Molecular pathology for tubular gastrointestinal tract

KOPANA seminar 2020

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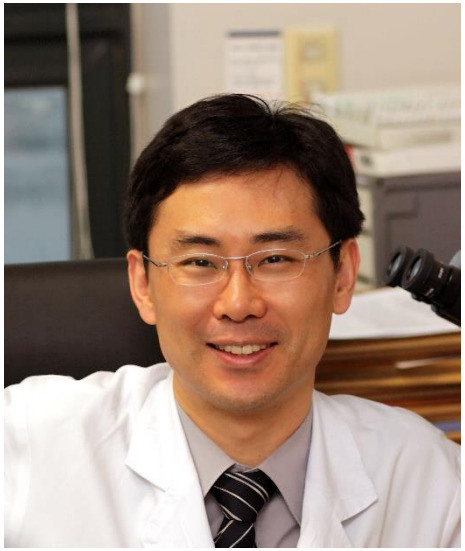

- **Education:**
  - Seoul National University (MD/PhD)
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Molecular pathology of esophago-gastric adenocarcinoma

Druggable target in gastric cancer: ERBB2

- ERBB2 overexpression / amplification
- Positive criteria: IHC 3+ or IHC 2+ and FISH (or SISH) +
- Prevalence of HER2 positivity in Asian countries (including Korea): 6~15%
- Overexpression predict drug response better than amplification.

HER2 testing guidelines in esophago-gastric cancer (last updated in Dec 2016)

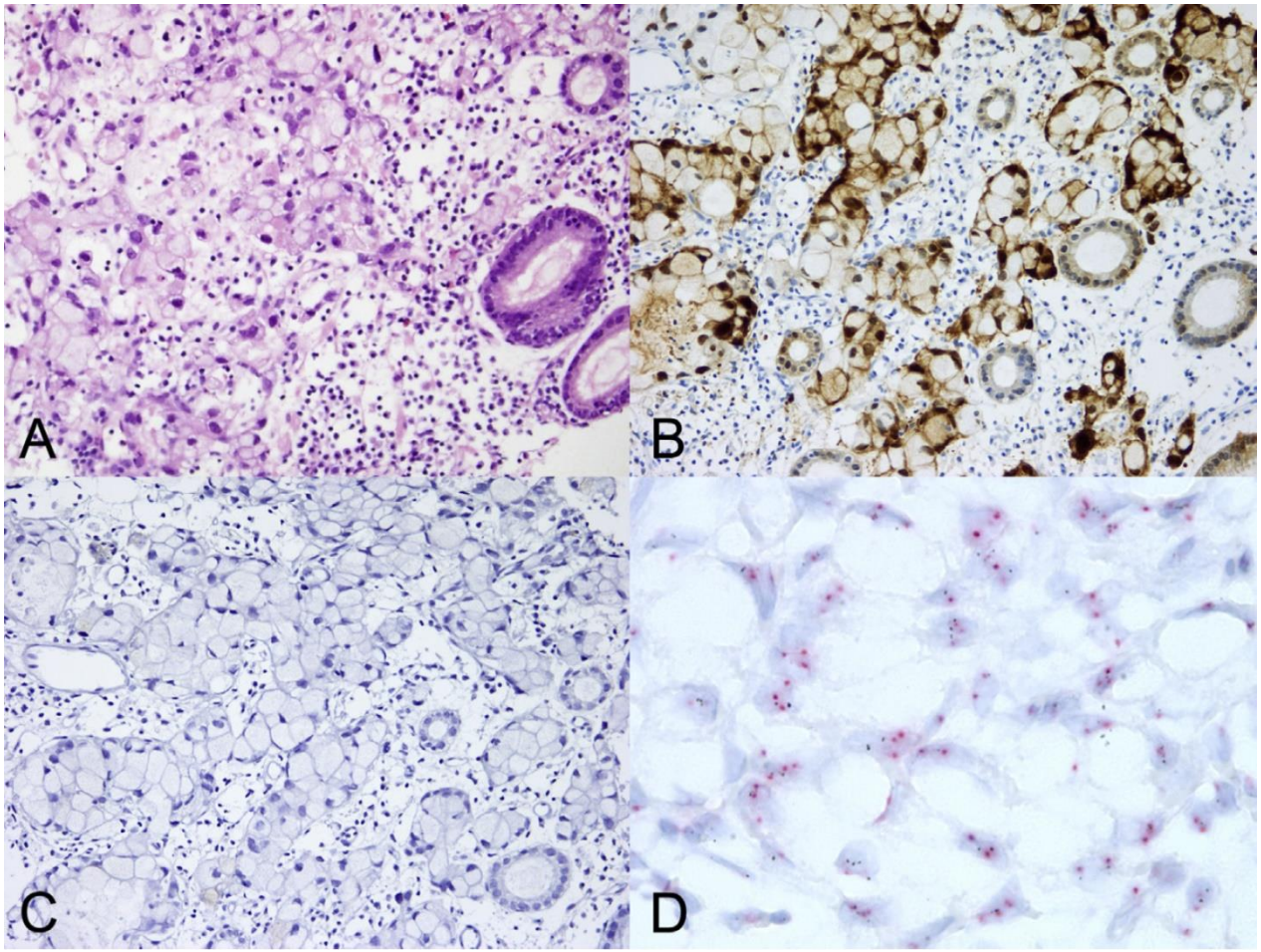
- Core tips:
  - Percentage cutoff: **10% (resection), a cluster of 5 or more tumor cells (biopsy)**
  - Staining pattern: basolateral or lateral membranous
  - Intensity: 3+ (strong, **complete**), 2+ (weak to moderate, **complete**), 1+ (Faint, not complete)
  - Final decision: 3+ (positive, no further testing), 2+ (equivocal, perform ISH), 1+ or 0 (negative)

