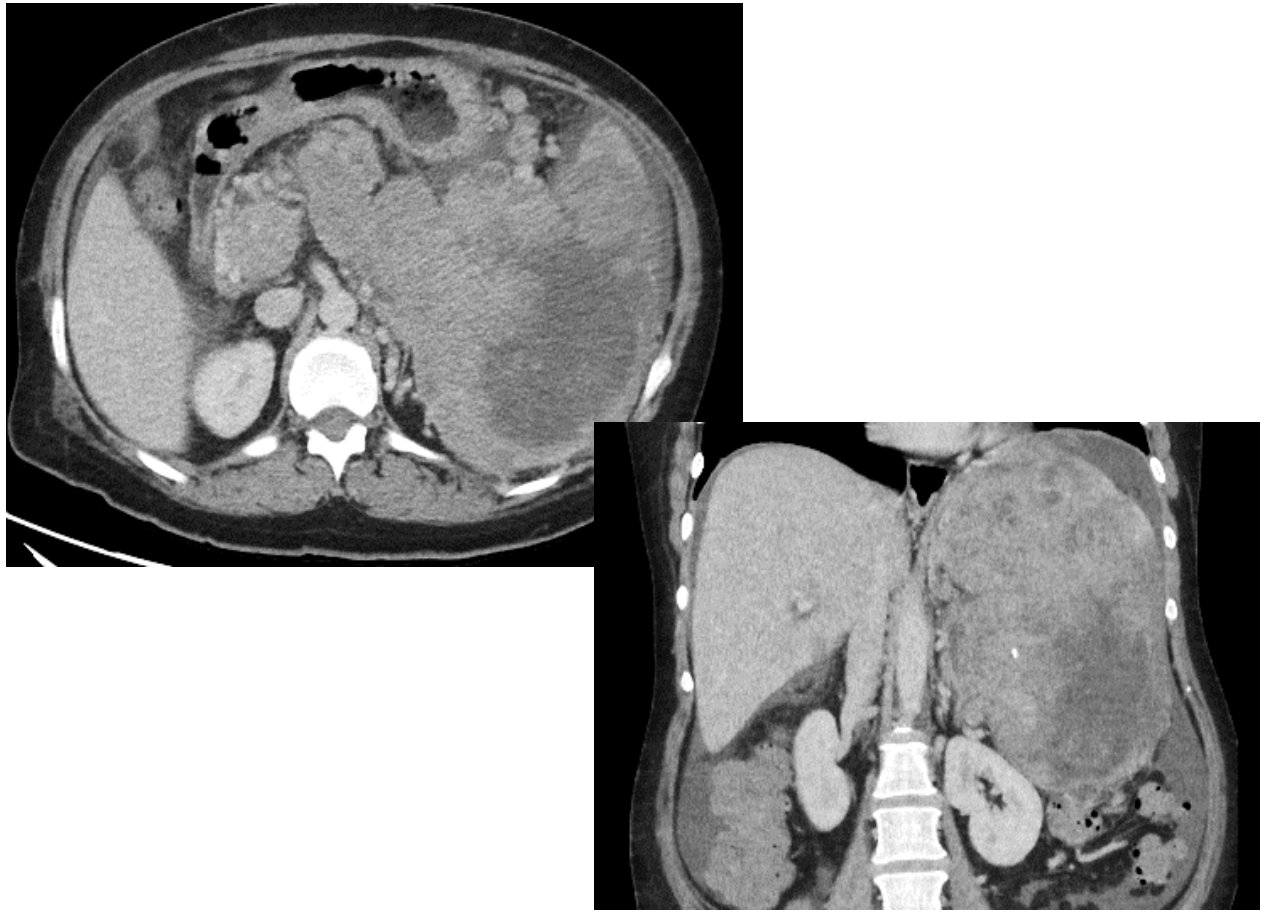


Cell *186*, April 13, 2023

Real World Cases

CASE #1

MEET CATHY



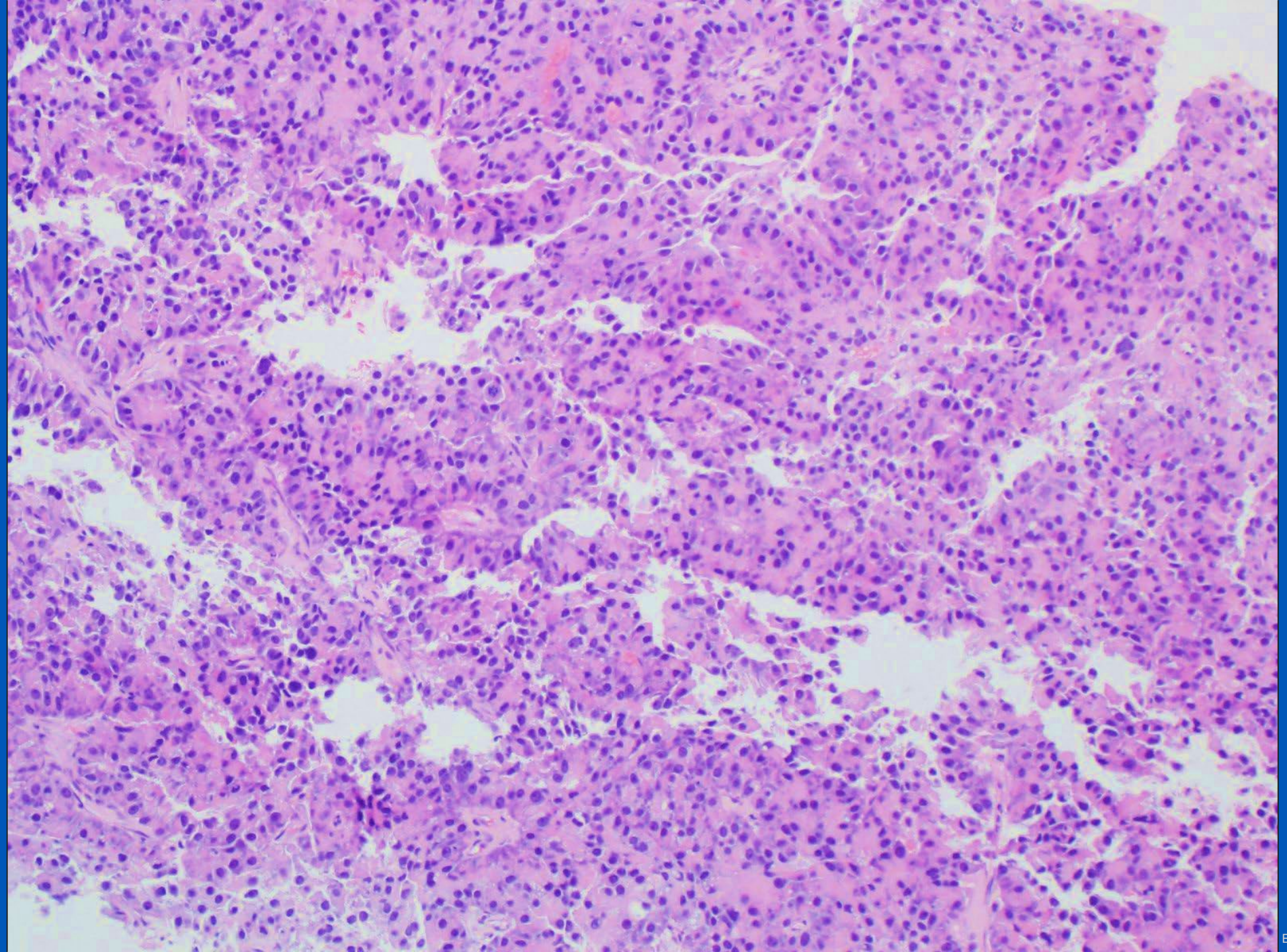
52 year old real estate agent who presented with abdominal pain and weight loss.

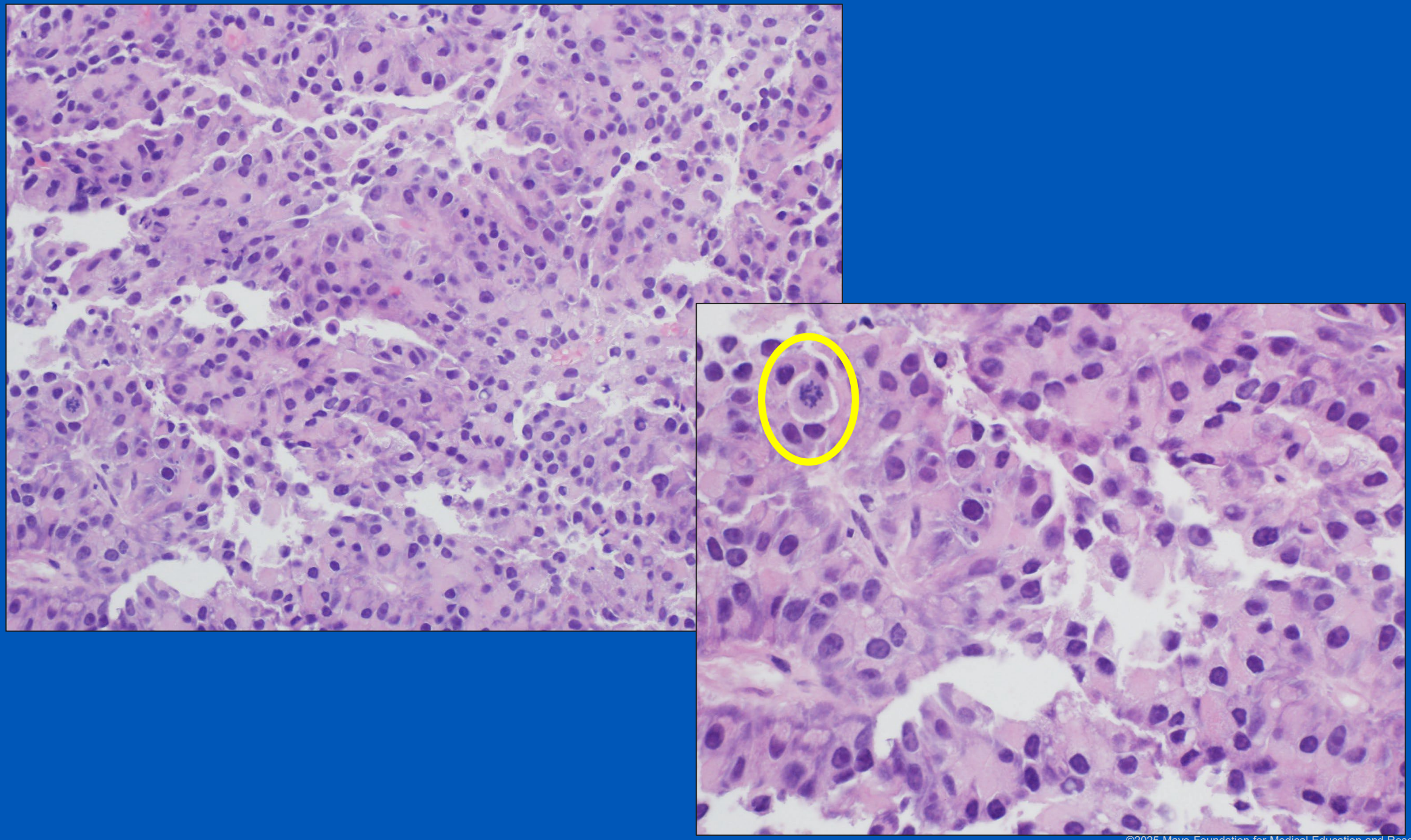
Ultrasound guided “splenic” lesion biopsy

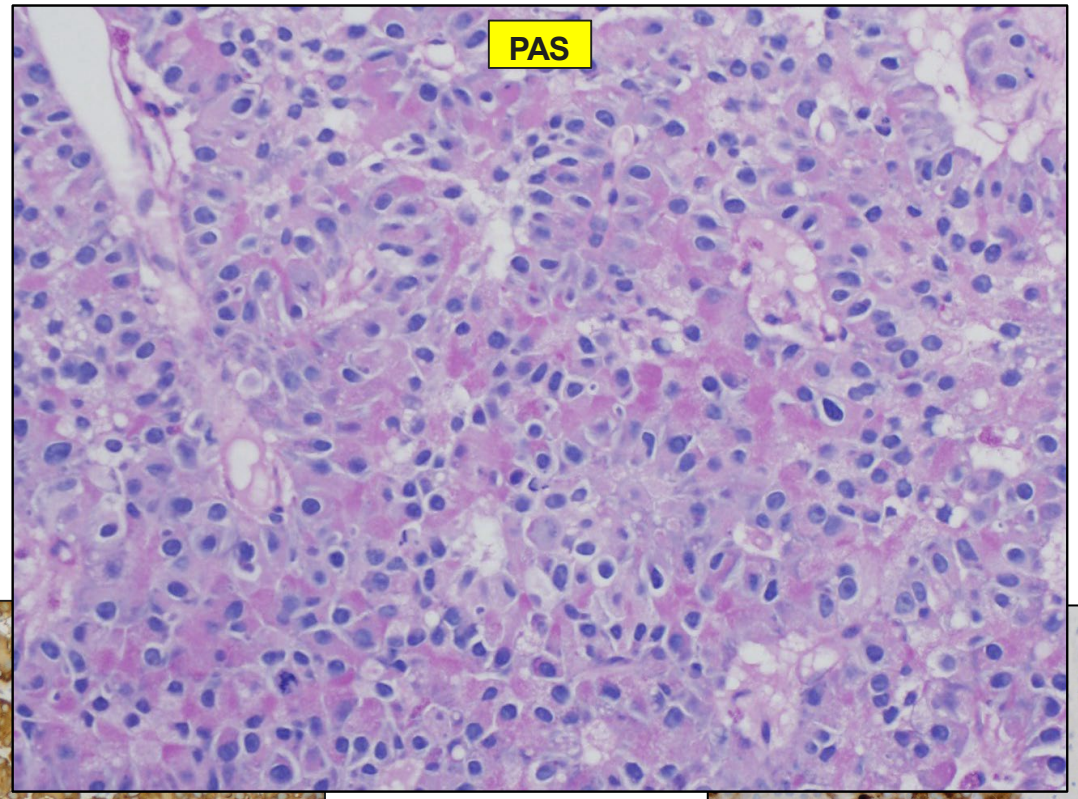
2 months of FOLFIRINOX

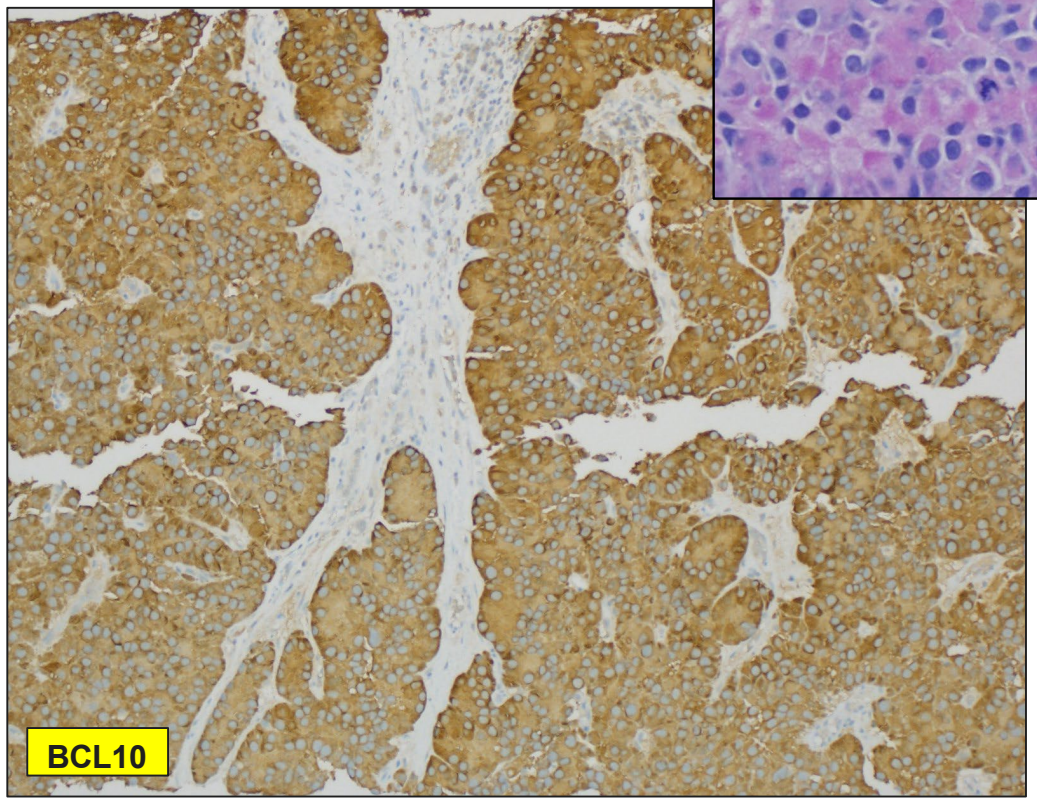
No response and developed ascites

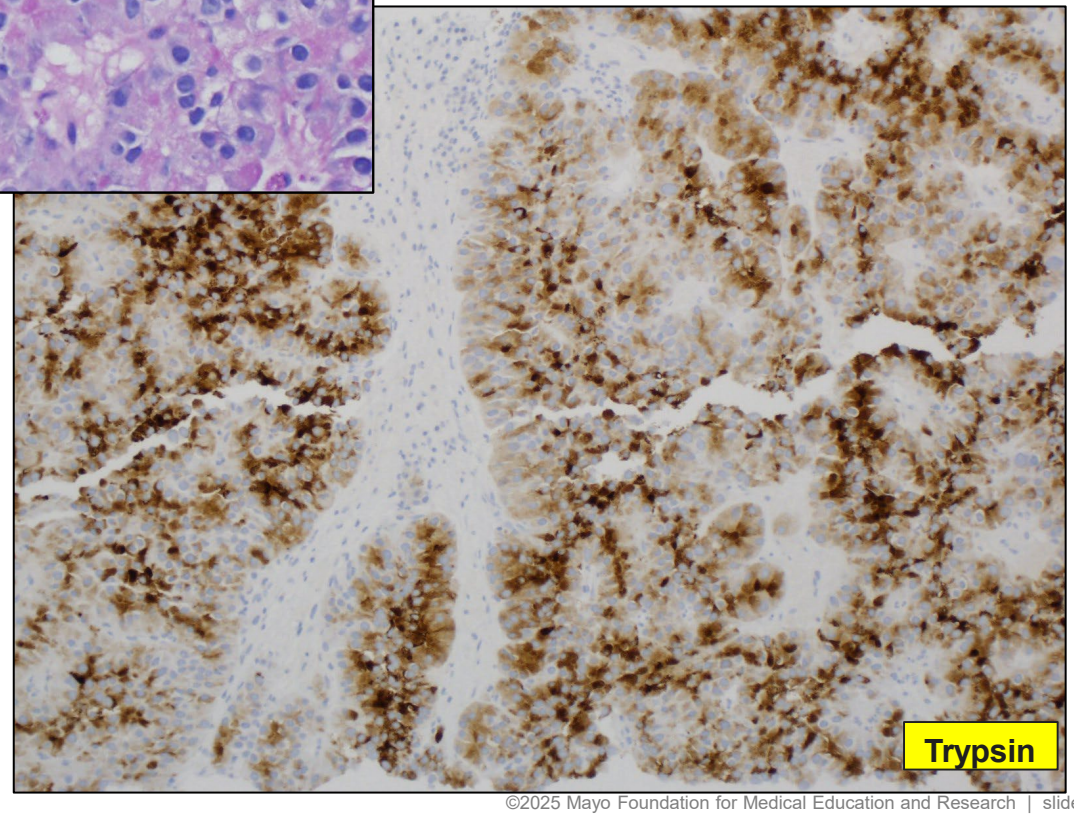
https://www.hellocancerfriends.com/











Treatment on Phase 2 Study with Anti-PD1 Agent

Genomic Findings

AKT1 p.E17K
NM_001014431.1:c.48G>A
Estimated variant allele frequency: 25%

ATM exon 8 splice acceptor mutation
NM_000051.3:c.902-1G>A
Estimated variant allele frequency: 41%