- Preanalytic guidelines: shorten cold ischemic time (ideally less than 1 hour), fixation (10% neutral buffered formalin for 6 to 72 hours)

Interpretation of SISH

- Score at least 20 non-overlapping nuclei. Scan areas with higher HER2 copy number (CN) or HER2 overexpression
- **HER2/CEP17≧2 → Positive**
- **HER2/CEP17<2, and HER2 CN>6 → Positive**
- HER2/CEP17<2, and HER2 CN 4~6 → Score 20 additional tumor cells
- Otherwise: Negative

Beware of false positives (clone 4B5 example)



D: SISH (black, ERBB2; red, CEP17)

B: Clone 4B5 (by Roche) C: Hercep test (by DAKO) (Woo CG et al. *Pathology* 2017; 49(1): 38-43).

Beware of false positives (clone 4B5 example)

Table 4 Distribution of Pathway immunohistochemistry staining patterns in signet ring cell carcinoma with score 2 and 3

IHC score	Cytoplasmic expression and/or nuclear stain	True membranous pattern	Total
Score 2	4 (36.4)[a]	7 (63.6)	11 [37.9][b]
Score 3	15 (83.3)	3 (16.7)[c]	18 [62.1]
Total	19 (65.5)	10 (44.5)	29

[a] Numbers in parentheses, percentage of cases in each row.
[b] Numbers in brackets, percentage of cases in the column.
[c] These cases are also silver *in situ* hybridisation (SISH) positive.

Signet-ring cell carcinoma cases that were initially scored as 3+ (N=29): 51.7% (15 cases) did not show *ERBB2* amplification by SISH.

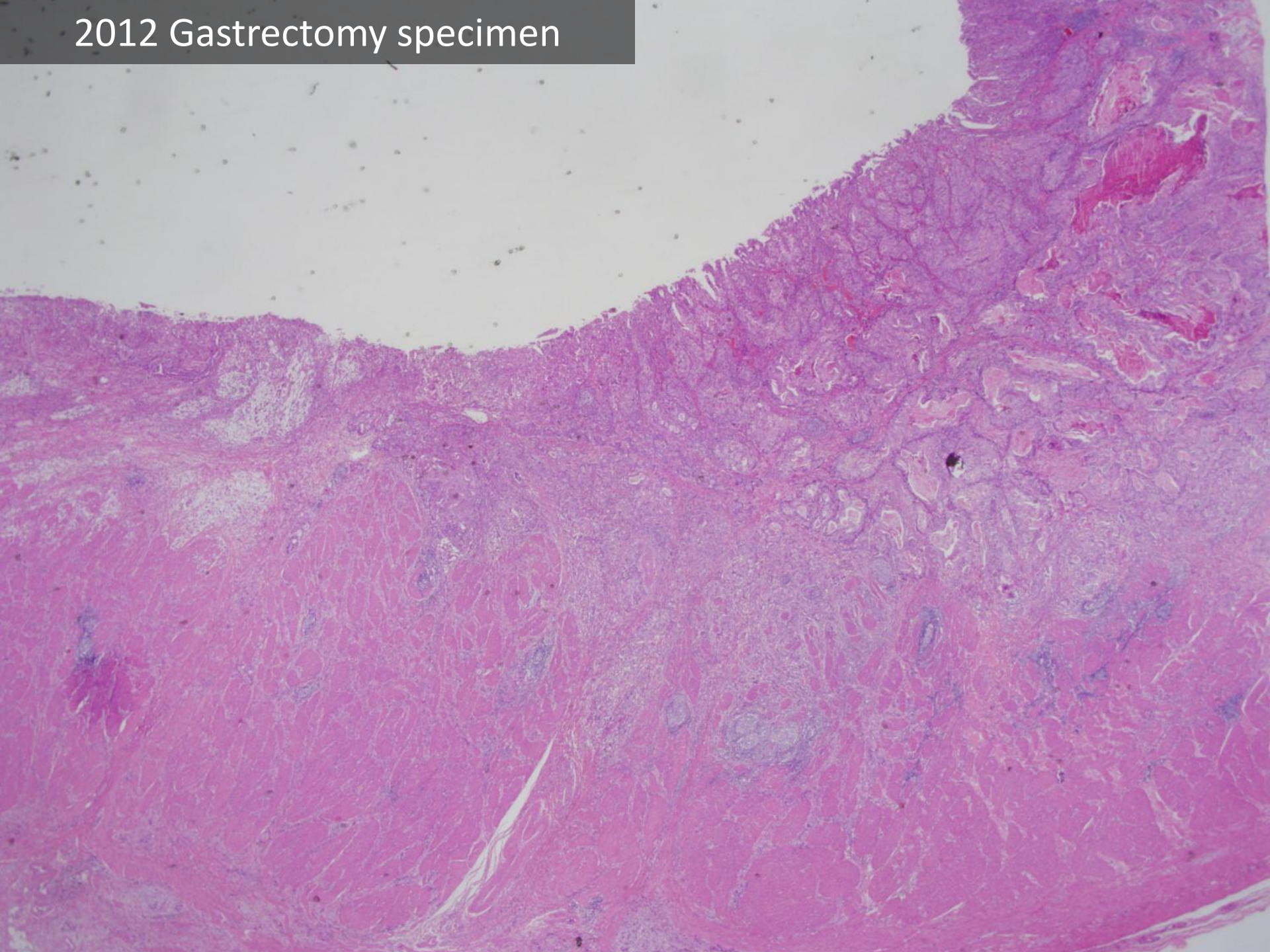
(Woo CG et al. *Pathology* 2017; 49(1): 38-43).