ATM exon 8 splice donor mutation
NM_000051.3:c.1065+1G>A
Estimated variant allele frequency: 39%

BRCA2 p.S2372*
NM_000059.3:c.7115C>G
Estimated variant allele frequency: 43%

CDKN2A p.D108N
NM_000077.4:c.322G>A
Estimated variant allele frequency: 39%

MSH6 exon 4 splice donor mutation
NM_000179.2:c.3172+1G>C
Estimated variant allele frequency: 42%

Genomic Findings

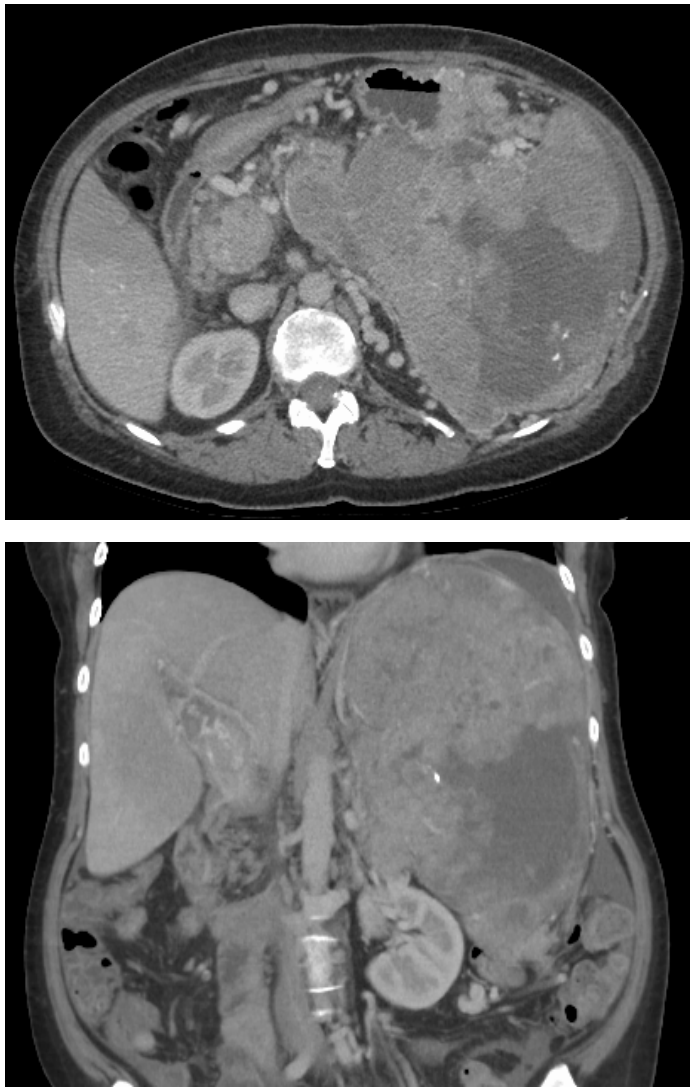
MSS
Microsatellite Stable

TMB - High
Mutations per MB: 332
Confidence interval: 299 - 367

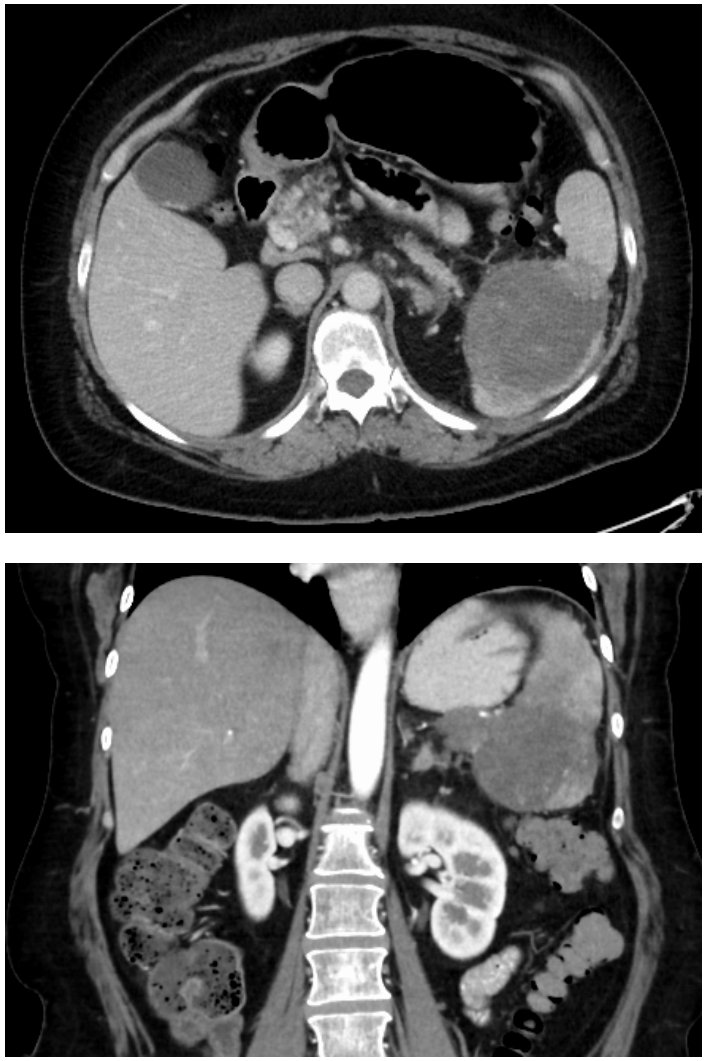
PD-L1 - Low
RNA expression score: 1

STRATA™ NGS

Initial Scan



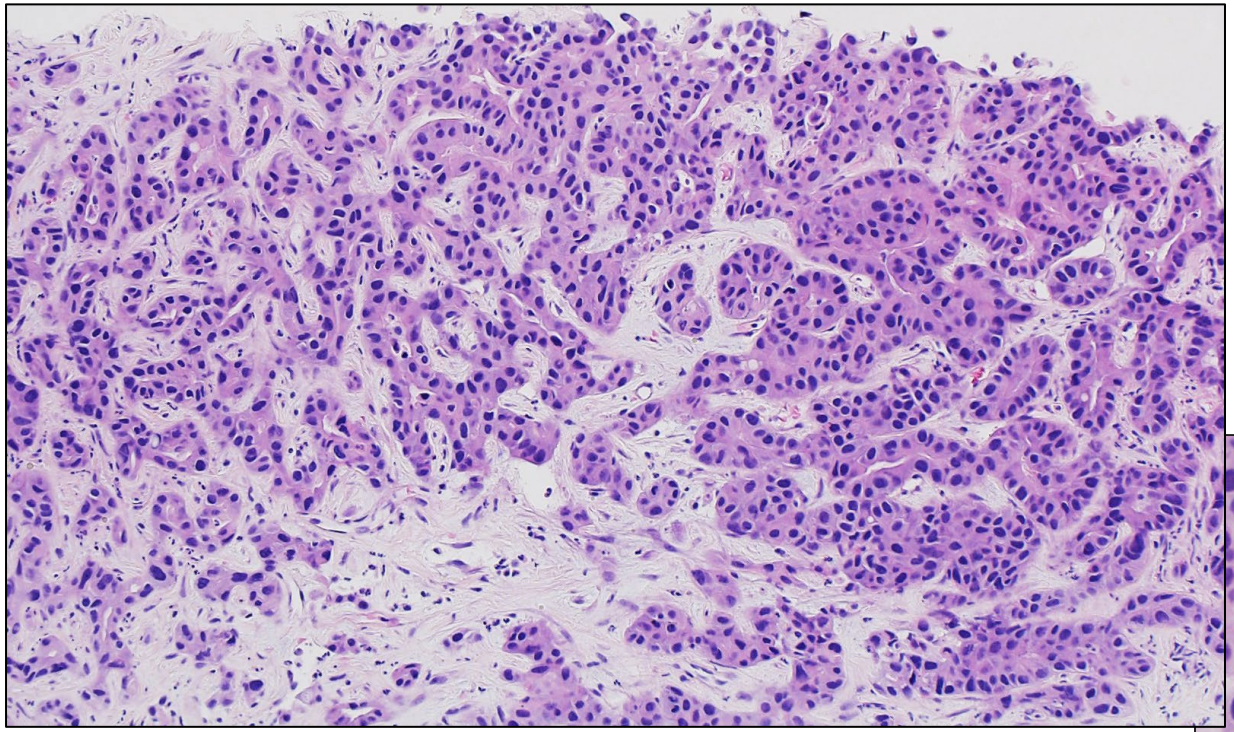
8 months later

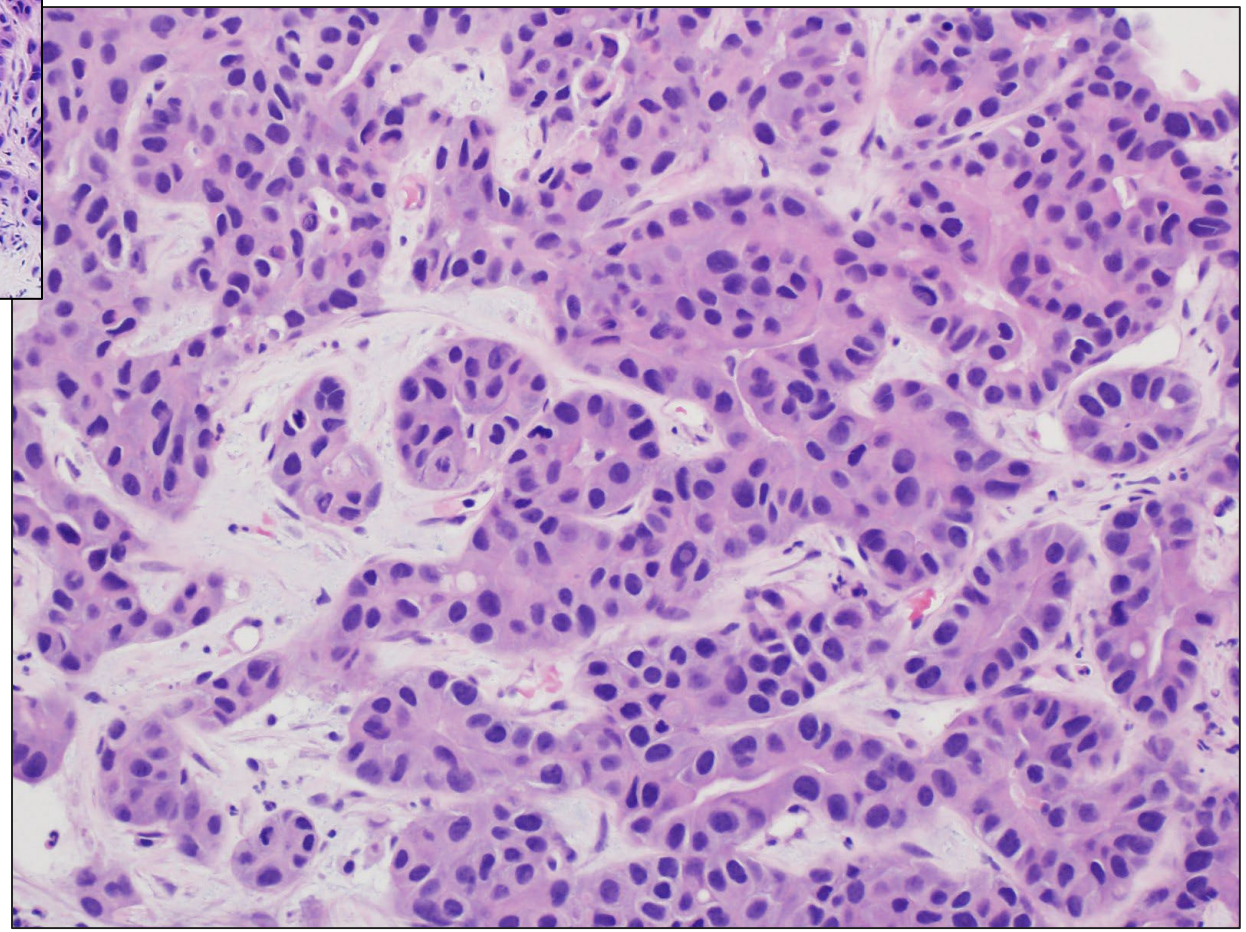


Patient still alive 5 years later!!

CASE #2

69 y/o gentlemen who presented with abdominal pain, bloating and 30 lb weight loss.