Regional heterogeneity of ERBB2 status in gastric cancer

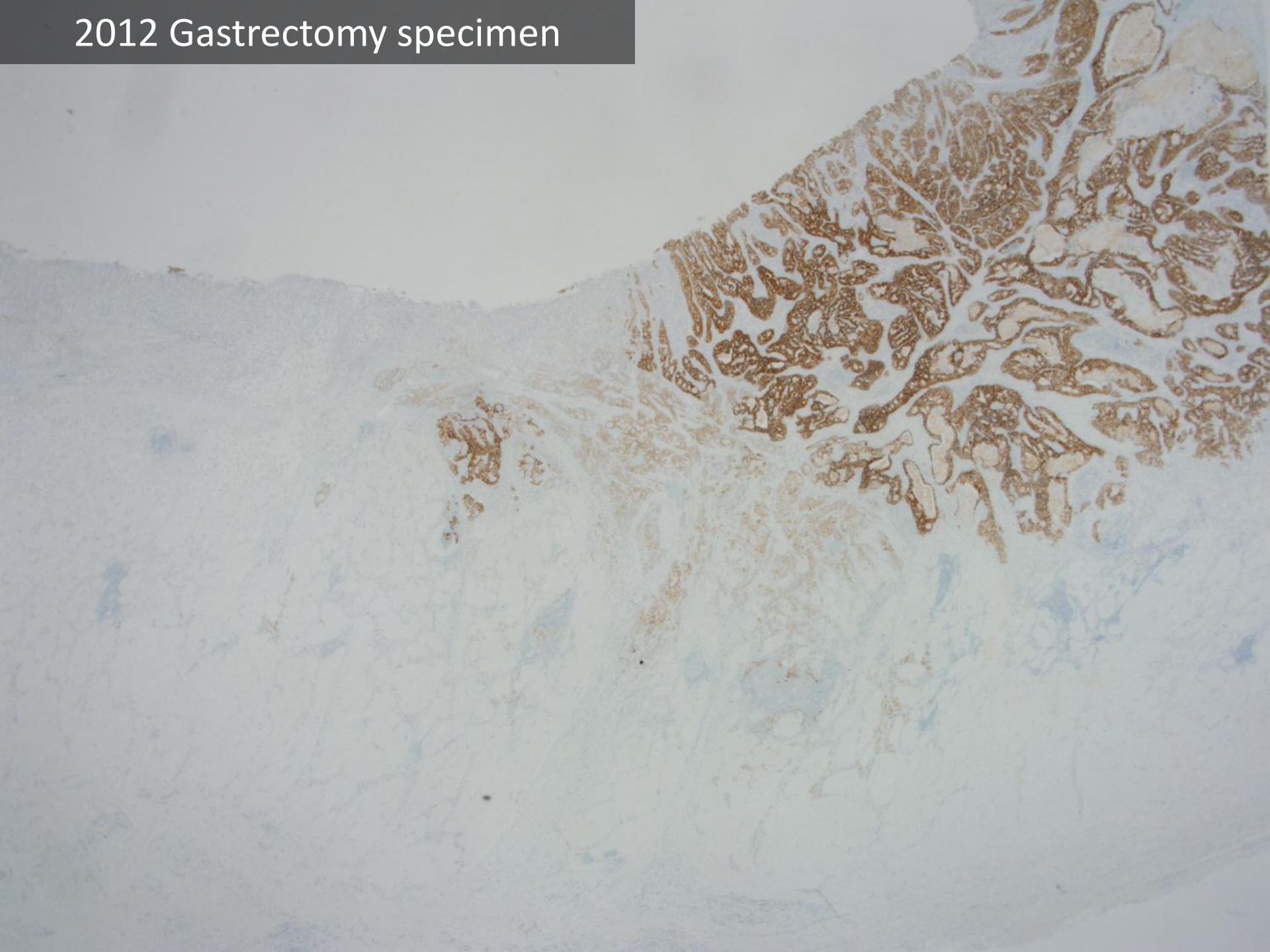
- Variable but comparable frequencies:
  - 74.0% for IHC 2+, 41.1% for IHC3+ (Lee HE et al., *Eur J Cancer* 2013)
  - 63.5% for IHC2+, 28.3% for IHC3+ (Nishida Y et al., *Gastric Cancer* 2015)

- Clinical significance:
  - HER2 negative fraction may not be responsive to anti-HER2 therapy.
  - Small biopsy may not be representative of the entire tumor.

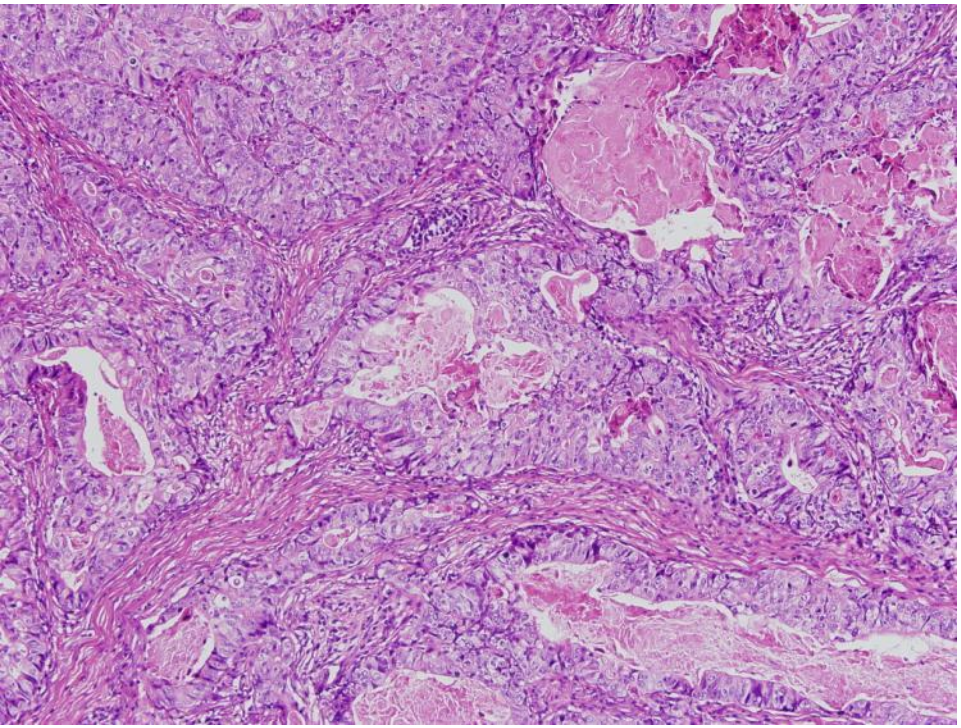
2012 Gastrectomy specimen

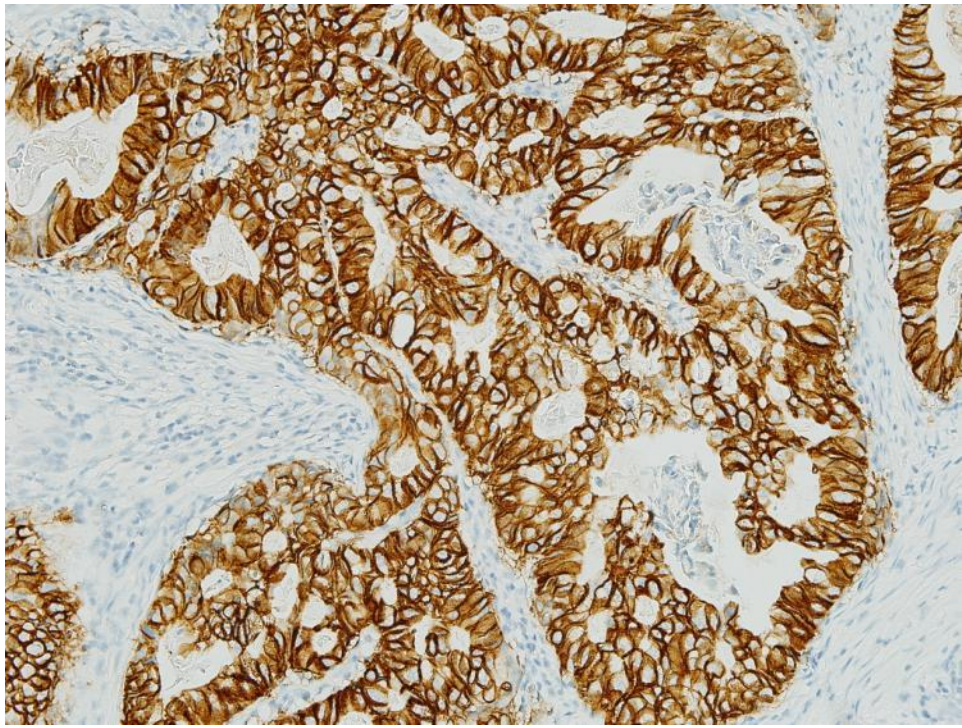


2012 Gastrectomy specimen

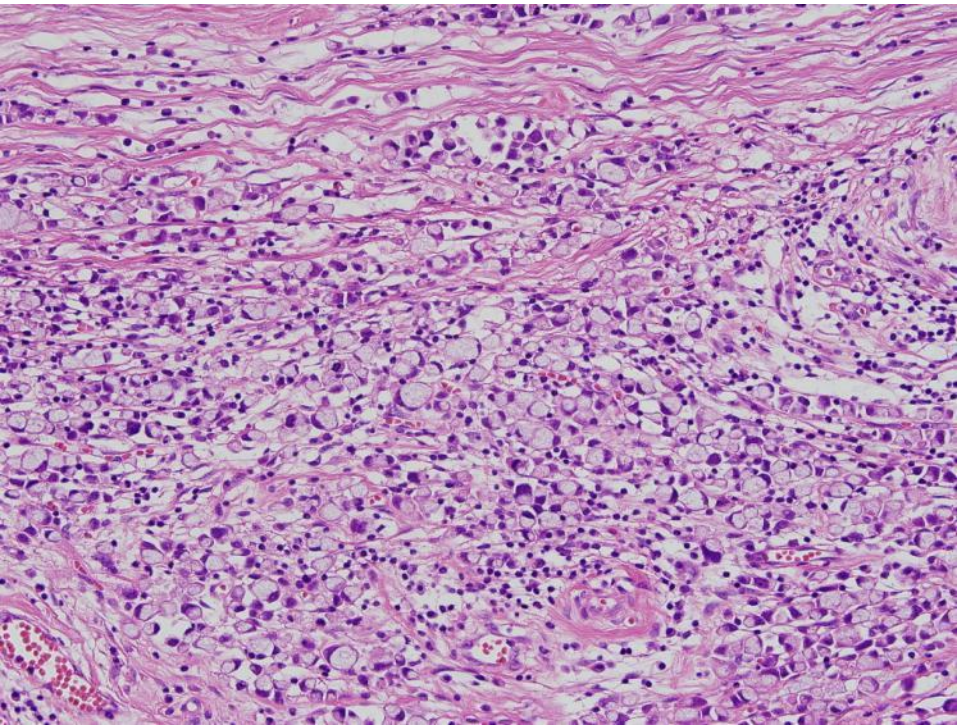


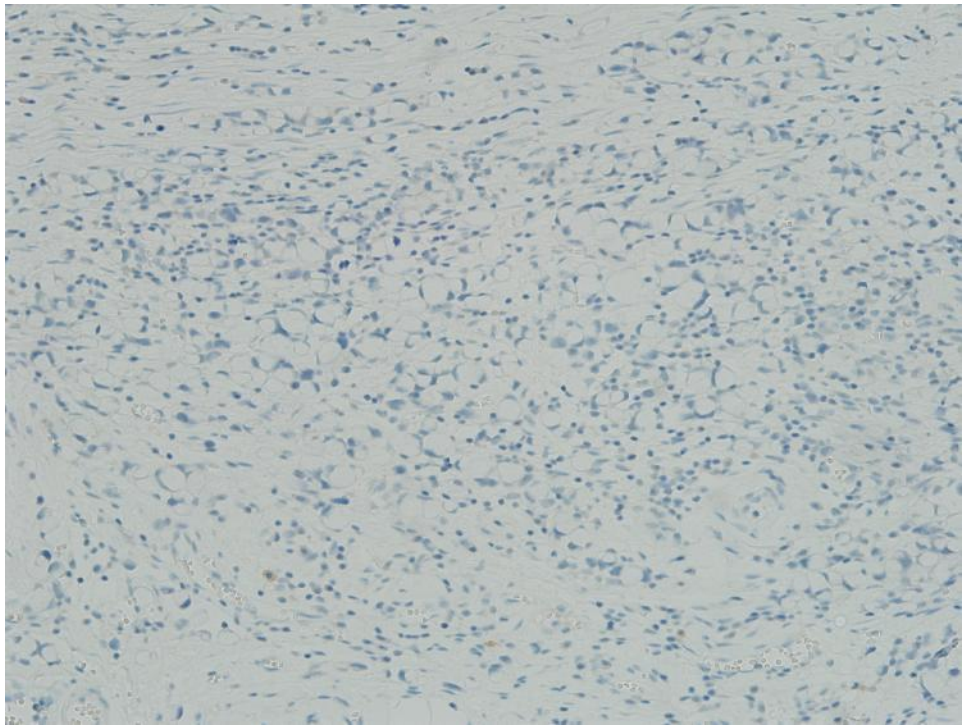