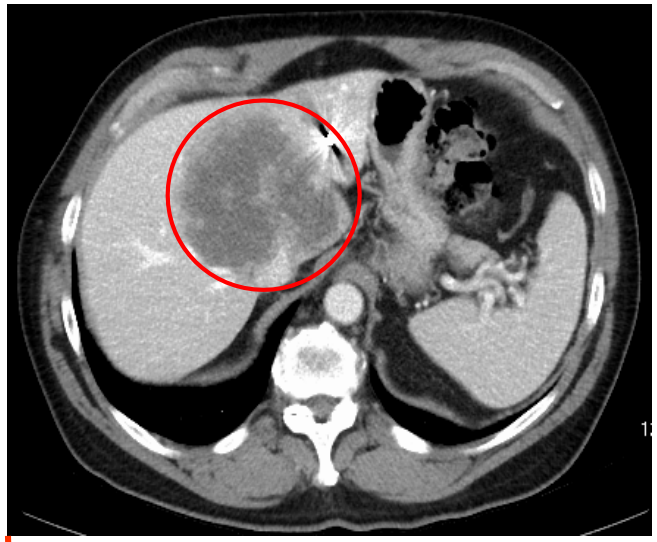




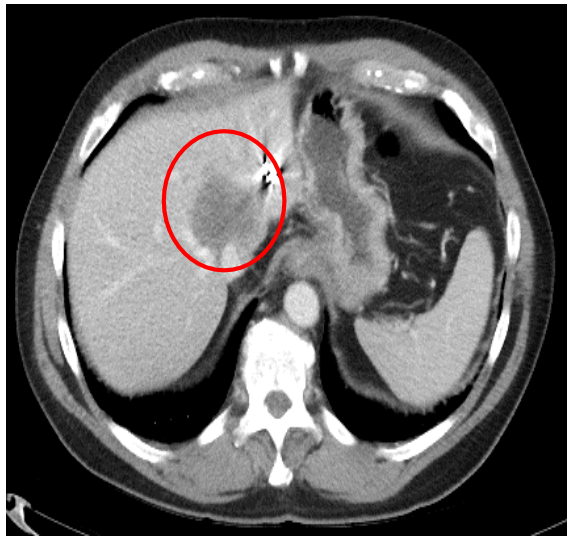
Tissue and Liquid Biopsies

Tumor Biopsy: FGFR2-BICC1

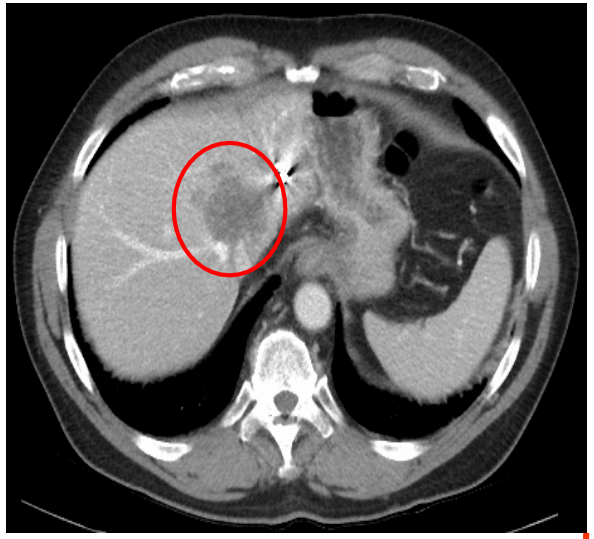
Initial Scan



5 months later



7 months later



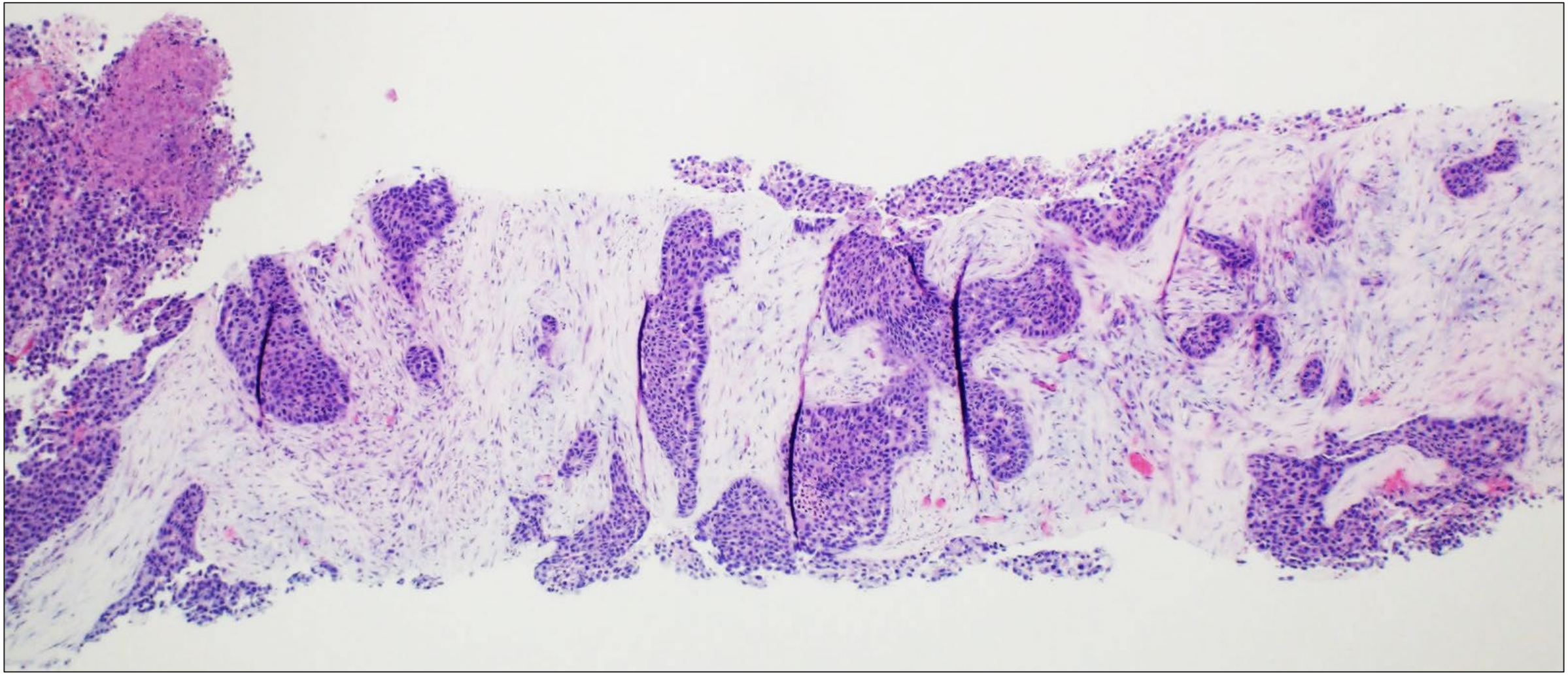
On-trial treatment with FGFR Inhibitor

New lesions noted on follow-up imaging

Liquid Biopsy:
FGFR2-BICC1
FGFR2 V564I
FGFR2 N549K
FGFR2 N549D

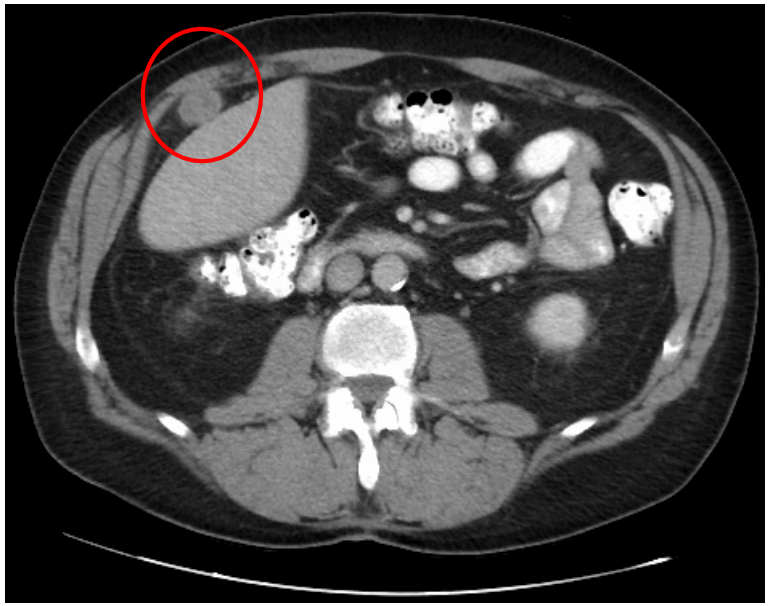
Consolidative Radiation Therapy

17 MONTHS LATER…PERITONEAL BIOPSY



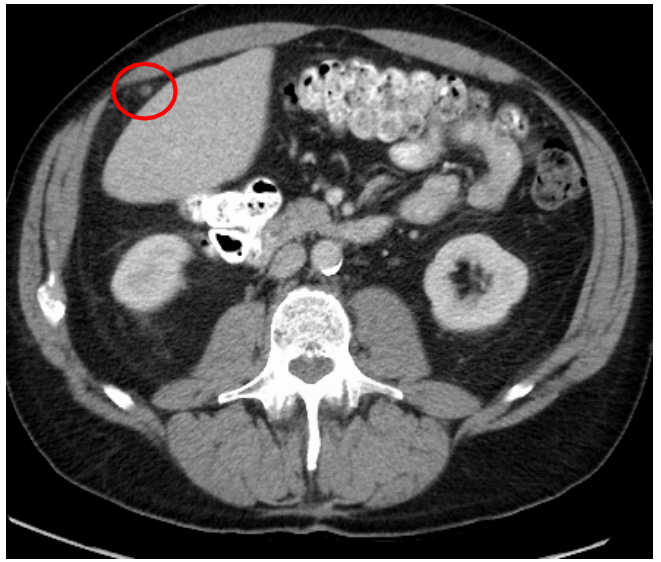
Tissue and Liquid Biopsies

17 months



Tumor Biopsy (new site): FGFR2-BICC1

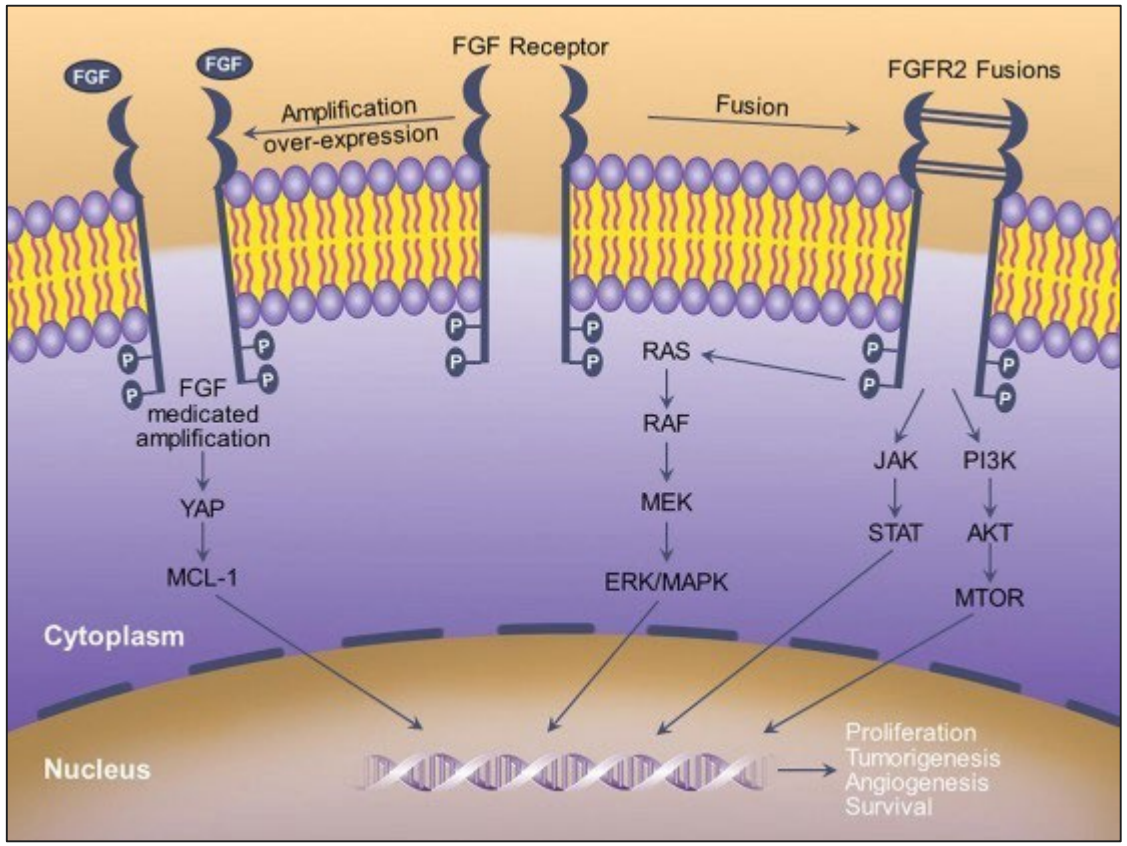
TAS-120
(futibatinib; FGFR inhibitor)
Compassionate Use



21 months

FGFR2 Fusion

- Genomic analysis has shown that fibroblast growth factor receptor 2 (FGFR2) fusions were identified in 13-50% of intrahepatic cholangiocarcinoma (iCCA) patients.
- Chromosomal translocation of FGFRs result in the formation of chimeric FGFR fusion proteins, which often cause aberrant signaling leading to the development and progression of the cancer.
- Over the last 5 years, the FDA has granted accelerated approval to at least 3 drugs for the treatment of adults with cholangiocarcinoma harboring an FGFR2 fusion or other rearrangement.

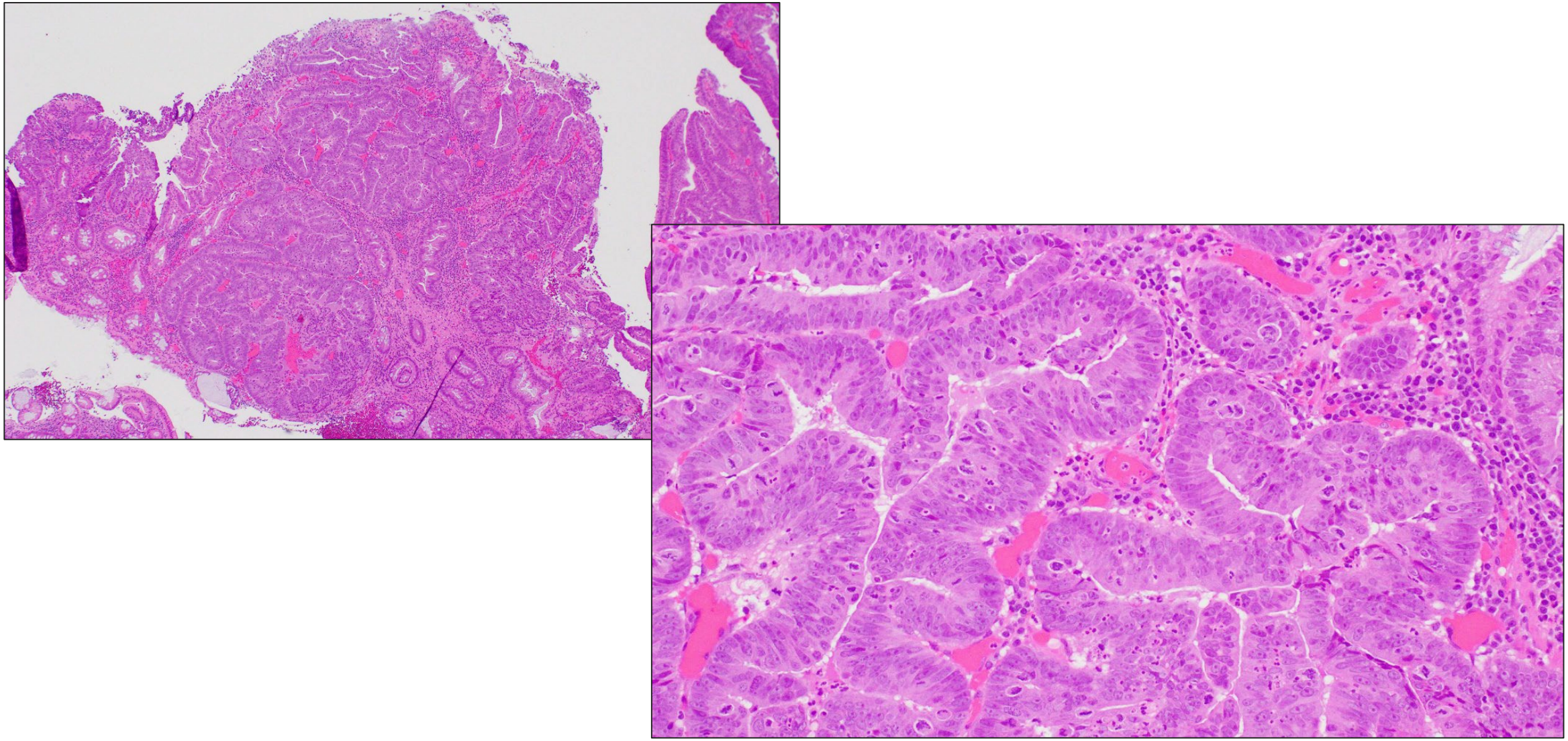


CASE #3…unfortunately, we are not perfect

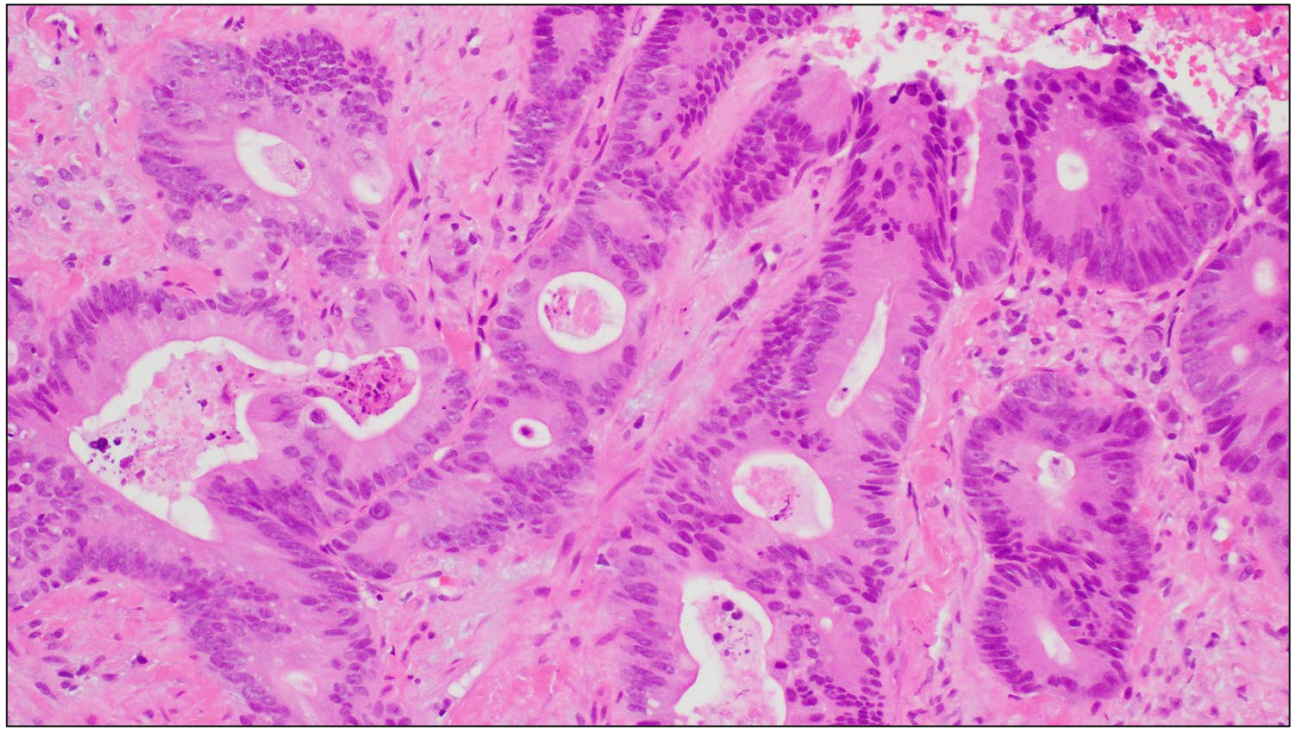
65 Y/O Gentleman who Presented for Continuation of Care

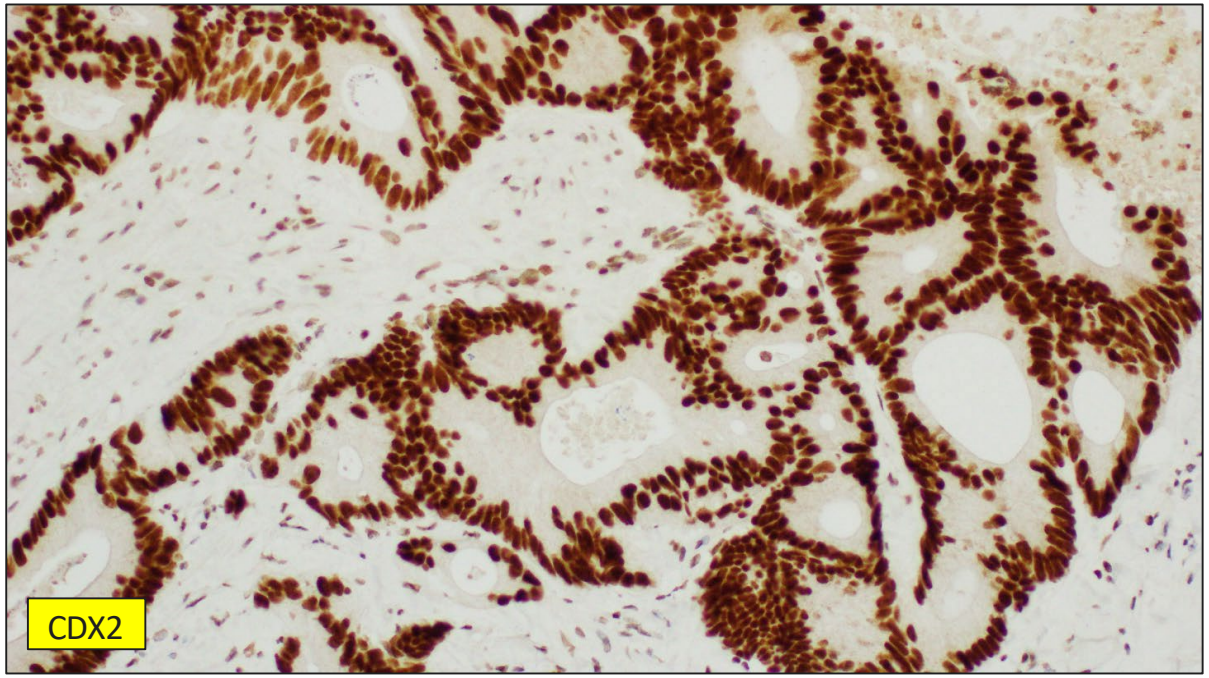
- Feb 2016: Rectal bleeding, colonoscopy, “possible” malignancy
- May 2016: Transanal resection and chemorads; rectal adencarcinoma
- Aug 2016: Anterior peritoneal resection
- Dec 2016: PET concerning for liver met; biopsy negative
- Aug 2017: PET shows increasing size of liver lesion; IHC c/w mCRC
- April 2018: NEW left lobe lesion (Seg III); IHC c/w mCRC
- July 2019: Segment III resection; mCRC
- Nov 2019: 2.4 cm Lung lesion (medial right lobe); mCRC