HER2 positive component



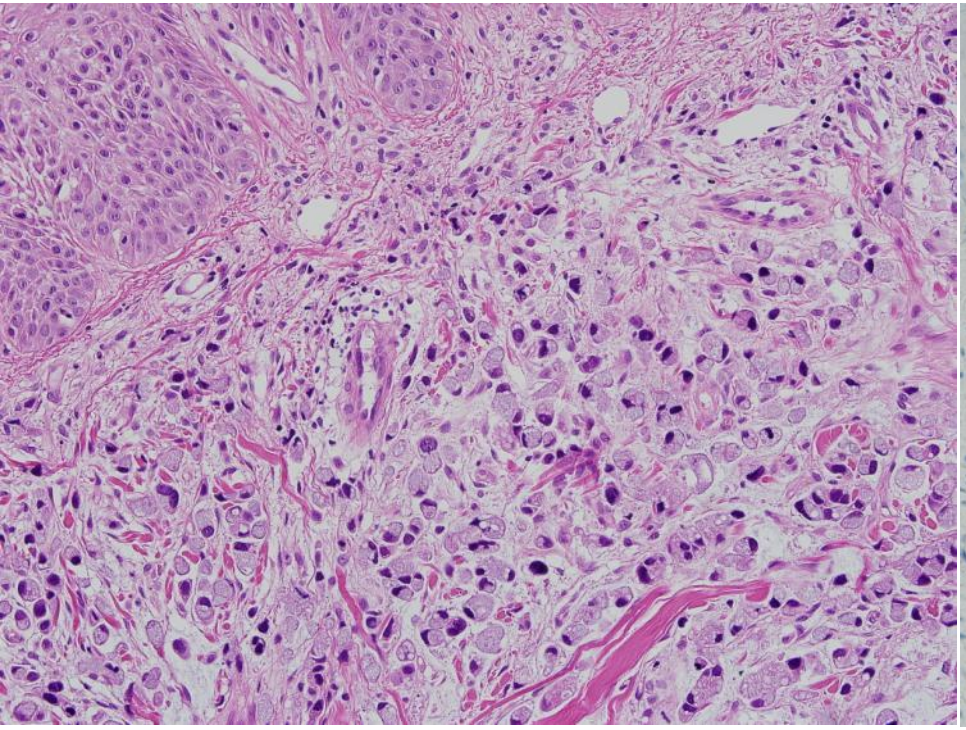


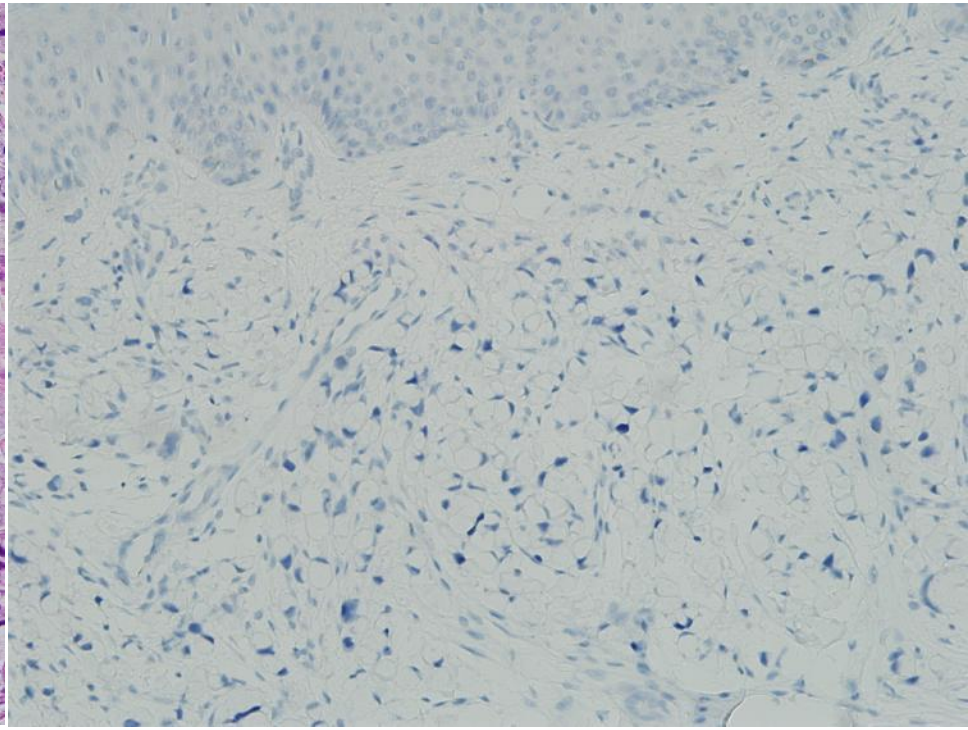
HER2 negative component



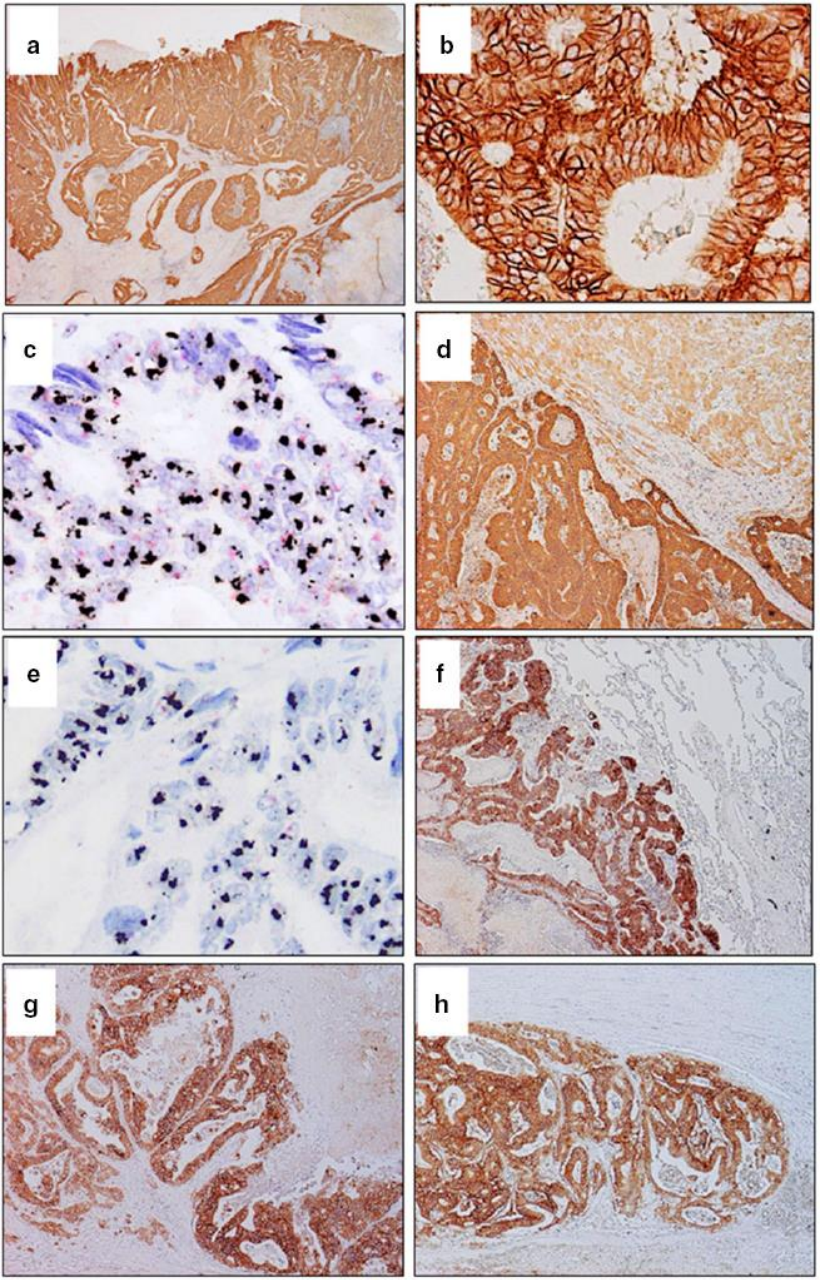


2016 skin metastasis after traditional cytotoxic chemotherapy