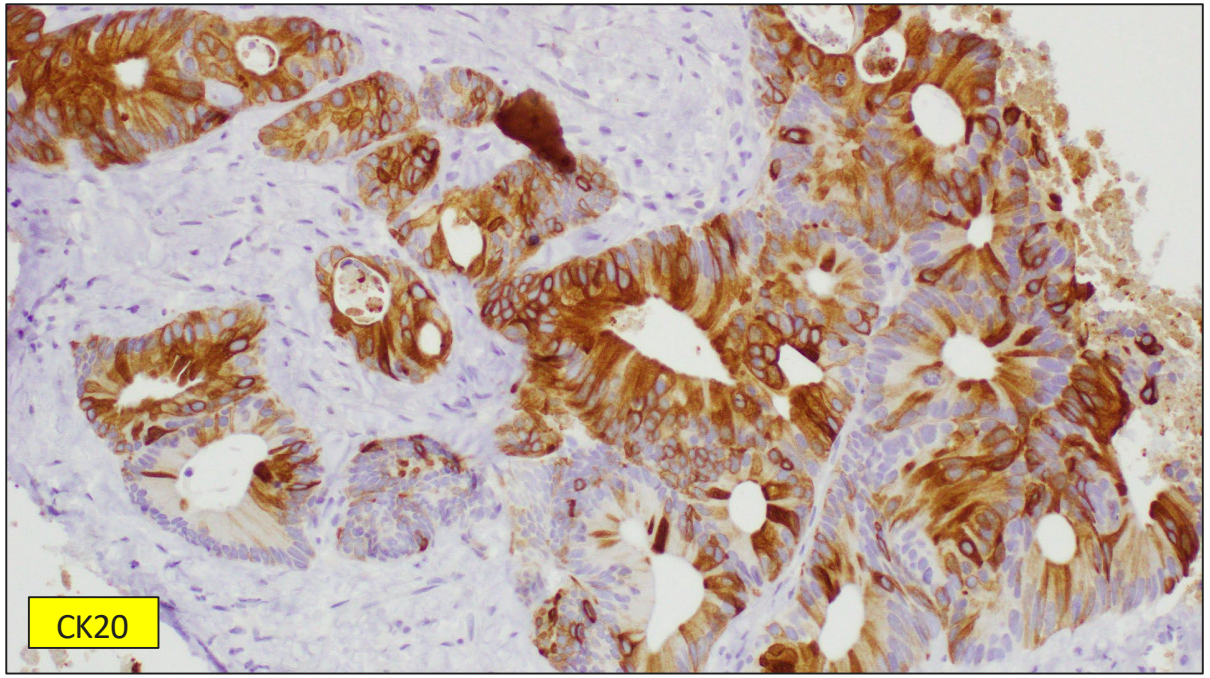
Rectal Biopsy in 2016



Liver Biopsy 2017



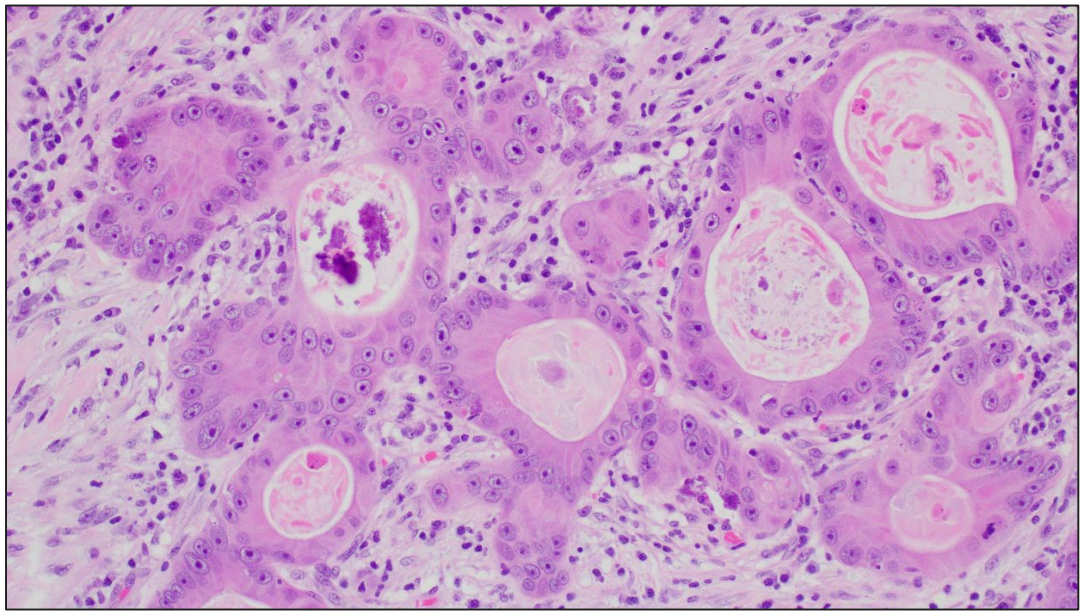


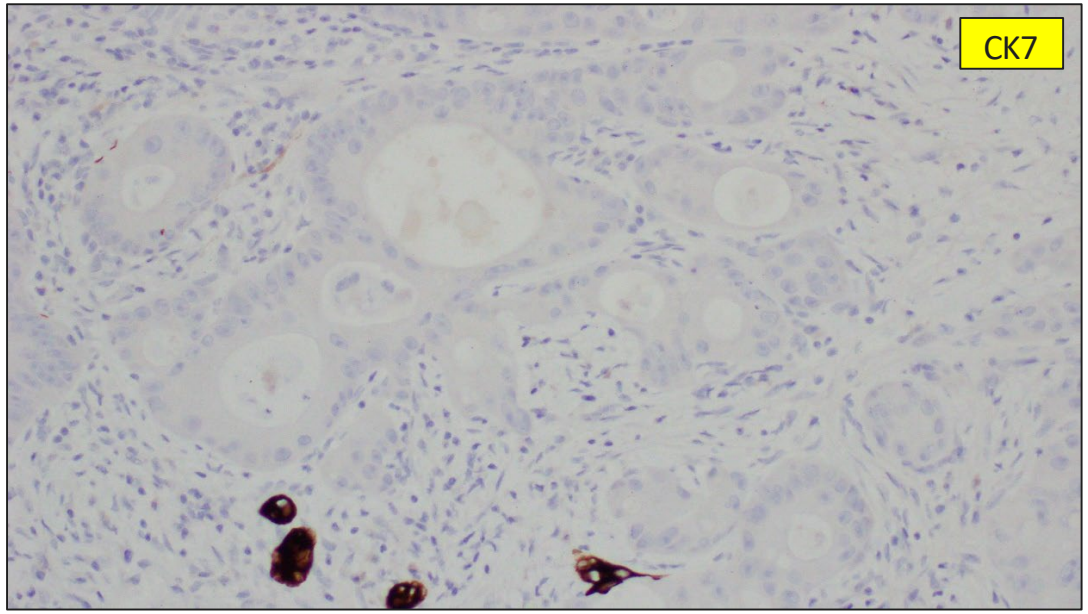


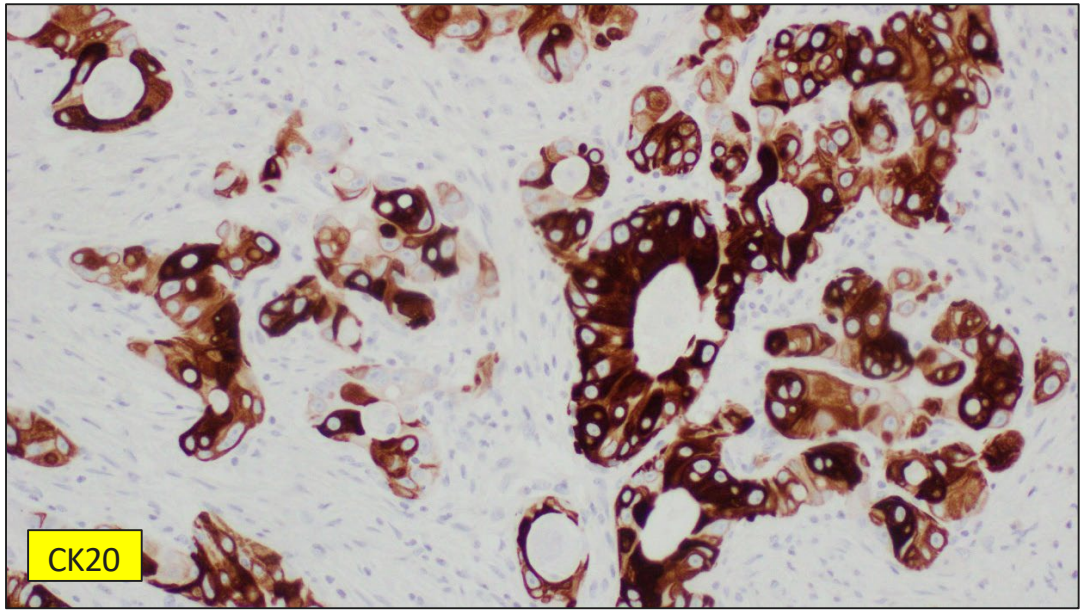
Liver met in 2018; same immunoprofile
Liver met in 2019; sent for molecular

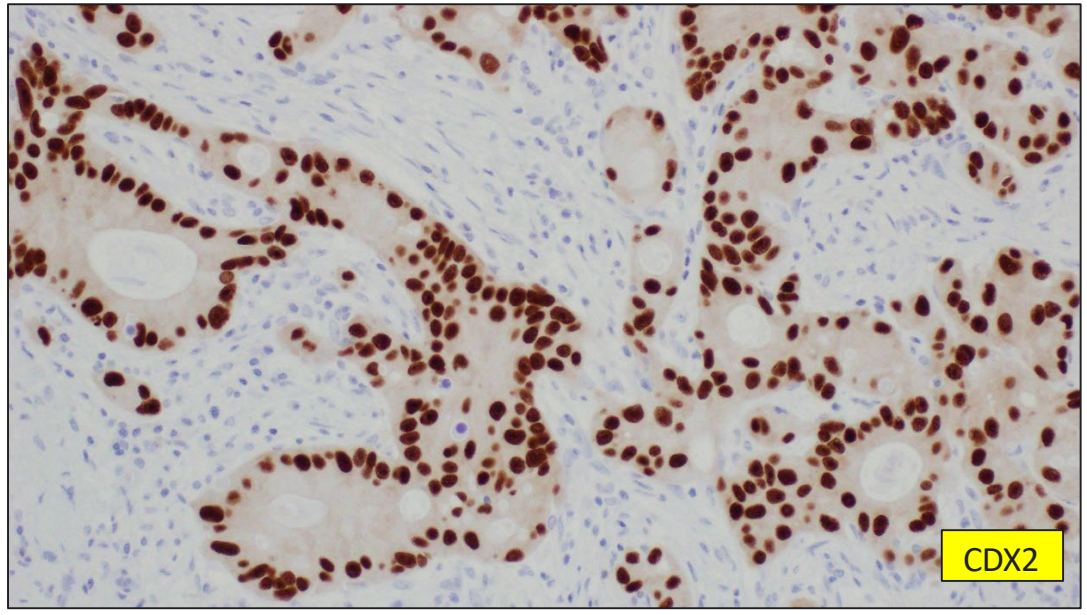
Date	History	Pathology Diagnosis	Molecular Findings
February 2016	Presented with rectal bleeding Colonoscopy: rectal polyp	Possible malignancy	
May 2016	Transanal resection + Chemoradiation	Colorectal adenocarcinoma (low-grade)	***NRAS* p.Q61K** ***TP53* p.R248W**
August 2016	Anterior peritoneal resection		
December 2016	PET CT: concerning liver metastasis	Liver biopsy: negative	
August 2017	PET CT: increase size of left lobe liver lesion Liver biopsy	Positive for metastatic adenocarcinoma of colorectal origin (CK7-/CK20+/CDX-2+)	
April 2018	Surveillance CT: new left lobe lesion (segment III) Liver biopsy	Positive for metastatic adenocarcinoma of colorectal origin (CK7-/CK20+/CDX-2+)	
July 2019	Segment III liver wedge resection	Metastatic adenocarcinoma, well-differentiated of colorectal origin (diagnosed based on morphology)	***IDH1* p.R132C** ***KRAS* p.G13D**
November 2019	Enlarging large cystic nodule (2.4 cm) in the medial right lower lobe Lung nodule biopsy	Metastatic colorectal adenocarcinoma (diagnosed based on morphology)	

Liver Resection 2019

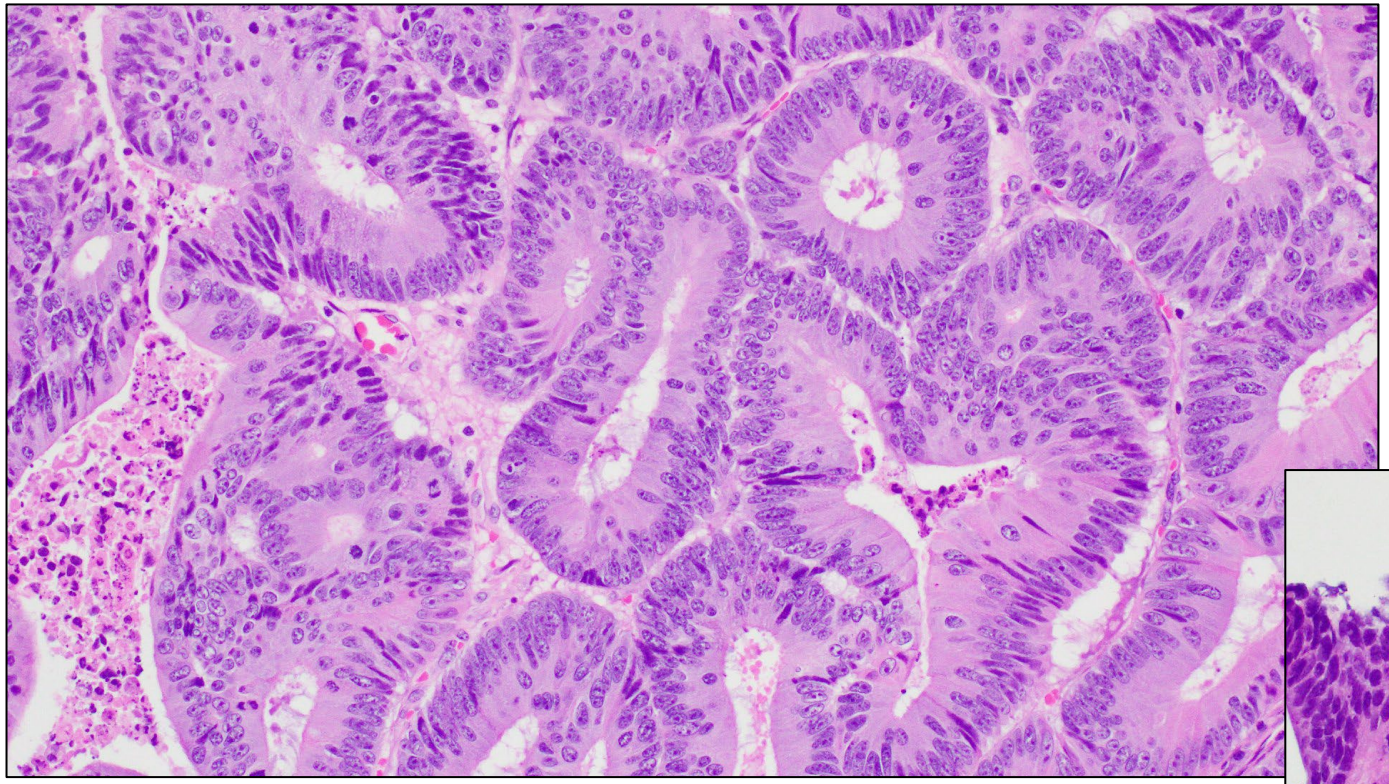


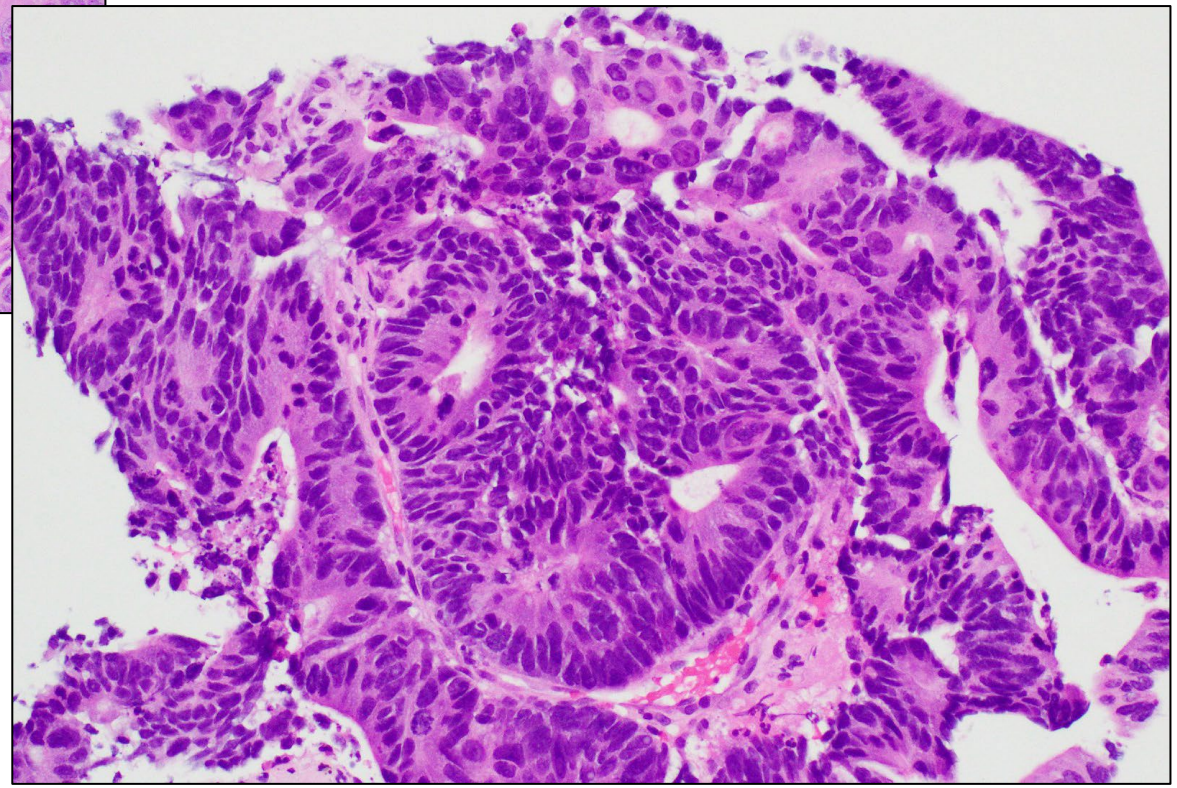






Liver and Lung Nodule from 2019





Date	History	Pathology Diagnosis	Molecular Findings
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May 2016	Transanal partial resection + Chemoradiation	**Colorectal adenocarcinoma** (low-grade)	***NRAS* p.Q61K** ***TP53* p.R248W**
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December 2016	PET CT: concerning liver metastasis	Liver biopsy: negative	
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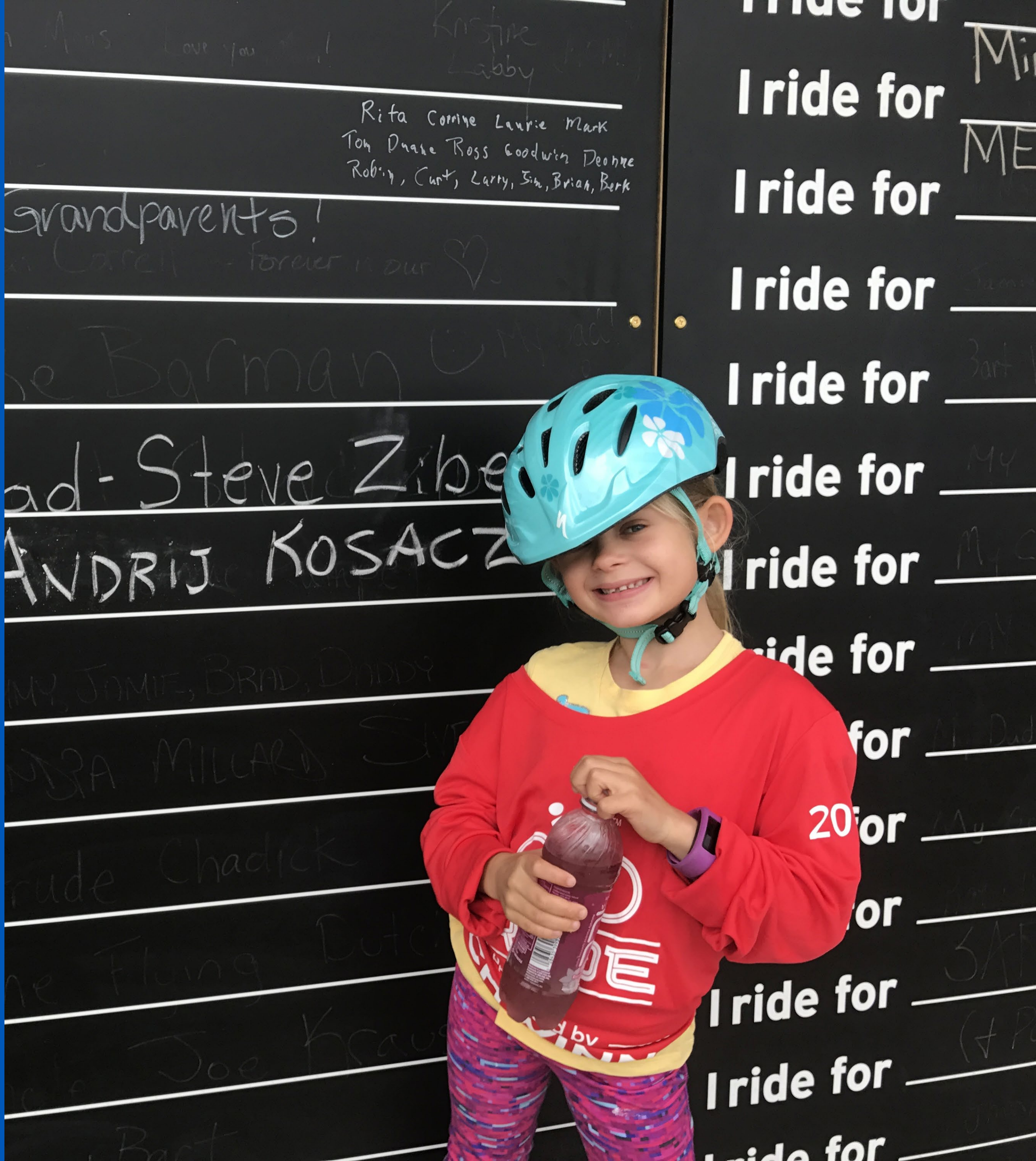
July 2019	Segment III liver wedge resection	**Metastatic adenocarcinoma, well-differentiated of colorectal origin** (diagnosed based on morphology)	***IDH1* p.R132C** ***KRAS* p.G13D**
November 2019	Enlarging large cystic nodule (2.4 cm) in the medial right lower lobe Lung nodule biopsy	**Metastatic colorectal adenocarcinoma** (diagnosed based on morphology)	***NRAS* p.Q61K** ***TP53* p.R248W**

This case highlights the importance of biomarker testing, especially in the setting of previously treated tumors.

RECENTLY APPROVED & EMERGING BIOMARKERS

We all have our reasons



A Novel Biomarker in the Gastric/GEJ Cancer

- Zolbetuximab received FDA approval on October 18, 2024 along with FDA approval for the VENTANA CLDN18 (43-14A) RxDx Assay as a companion diagnostic device to help identify patients who may be eligible for treatment.
- Initially invited to sit on a zolbetuximab Advisory Board.
- Unique approach by a pharma company asking pathologists to provide feedback/guidance on what is considered a good biomarker and to evaluate CLDN18.2.
- Guidance on what **Pathologists** are interested in knowing about when a new drug and it's companion diagnostic biomarker is nearing approval.
    - ***Proportion of patients expressing the biomarker***
    - ***Reproducibility of staining***
    - ***Timing of analysis***
    - ***CDx vs LDT***
    - ***Scoring algorithm***
    - ***Training resources and general dissemination of information to pathologists***
- Invited to be part of an international consensus committee to review and create a CLDN Path Hub.

Mod Pathol 37 (2024) 100589

MODERN PATHOLOGY

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY
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Journal homepage: https://modernpathology.org/

Review Article

Claudin-18.2 Immunohistochemical Evaluation in Gastric and Gastroesophageal Junction Adenocarcinomas to Direct Targeted Therapy: A Practical Approach

Matteo Fassan[a,b,*], Takeshi Kuwata[c], Kristina A. Matkowskyj[d], Christoph Röcken[e], Josef Rüschoff[f]

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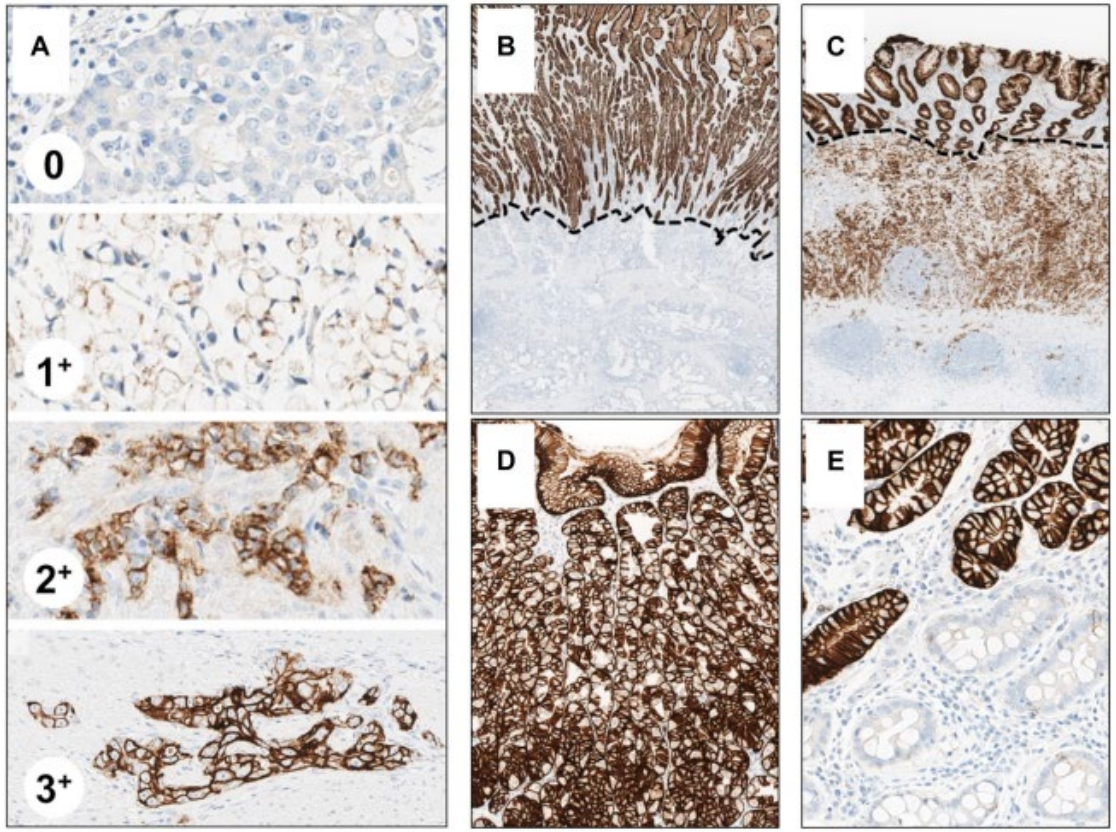


Claudin Proteins

- **Family of at least 27 transmembrane proteins**
- **Composed of 4 transmembrane helix domains and 2 extracellular loops**
- **Major structural components of tight junctions**
  - Play a critical role in maintenance of cell polarity and in selective paracellular permeability
- **Expressed throughout the body and display tissue specific expression patterns**

Basic Structural Components of Tight Junctions

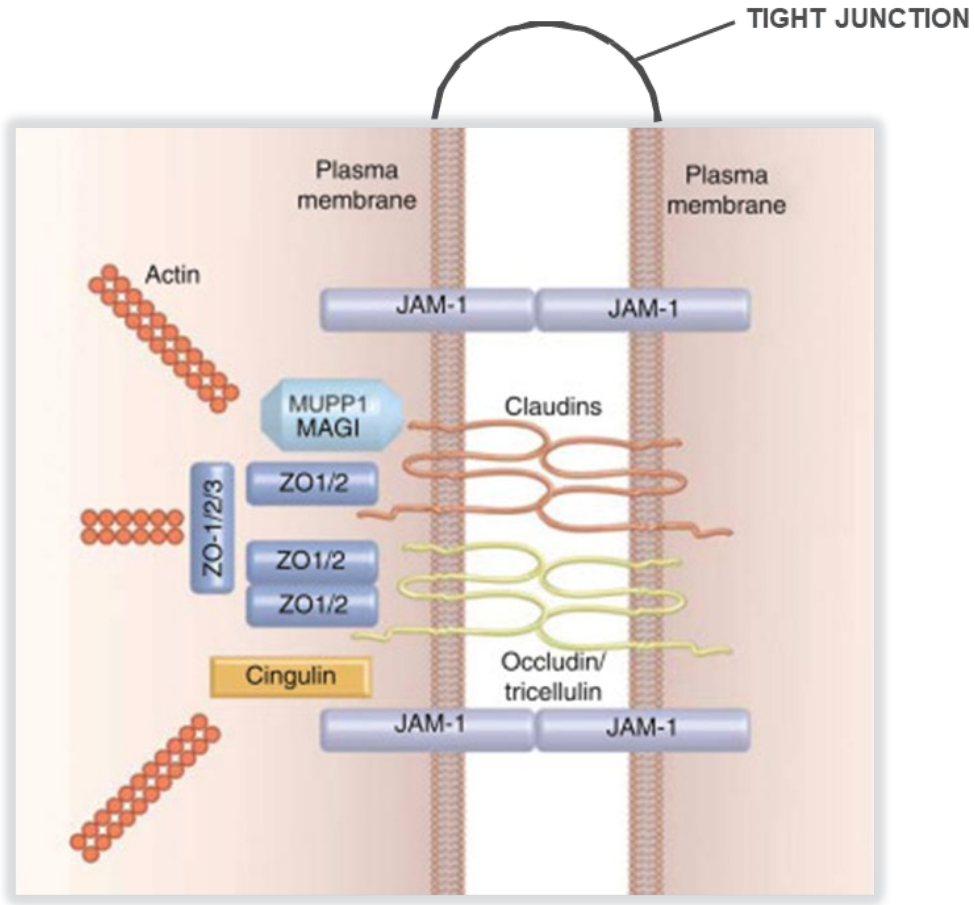
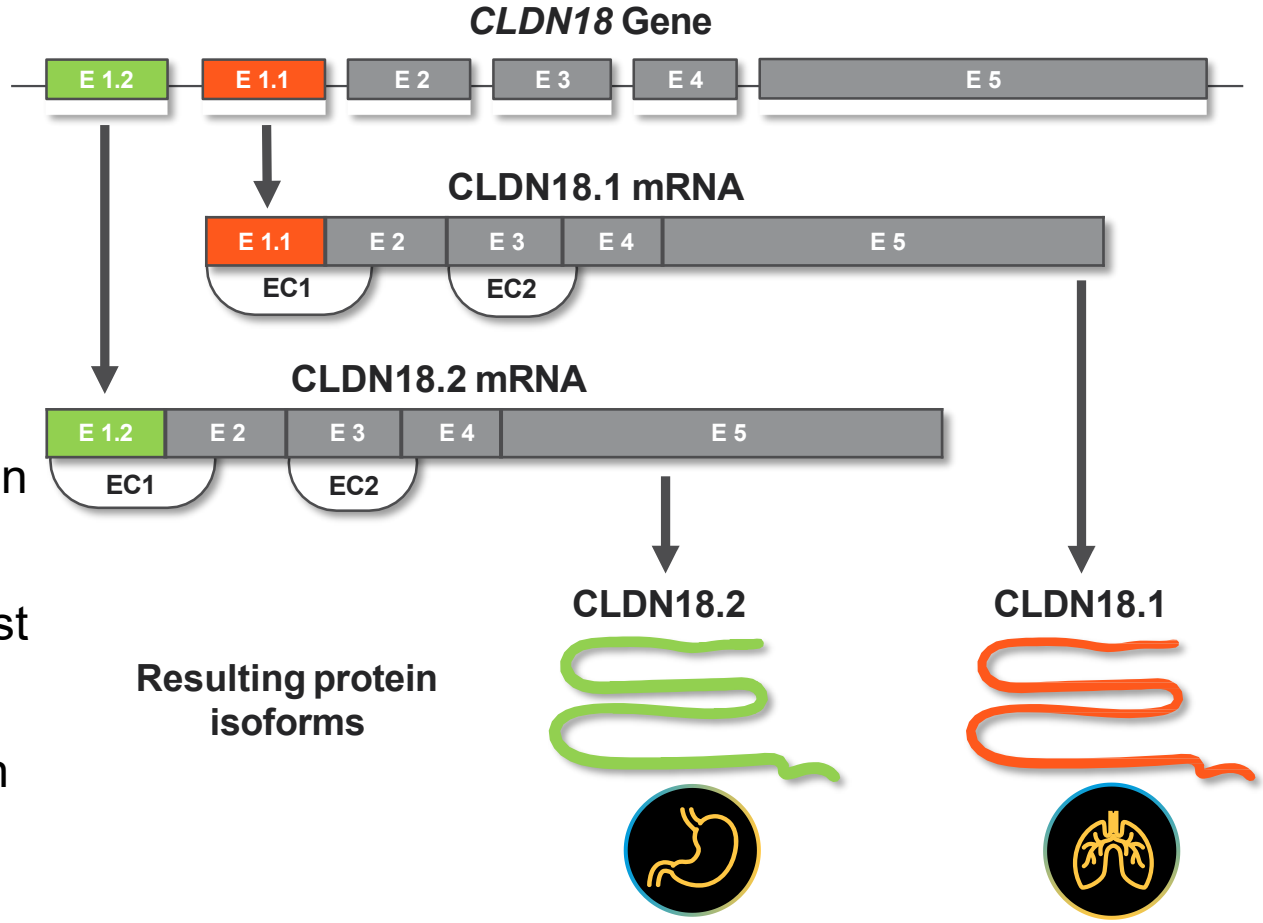


Image from Niessen CM. *J Invest Dermatol*. 2007 Nov;127(11):2525-32.

1. Singh AB, et al. *Semin Cell Dev Biol*. 2015;42(1096-3634 (Electronic):58-65.
2. Mineta K, et al. *FEBS Lett*. 2011;585(4):606-12.
3. Sahin U, et al. et al. *Clin Cancer Res*. 2008;14(23):7624-7634.
4. Otani T, et al. *Trends Cell Biol*. 2020;30(12):1014.
5. Tsukita S, et al. *Trends Biochem Sci*. 2019;44(2):141-152.
6. Hu YJ, et al. *Mol Biol Rep*. 2013;40:6123-42.
7. Hewitt KJ, et al. *BMC Cancer*. 2006;6:186. doi: 10.1186/1471-2407-6-186.
8. Image adapted from Niessen CM. *J Invest Dermatol*.2007;127(11):2525-32.

CLDN18.1 AND CLDN18.2: TWO EXISTING ISOFORMS OF CLDN18

- Claudins are present throughout the body.
- The *CLDN18* gene has two alternative first exons, E1.1 and E1.2.
- Leads to splice variants encoding two protein isoforms:
  - CLDN18.1
  - CLDN18.2
- A high degree of homology is shared between CLDN18.1 and CLDN18.2.
- Differ by 8 of 51 amino acids in the first extracellular domain.
- When evaluating gastric tumor tissue, staining can be attributed to the presence of CLDN18.2.

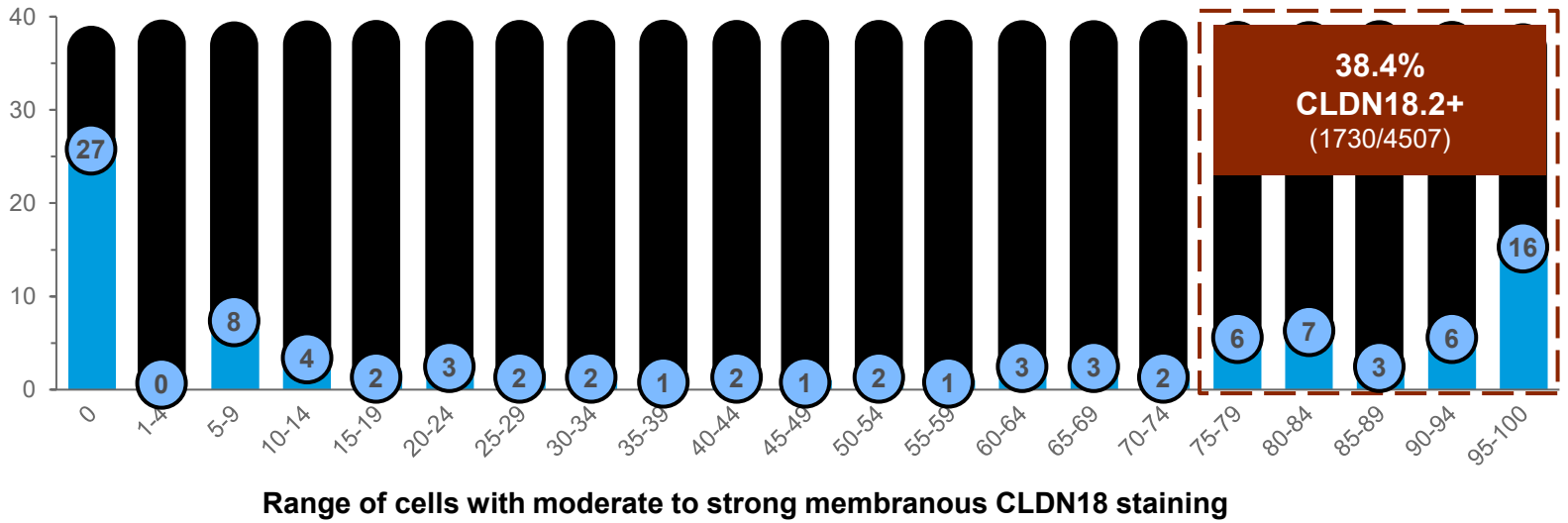


1. Sahin U, et al. *Clin Can Res*. 2008;14(23):7624–7634.
2. Niimi T, et al. *Mol Cell Biol*. 2001;21(21):7380–7392.
3. Saito T, et al. *ESMO World Congress on Gastrointestinal Cancer*. 2021.

Global Prevalence of CLDN18.2 Across two Phase 3 Clinical Trials

Distribution of CLDN18 Expression (n=4507)

Screened patients in two phase 3 clinical trials



Range of cells with moderate to strong membranous CLDN18 staining

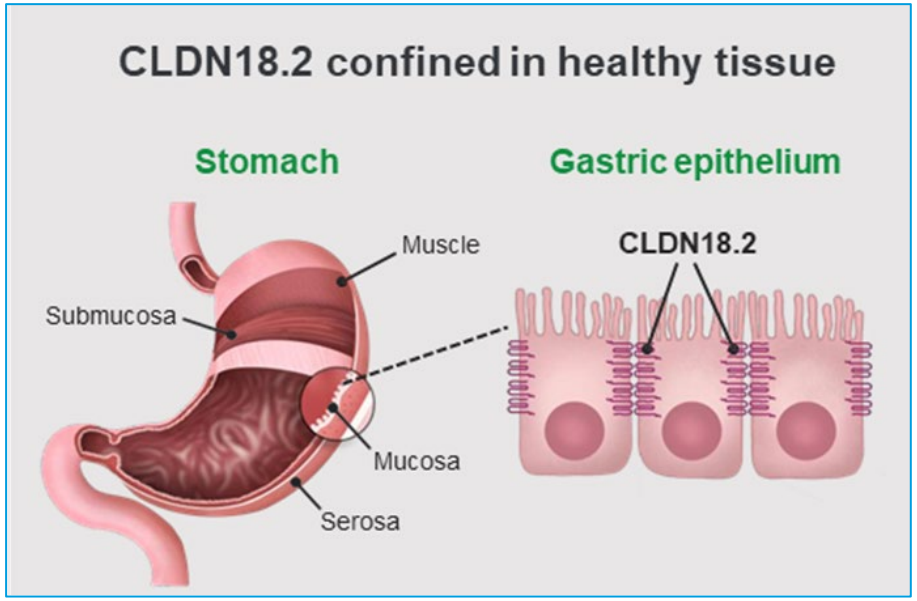
- 38.4% of screened patients across two global phase 3 clinical trials had CLDN18.2+ tumors.
- 42.3% (1568/3705) of HER2-negative patients were CLDN18.2+.
- In comparison to other biomarkers, CLDN18.2 is quite prevalent.