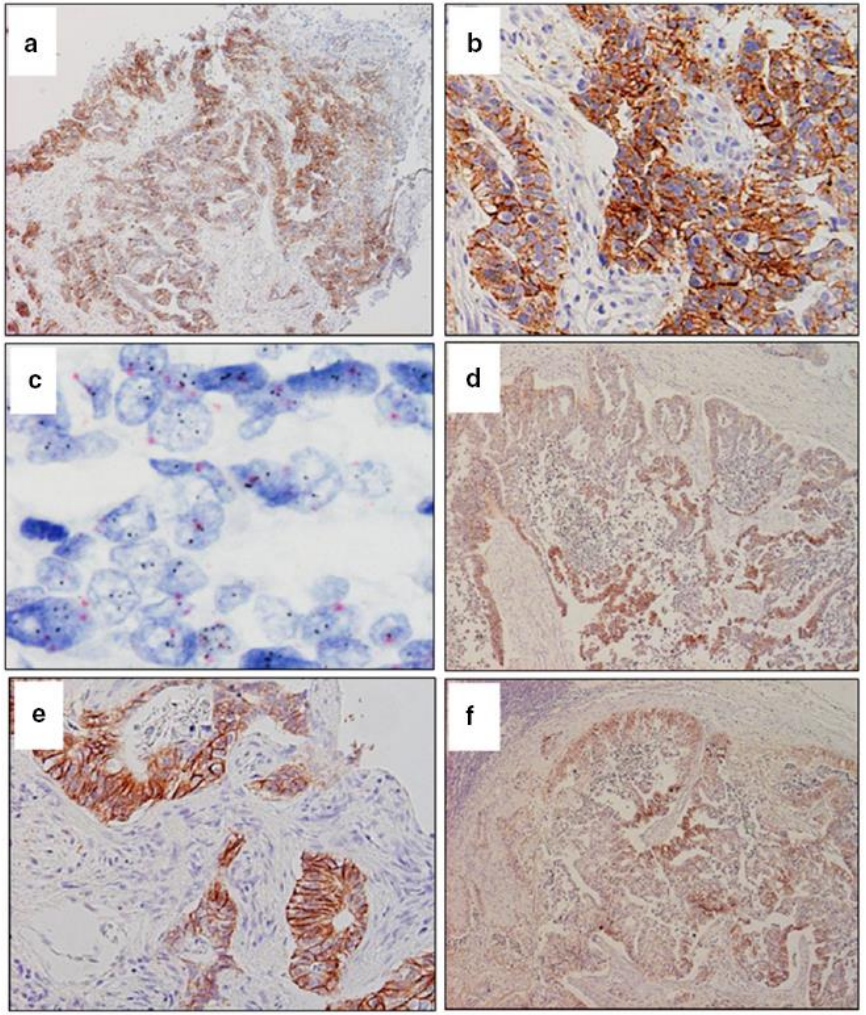




Autopsy study for HER2 heterogeneity



Homogeneous pattern with high level of ERBB2 amplification (**Case #1**)



Heterogeneous pattern with focal ERBB2 IHC 3+ and equivocal ERBB2 copy-number (**Case #4**)
(Saito T et al., *Pathol Int* 2015)

Correlation with drug response

Table 1 Clinicopathological characteristics of HER2-positive autopsied and resected gastric cancer patients