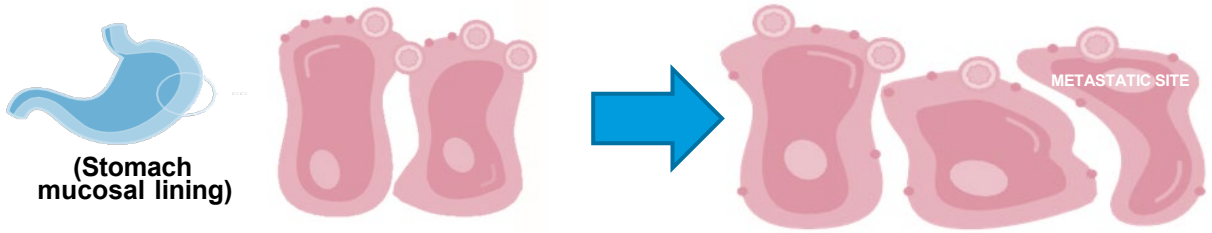
CLDN18.2 positivity was defined as ≥75% of tumor cells demonstrating moderate to strong, membranous CLDN18 staining

CLDN18 staining by IHC using the investigational VENTANA CLDN18 (43-14A) RxDx Assay (Ventana Medical Systems, Inc. [Roche Tissue Diagnostics]) on the BenchMark ULTRA instrument.
All IHC testing was performed by central laboratory testing (Q2 Solutions).
Includes both HER2+ and HER− patients.

1. Shitara K, et al. *Gastric Cancer*. 2024;27:1058-1068.

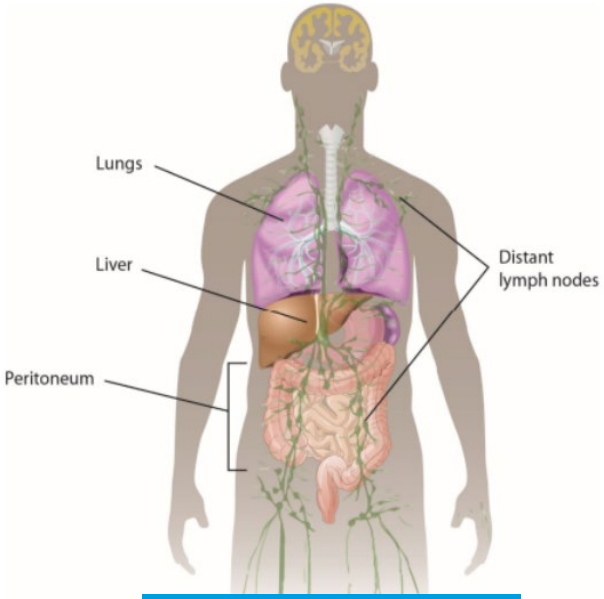
CLAUDIN 18.2 EXPRESSION IN PRIMARY AND METASTATIC GASTRIC CANCER





De Novo CLDN18.2 expression in Gastric Cancer

CLDN18.2 expression in both PRIMARY and METASTATIC SITES



Most common sites of metastasis for Gastric/GEJ Cancer

Image adapted from: Verstegen MHP, Harker M, van de Water C, et al. Metastatic pattern in esophageal and gastric cancer: influenced by site and histology. *World J Gastroenterol* 2020.

This data suggests that accurate CLDN18.2 assessment can be performed from either the primary tumor and/or sites of metastases.

1. Pellino A, Brignola S, Riello E, et al. *J Pers Med*. 2021;11(11):1095.
2. Rohde C, Yamaguchi R, Mukhina S, Sahin U, Itoh K, Türeci Ö.*J Clin Oncol*. 2019;49(9):870-876.
3. Sahin U, Koslowski M, Dhaene K, et al. *Clin Cancer Res* 2008;14(23):7624-34.
4. Verstegen MHP, Harker M, van de Water C, et al. *World J Gastroenterol* 2020;26(39):6037-46.

Assays/antibodies to test for CLDN18.2

- CLDN18 antibodies can identify both CLDN18 isoforms (CLDN18.1 and CLDN18.2).
- When evaluating G/GEJ tumour tissue, staining can be attributed to the presence of CLDN18.2 because:
    - * CLDN18.2 is normally present in gastric epithelium and is often retained in malignant gastric tissue
    - * CLDN18.1 is primarily expressed in lung adenocarcinoma and its expression is negligible or absent in G/GEJ cancers
- Several assays, antibodies, and platforms are available for the assessment of CLDN18.2 expression.
- The choice of assays and antibodies includes but is not limited to*:

Name	Company	Clone/catalog #	Clonality	Host	Field of Use
VENTANA® CLDN18 (43-14A) RxDx Assay	Roche Tissue Diagnostics	43-14A (08285918001)	Monoclonal	Mouse	IVD (CDx assay)
VENTANA® CLDN18 (43-14A) Assay	Roche Tissue Diagnostics	43-14A (08504148001)	Monoclonal	Mouse	IVD (analytical assay)
Anti-Claudin18 antibody	abcam	43-14A (ab314690)	Monoclonal	Mouse	Research Use only
PathPlus™ Monoclonal Mouse anti-Human CLDN18 / Claudin 18 Antibody	LSBio	LS-B16145	Monoclonal	Mouse	Research Use only
Claudin-18 antibody	Novus Biologicals	NBP2-32002	Polyclonal	Rabbit	Research Use Only

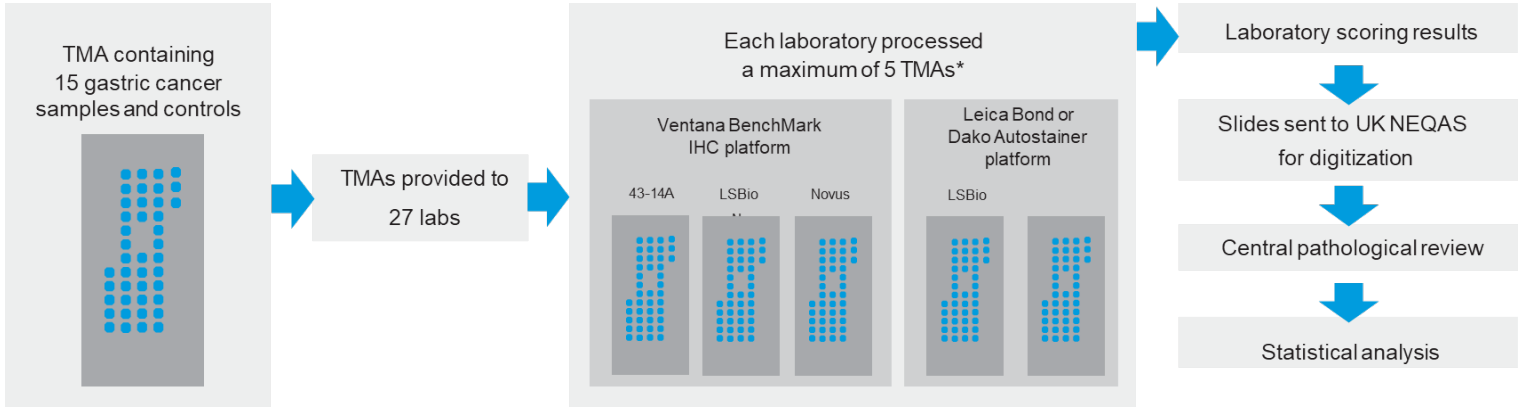
Saito T, Matsuda T, Moran D. ESMO World Congress on Gastrointestinal Cancer 2021; Poster 286

Current Claudin18.2 targeted therapies under investigation in gastric cancer

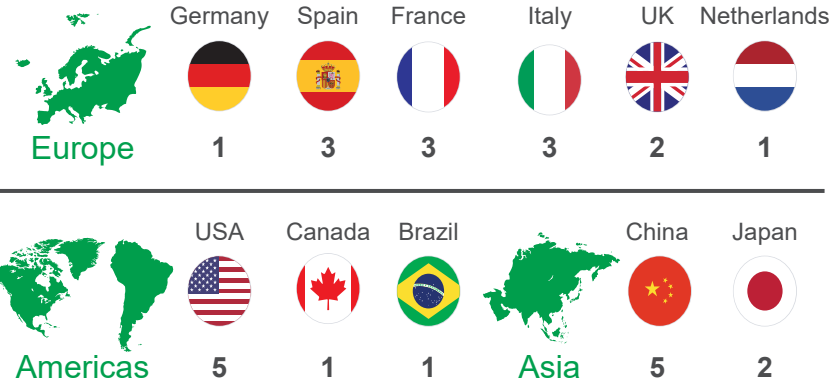
Therapy name (Company)	Class	Stage	NCT	Estimated study completion	Indication/Description	Definition of CLDN18.2 positivity	Key findings from Phase I/II studies to date
Zolbetuximab (Astellas)	mAb	Phase III	NCT03504397	March 2025	1L+ CLDN18.2+ HER2- GC/GEJC/PC	IHC 2+/3+ in TC≥75% ~35-40% of patients	Based off promising efficacy data from Phase III trials, Zolbetuximab has received approval in Japan and by the EMA, it is currently awaiting an FDA decision following refiling
Osemitamab (Mabspace/Transcenta/ BMS)	mAb	Phase III	NCT06093425	Jan 2026	1L CLDN18.2+ GC/GEJC HER2 negative, known PD-L1 CPS status	IHC membrane staining ≥10% tumour cells with ≥1+ intensity per LDT assay[1]	Preliminary efficacy data indicate good anti-tumour activities; especially for the patients with H/M CLDN18.2 expression.
ASKB589 (AskGene Pharma)	mAb	Phase III	NCT06206733	Dec 2028	1L+ CLDN18.2+ GC/GEJC	Medium-high CLDN18.2 expression IHC 2+/3+ membrane staining in tumour cells ≥40%[2]	ASKB589 combined with CAPOX and PD-1 inhibitor has manageable safety and promising antitumor activity.
M108 (FutureGen Biopharma)	mAb	Phase III	NCT06177041	Nov 2027	1L CLDN18.2+, HER2- GC/GEJC PD-L1 CPS < 5	IHC 1+/2+/3+ ≥10%*[3]	N/A
AZD0901/CMG901 (AstraZeneca)	ADC	Phase III	NCT06346392	Oct 2026	2L+ CLDN18.2+ HER2- GC/GEJC	≥ 2+ intensity in ≥ 20% tumour cells*[4]	Preliminary data indicate promising clinical efficacy. The Phase 1b dose-expansion trial is rapidly enrolling, with trials planned.
SHR-A1904 (Hengrui Medicine**)**	ADC	Phase I/III	NCT06350006	Dec 2028	1L+ CLDN18.2+ HER2- GC/GEJC/PC	IHC 2+/3+ in tumour cells ≥ 50%*[6]	N/A
LM-302 (LaNova Medicines)	ADC	Phase I/II	NCT06351020	Dec 2026	2L+ CLDN18.2+ HER2- GC/GEJC	Tumour cells ≥ 50%, IHC ≥ 2+[7]	LM-302 was well-tolerated and demonstrated promising anti-tumour activity in CLDN18.2-positive patients with 3L+ and beyond GC/GEJC in Phase I/II trials.
CT041 (CARsgen)	CAR-T	Phase II	NCT04581473	June 2038	3L+ CLDN18.2+ HER2- GC/GEJC	IHC 2+/3+ tumour cells ≥ 40% patients[8]	CT041 demonstrated a promising safety profile and highly encouraging efficacy in heavily pretreated patients with CLDN18.2-positive advanced GI cancers.
AZD5863 (AstraZeneca)	BsAb†	Phase I/II	NCT06005493	Nov 2026	2L+ GC/GEJC/PC	Must show positive CLDN18.2 expression in tumour cells as determined by IHC[9]	Study data expected in 2025 or later.
IBI343 (Innovent)	ADC	Phase III	NCT06238843	Dec 2027	2L+ CLDN18.2+ HER2- GC/GEJC	IHC 2+/3+ on at least 40% of tumour cells*[5]	IBI343 was well tolerated and demonstrated signs of efficacy in patients in Phase Ia/Ib trials.

Global Ring Study: Method

- Up to **5 sets of a TMA comprising 15 FFPE gastric cancer samples**
- Represented in triplicate by 1.5 mm cores
- Distributed to **27 laboratories** in **11 countries**



Number of laboratories



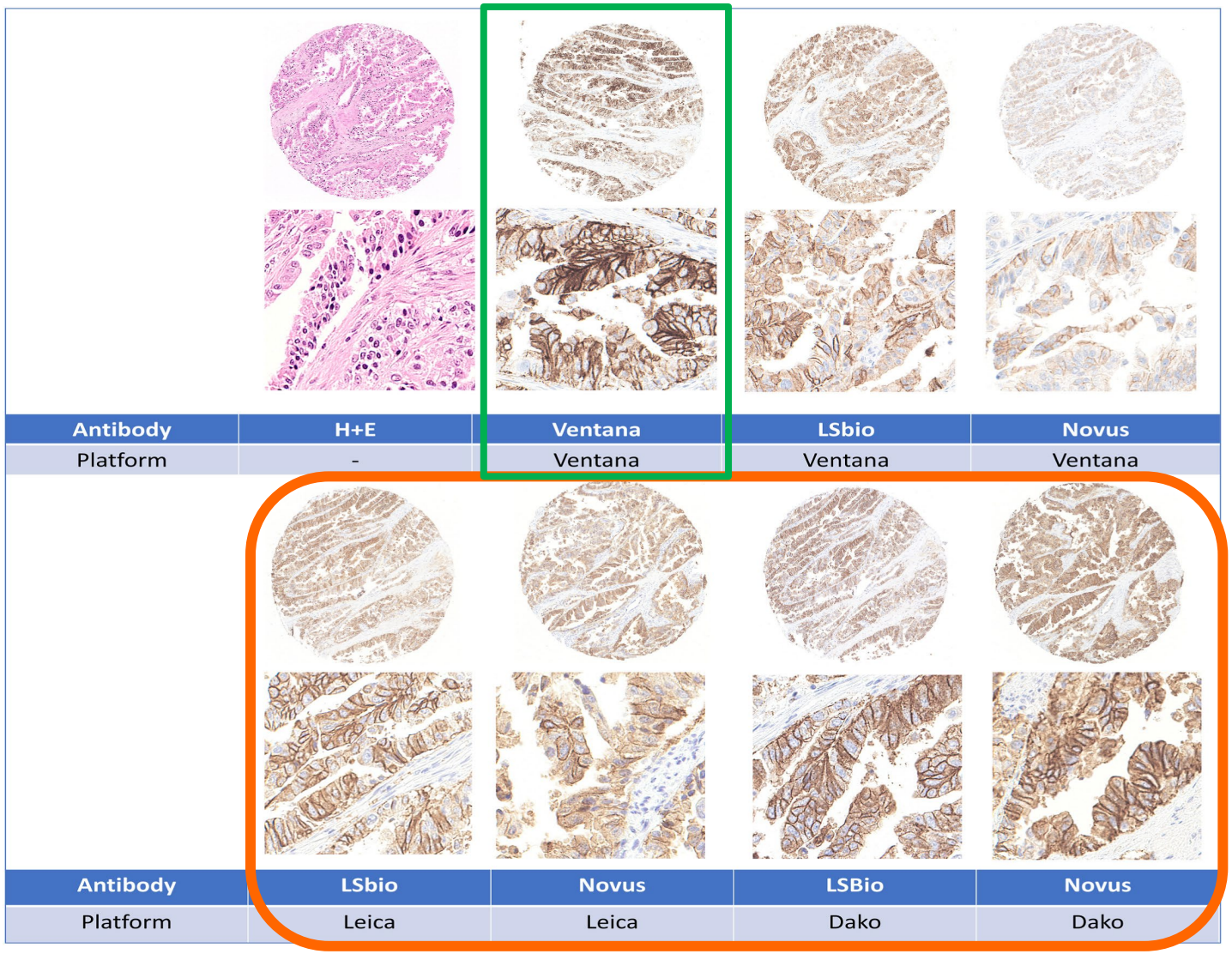
- Laboratories were provided with staining protocols, an instructional video, and reference guide for understanding of CLDN18.2 IHC staining characteristics.
- **Expected reference** results were obtained for each sample stained by a central laboratory using the VENTANA® CLDN18 (43-14A) Assay.
- CLDN18.2 positivity was defined as ≥75% of tumor cells demonstrating moderate to strong (≥2+) membranous CLDN18 staining intensity by immunohistochemistry, as used in two clinical trials.

Antibodies and platforms		Number of laboratories
Antibodies	Ventana 43-14A	26
	LSBio	25
	Novus	27
Platforms	Ventana BenchMark	27
	Dako AutoStainer	10
	Leica BOND	13

*This assay is an antibody reagent product that is classified as Class I in the USA and General IVD (CE-IVD Analytic) under IVDD in the EU. It is not a CDx assay.
CDx, companion diagnostic; CE, conformité européenne; CLDN18, claudin 18; FFPE, formalin-fixed paraffin-embedded; GC, gastric cancer; IVD, *in vitro* diagnostic; IVDD, *in vitro* diagnostic directive; NEQAS, National External Quality Assessment Services; TMA, tissue microarray.

Bharat Jasani, Philippe Taniere, Hans-Ulrich Schildhaus, Kevin Blighe, Suzanne Parry, Dawn Wilkinson, Neil Atkey, Scott Clare-Antony, Clare McCabe, Christine Quinn, Steven Gibney, Andrew Dodson, Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer, Laboratory Investigation, Volume 104, Issue 1, 2024, 100284.

Global Ring Study: Results



Antibody	H+E	Ventana	LSbio	Novus
Platform	-	Ventana	Ventana	Ventana

Antibody	LSbio	Novus	LSBio	Novus
Platform	Leica	Leica	Dako	Dako

- The **Ventana assay shows the darkest** (brown/black) positive staining, with clear and sharp membranous staining.
- Both the **LSBio** and **Novus** show weaker staining on the Ventana platform and ***more defined membranous staining on the Leica and Dako platforms.***
- Analytical performance (**accuracy, sensitivity, and specificity**) of the **VENTANA CLDN18 (43-14A) IVD Assay was >95%** and reproducible across the 27 laboratories when compared to the consensus reference results.
- **Analytical performance was equivalent** to the VENTANA (43-14A) IVD Assay for the **LSBio antibody** when stained on the Dako or Leica platform. Staining was also reproducible for the **Novus antibody.**

Bharat et. al. Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer. Laboratory Investigation, Volume 104, Issue 1, 2024, 100284.

Evaluation of CLDN18.2 Expression

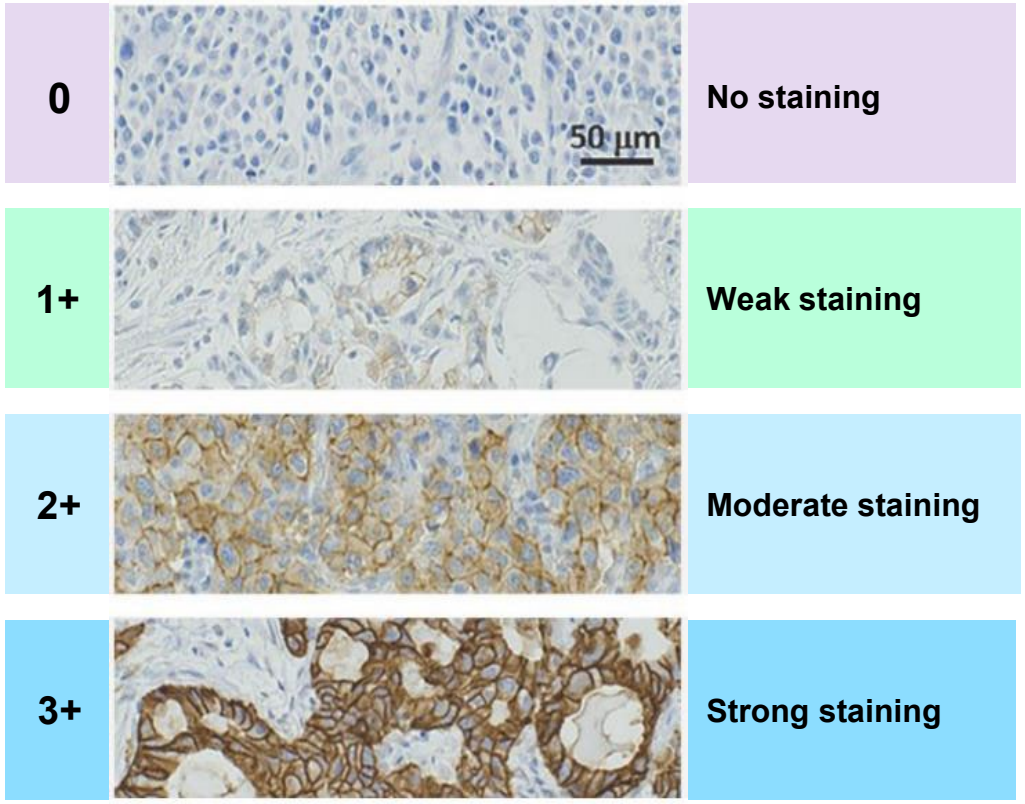
Analysis includes both Membranous Stain Intensity and Percentage of Tumor

General Rules for CLDN18 Staining Evaluation

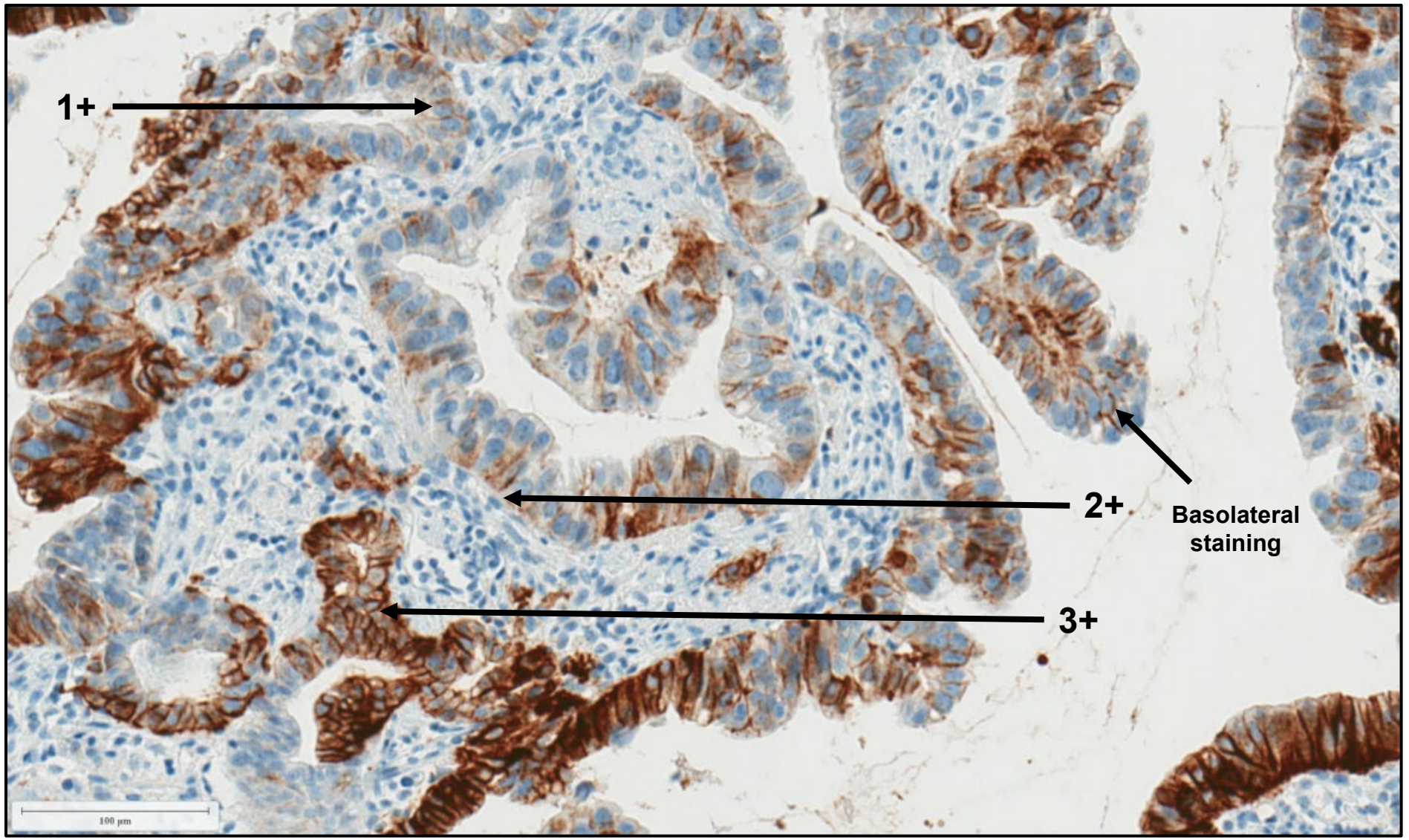
Cells included	Greater than 50 viable tumor cells
Subcellular localization	Cell membrane
Staining intensity	Moderate (2+) to strong (3+)
Staining pattern	Apical, circumferential (partial & complete), basolateral/lateral and micro-luminal
Scoring denominator	Total number of viable invasive tumor cells

NOTE: The entire tumor section should be considered for the scoring.

IHC scoring for CLDN18 in gastric cancer (membrane staining of tumor cells)



1. Pellino A, et al. *J Pers Med*. 2021;11(11):1095.
2. Rohde C, et al. *Jpn J Clin Oncol*. 2019;49(9):870-876.
3. Sahin U, Türeci Ö, et al. *Ann Oncol*. 2021;32(5):609–619. Supplementary appendix.

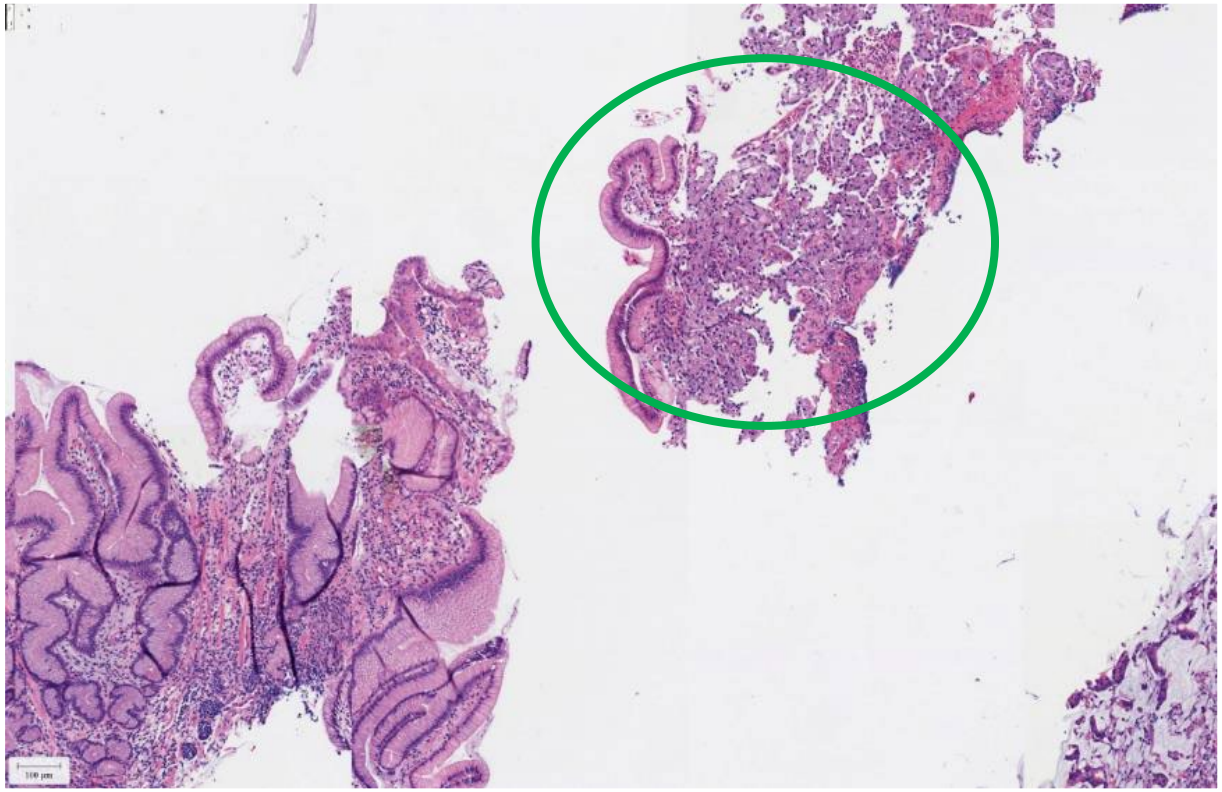


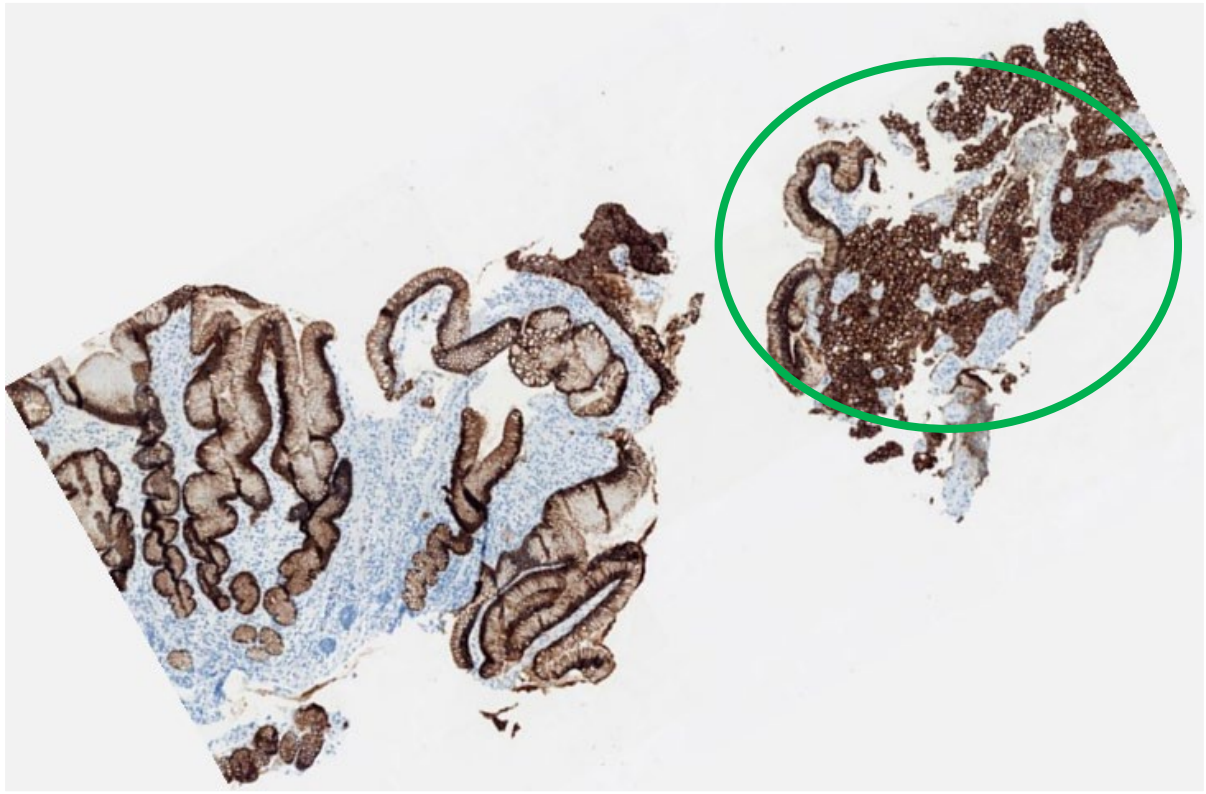
1+, 2+, and 3+ CLDN18 staining intensities* (20X magnification). Incomplete basolateral staining may be present.

*In two phase III clinical trials, CLDN18.2 positivity is defined as 2+/3+staininig intensity in ≥75% of tumour cells using the investigational Ventana CLDN18 (43-14A) RxDX assay.[2,3]

1. Astellas Data on File. Images of Claudin18.2 staining. MA-DOF-00276.
2. Shitara K, et al. *Lancet*. 2023;401(10389):1655–1668. 3. Shah MA, et al. *Nat Med*. 2023;29(8):2133–2141.

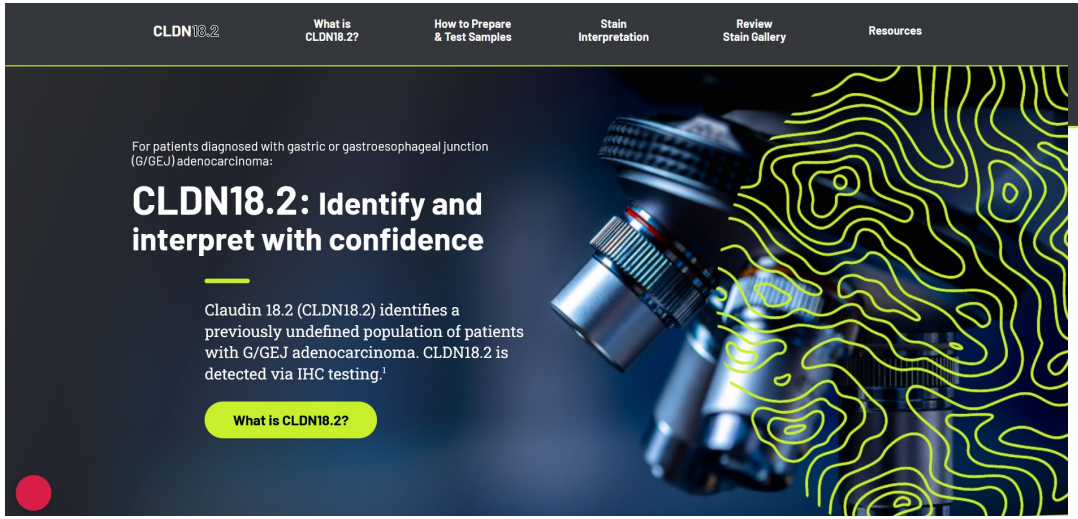
MUST DISTINGUISHING BENIGN VERSUS INVASIVE TUMOR FOR CLDN18 STAINING

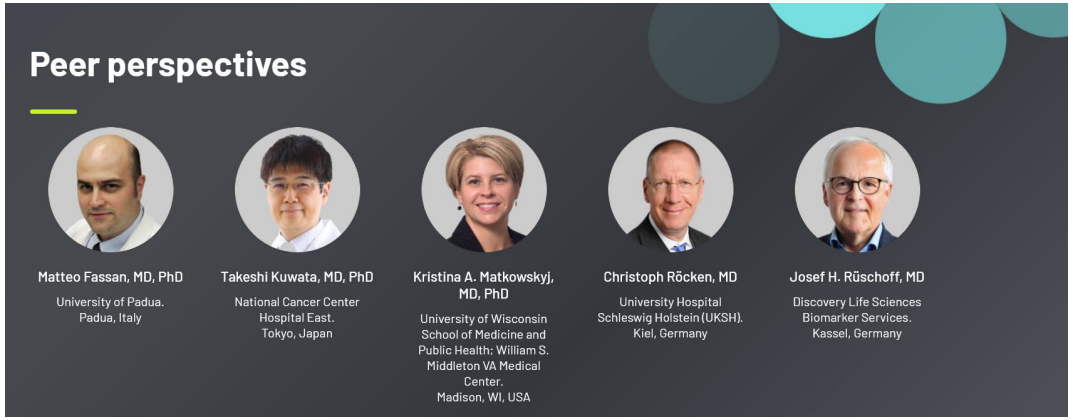


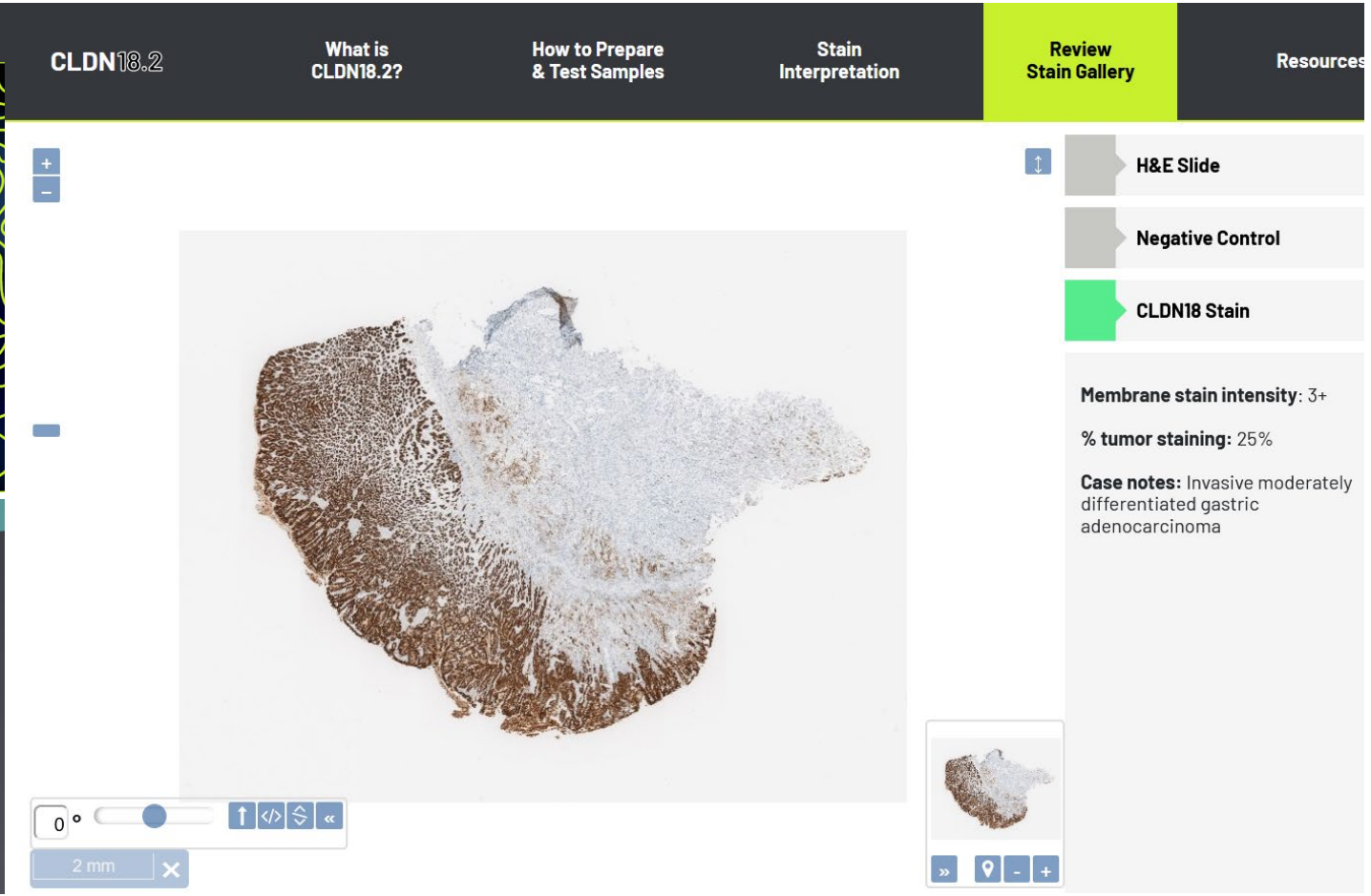


THE CLDN18.2 PATHOLOGY HUB EXPERIENCE:

AN INTERPRETATION GUIDE & TUTORIAL



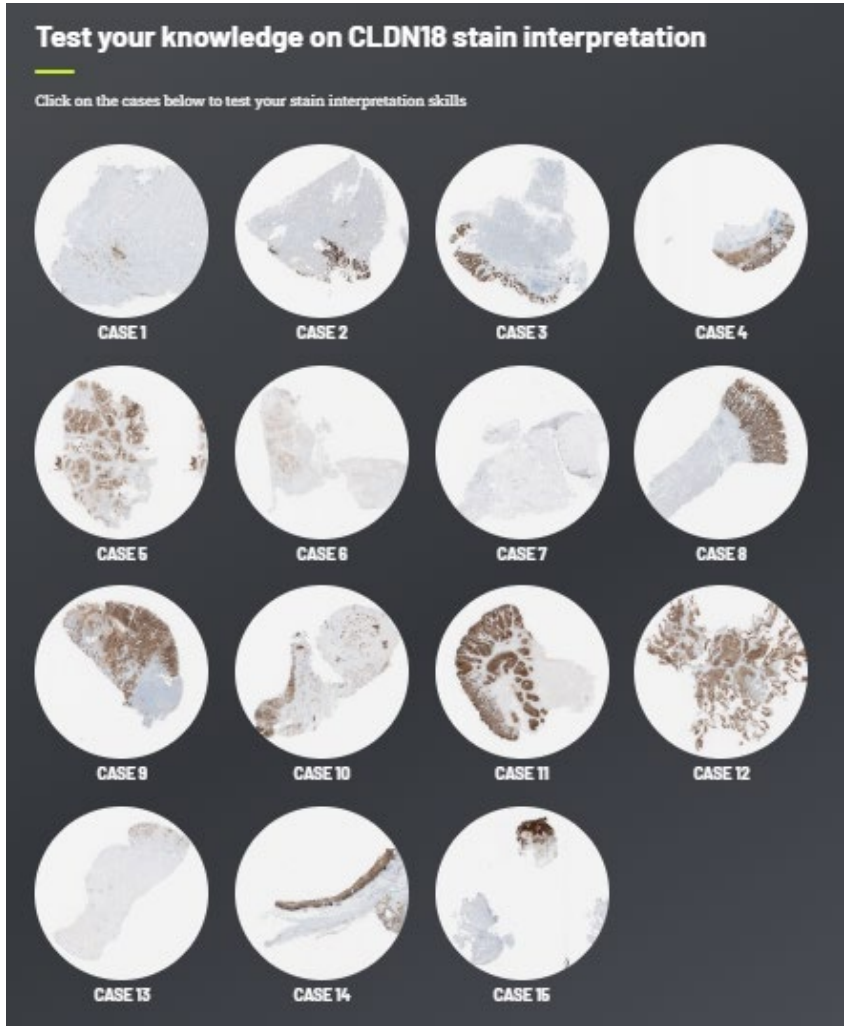




https://www.claudin182.com/

Stain Interpretation Quiz

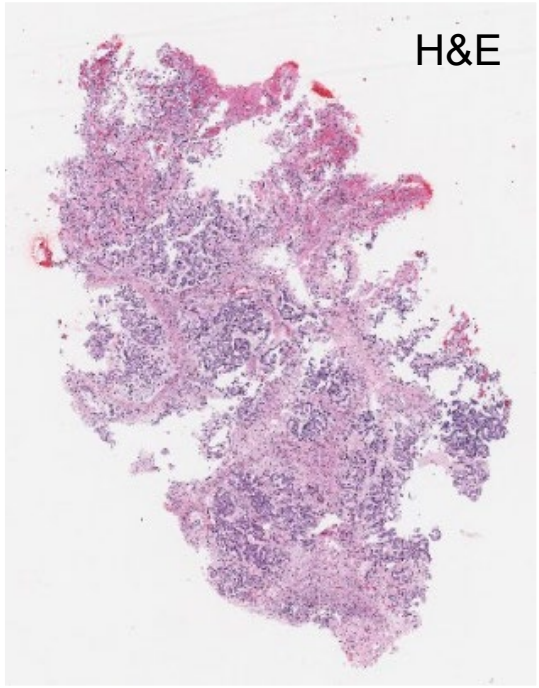
- Review and test your interpretative skills through matched, consensus scored whole slide images
- 15 cases to test your skills
- Expert commentary on all cases

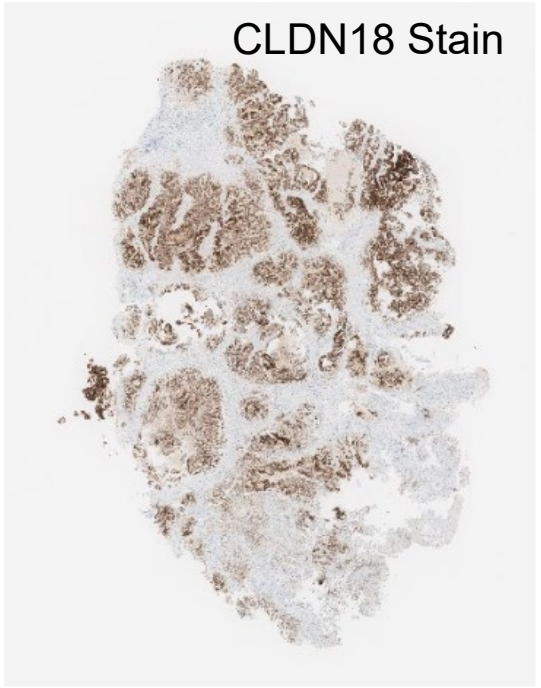


What is the percentage of tumour cells stained with moderate-to-strong (2+/3+) membranous stain intensity?

- 70%
- 75%
- 85%
- 95%
- 100%

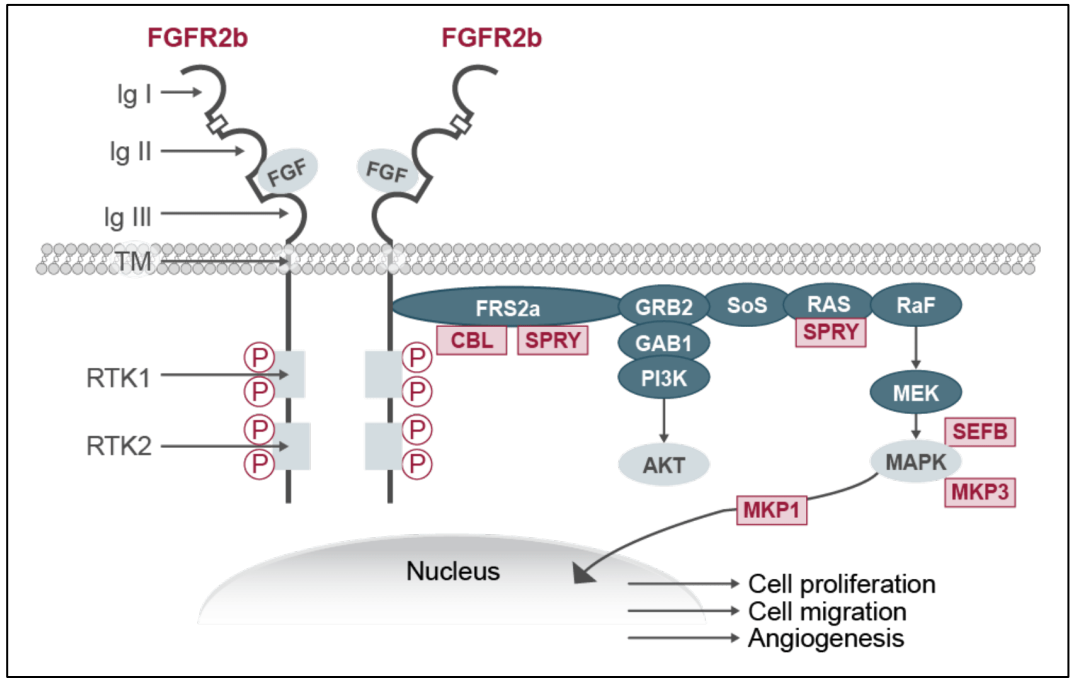
Submit





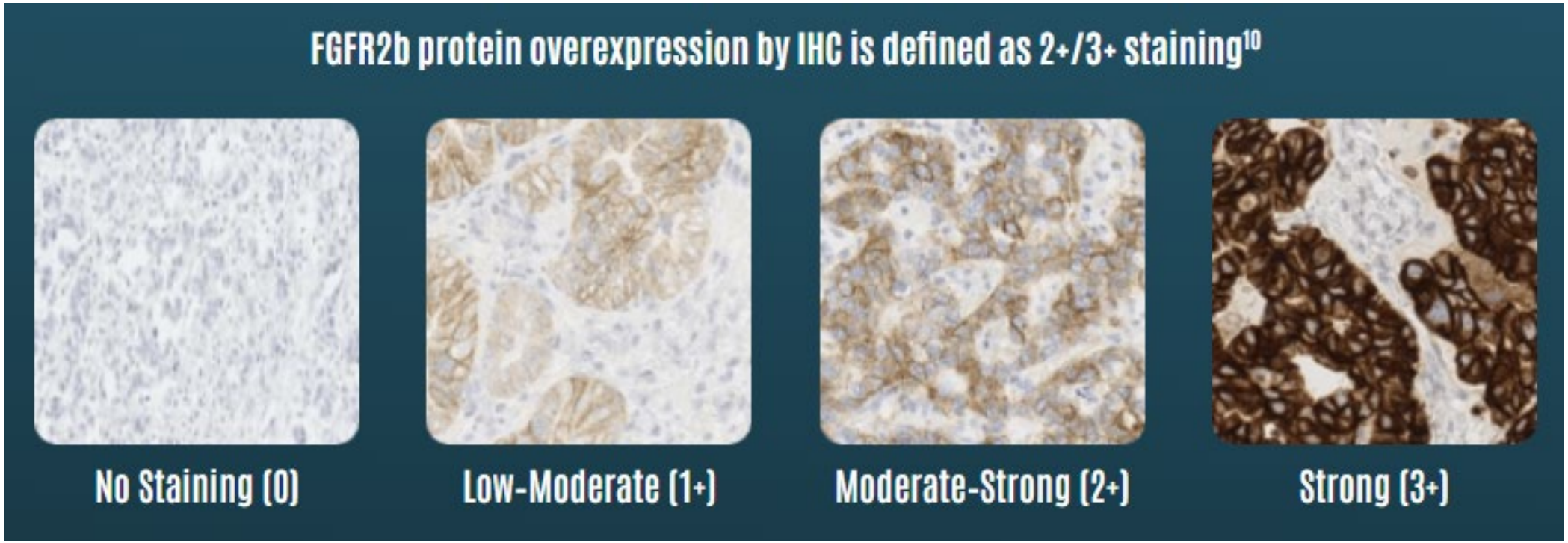
FGFR2B

- Fibroblast growth factor receptors (FGFRs) are transmembrane tyrosine kinase (TK) receptors.
- Binding leads to activation of the downstream PI3K-AKT and MAPK-ERK pathways.
- Amplification of the gene and overexpression of the splice variant occurs in gastroesophageal cancers, and several studies have found that both alterations are associated with **poor prognosis**.
- Current testing modalities to assess for FGFR2b alterations include:
  - Immunohistochemistry
  - mRNA ISH/FISH
  - PCR-based copy number assays
  - Liquid biopsy for *FGFR2* gene amplification
- These testing platforms have varied across studies and therapeutic agents investigated.



Int J Oncol. 2006;29:163–8.
Cancer Res. 2005;65:7591–5.
Nat Rev Cancer. 2010;10:116–29.
Pathobiology. 2015;82:269–79.

- Most recently, ***immunohistochmistry*** for FGFR2b expression has emerged as the most relevant modality in clinical trials utilizing the anti-FGFR2 antibody, bemarituzumab.
- According to data from the FIGHT trial (NCT03694522) that assessed this medication in combination with mFOLFOX6 during first line treatment of patients with unresectable/metastatic tumors:
  - 29% of patients displayed overexpression of FGFR2b, determined by IHC and defined as 2+ (moderate to strong) or 3+ (strong) reactivity in any percentage of tumor cells.
  - Only a small fraction were identified using molecular-based assays.
- Both PFS and OS appeared longer with bemarituzumab addition (although PFS improvement did not reach statistical significance).



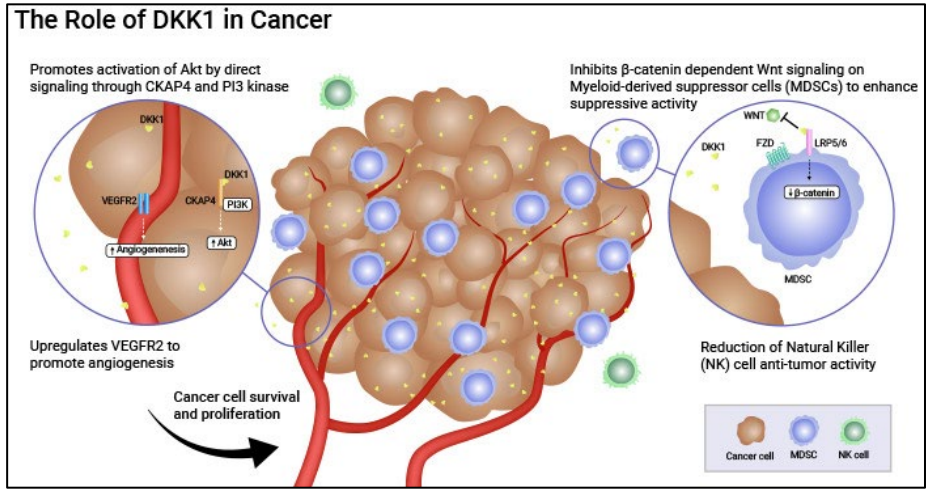
Can Res. 2014;74:5446–5446.
J Clin Oncol. 2020;38:2418–26.
Can Res. 2016;76:1407–1407.
MAbs. 2021;13:1981202.
Lancet Oncol. 2022;23:1430–40.

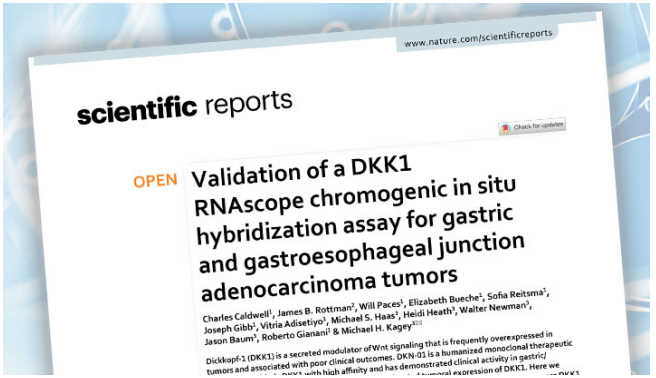
FGFR2B ON THE HORIZON…

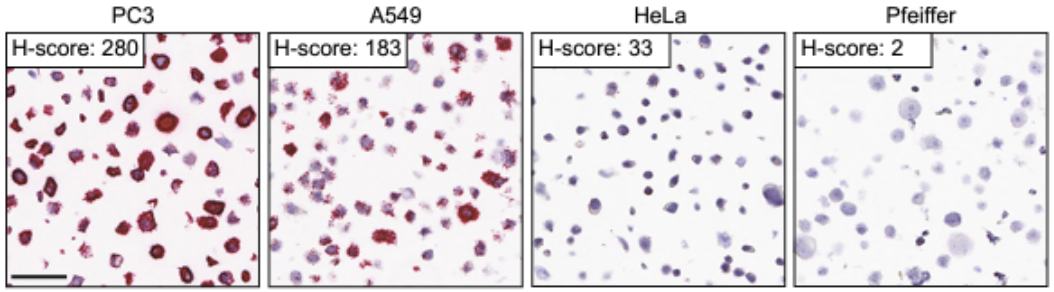
- Ongoing global phase 3 studies FORTITUDE-101 (NCT05052801) and FORTITUDE-102 (NCT05111626) should further validate the observed signal, as well as provide information regarding combination strategy with an immune checkpoint inhibitor.
- Several TKIs are being evaluated in esophageal and gastric adenocarcinomas with aberrant FGFR2 signaling detected by ***molecular testing*** (NCT04604132, NCT04189445, NCT02699606).
- Further investigations will be needed if a signal of activity is observed.
- Due to the low incidence of *FGFR2* gene alterations in this cohort of patients, the feasibility of larger studies will be challenging.
- At present, there are no formalized guidelines regarding FGFR2b evaluation.

DKK1

- Dickkopf-1 (DKK1) is a secreted protein that modulates Wnt signaling and contributes to an immune suppressive tumor microenvironment.
- DKK1 overexpression has been associated with ***poor prognosis and aggressive tumor biology***.
- Genetic/epigenetic changes can contribute to DKK1 dysregulation and increased expression in cancer cells.
- DKN-01 is an anti-DKK1 mAb which has demonstrated anti-tumor activity in patients with advanced GI adenocarcinoma with low tumor PD-L1 expression.
- High tumoral DKK1 mRNA expression can be assessed by chromogenic ISH RNAscope assay.
- Tumoral *DKK1* mRNA expression was evaluated in the DisTinGuish trial and assigned an H-score of 0 to 300 (high defined ≥ 35).







Cancer Immunol Res. 2022;10:1506–24.
J Clin Oncol. 2023;41:4027–4027.
Pathol Int. 2012;62:728–34.
J Clin Pathol. 2011;64:880–3.

DKN-01

- DKN-01 is a humanized IgG4 mAb targeting DKK1.
- This drug has been shown to activate NK cells, decrease Akt signaling, and upregulate PD-L1 expression.
- Clinical studies use in combination with immune checkpoint inhibitors (ICI) to take advantage of potential synergism.
- In the Phase 2 DisTinGuish trial (NCT04363801), DKN-01 in combination with ICI resulted in promising activity across different treatment lines.
- In a cohort of 25 treatment-naive patients with HER2 negative esophageal or gastric adenocarcinoma, DKN-01, tislelizumab (anti-PD1 antibody), and CAPOX chemotherapy resulted in ORR of 73%.
- Efficacy signals were explored in the prespecified subgroup analysis based on DKK1 mRNA levels.
  - In DKK1-high (*n* = 10) and DKK1-low (*n* = 9) patients, the ORR was 90% and 67%, respectively.
  - Durable responses were notably observed in patients with PD-L1-low (CPS < 5) tumors.
- Given these promising results, the DisTinGuish trial will explore in the first-line setting (NCT04363801):
  - DKN-01 in combination with tislelizumab and chemotherapy (CAPOX or mFOLFOX6)

VERSUS

  - Tislelizumab plus chemotherapy in patients with advanced EGA in the first-line setting (NCT04363801)

Mol Cancer Res. 2021;19:717–25.
J Clin Oncol. 2023;41:TPS484-TPS484.

IN SUMMARY…

- Targeted agents are revolutionizing cancer therapy.
- Biomarker testing is integral for the selection of an appropriate treatment plan to ensure that patients are not exposed to unnecessary toxicities associated with systemic therapies.
- Novel biomarkers will be investigated in future clinical studies and we (pathologists) need to work more closely with our clinical trialist colleagues to ensure consistency of emerging biomarkers along with reporting/scoring algorithms.
- To facilitate the growth of this rapidly developing multidisciplinary field, it is important that oncologists and pathologists work in synergy to ensure patients have all the information they need for the best possible outcome.
- While biomarker detection by immunohistochemistry is part of routine pathology practice, feasibility is limited by amount of tumor tissue and new approaches (multiplex IHC) may need to be considered.

We need to partner together to help patients get the personalized care they deserve!!!

Thank you for your time and attention