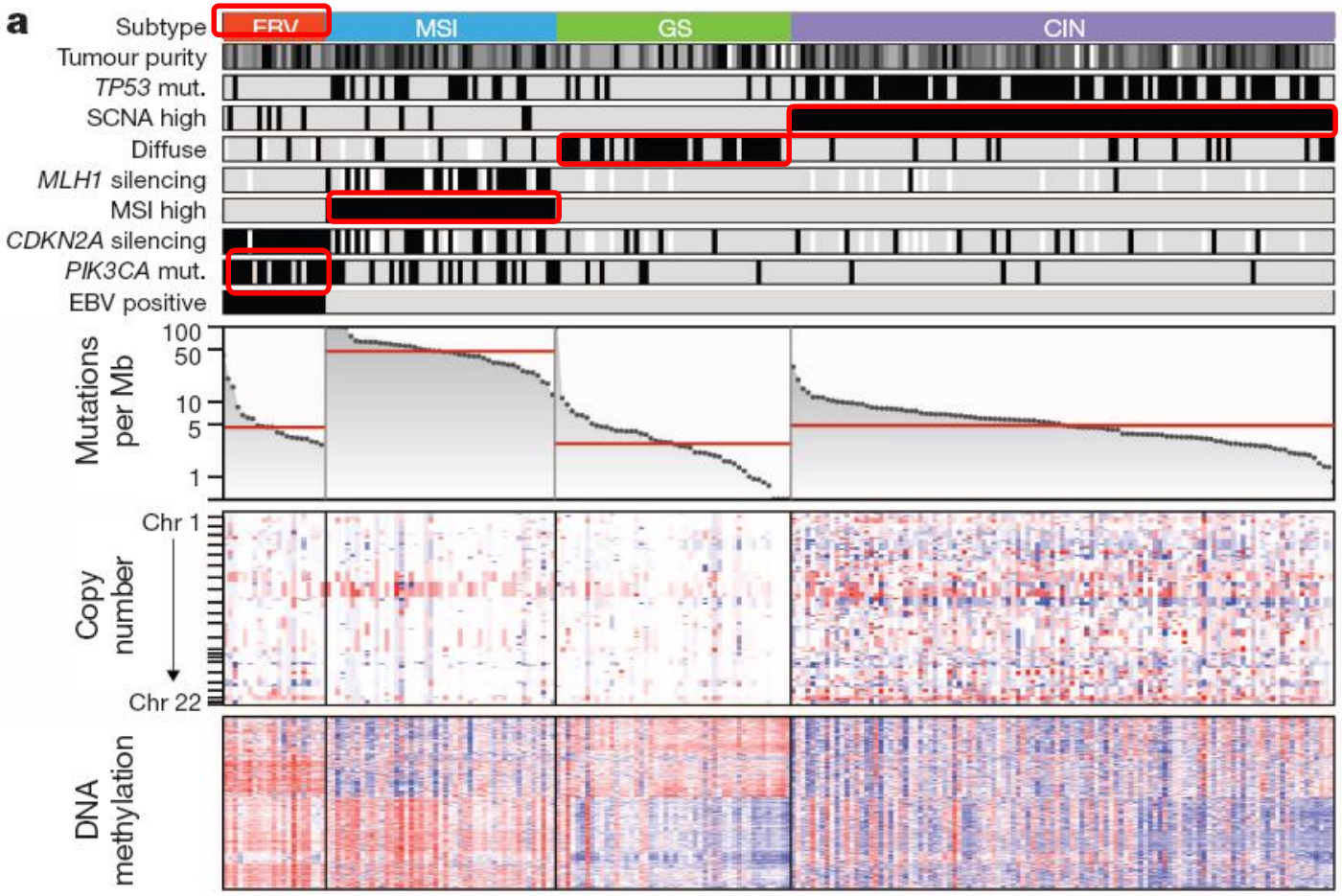
Variable	Case 1	Case 2	Case 3	Case 4
Age/sex	69/M	67/M	43/M	78/M
Source of Specimen	Autopsy	Resection	Autopsy	Autopsy
HER2 status in primary tumor IHC/DISH (Ratio†)	IHC3+/DISH+(>3) (RS)	IHC3+/DISH+(>3) (RS)	IHC3+/DISH+(>2.2) (Biopsy)	IHC3+/DISH+/(Biopsy)
Site of metastasis	Liver, lung, intestine, bone, LN	Liver, LN	Liver, lung, kidney, LN	Liver, LN
HER2 heterogeneity in primary tumor (% of IHC3+ area)	Homogeneity (100%)	Heterogeneity (20–30%)	Heterogeneity (70–80%)	Heterogeneity (10–20%)
HER2 heterogenity in metastatic tumor (% of IHC3+ area)	Homogeneity (100%)	Homogeneity (100%)	Heterogeneity (70–80%)	Heterogeneity (about 10%)
Clinical Tmab therapy (Effect)	Non-treated	Non-treated	Treated (PR)	Treated (PD)
Preclinical Tmab therapy	Strong response	Strong response	Not tested	No significant response

Ratio†: HER2/CEP17; LN, lymph node; PD, progressive disease; PR, partial response; RS, resected specimen; Tmab, Trastuzumab.

Strong, clonal HER2 amplification/overexpression predicts clinical response to anti-HER2 therapy.

(Saito T et al., *Pathol Int* 2015)

Genomic landscape of gastric cancer



(The Cancer Genome Atlas Network, *Nature* 2012)

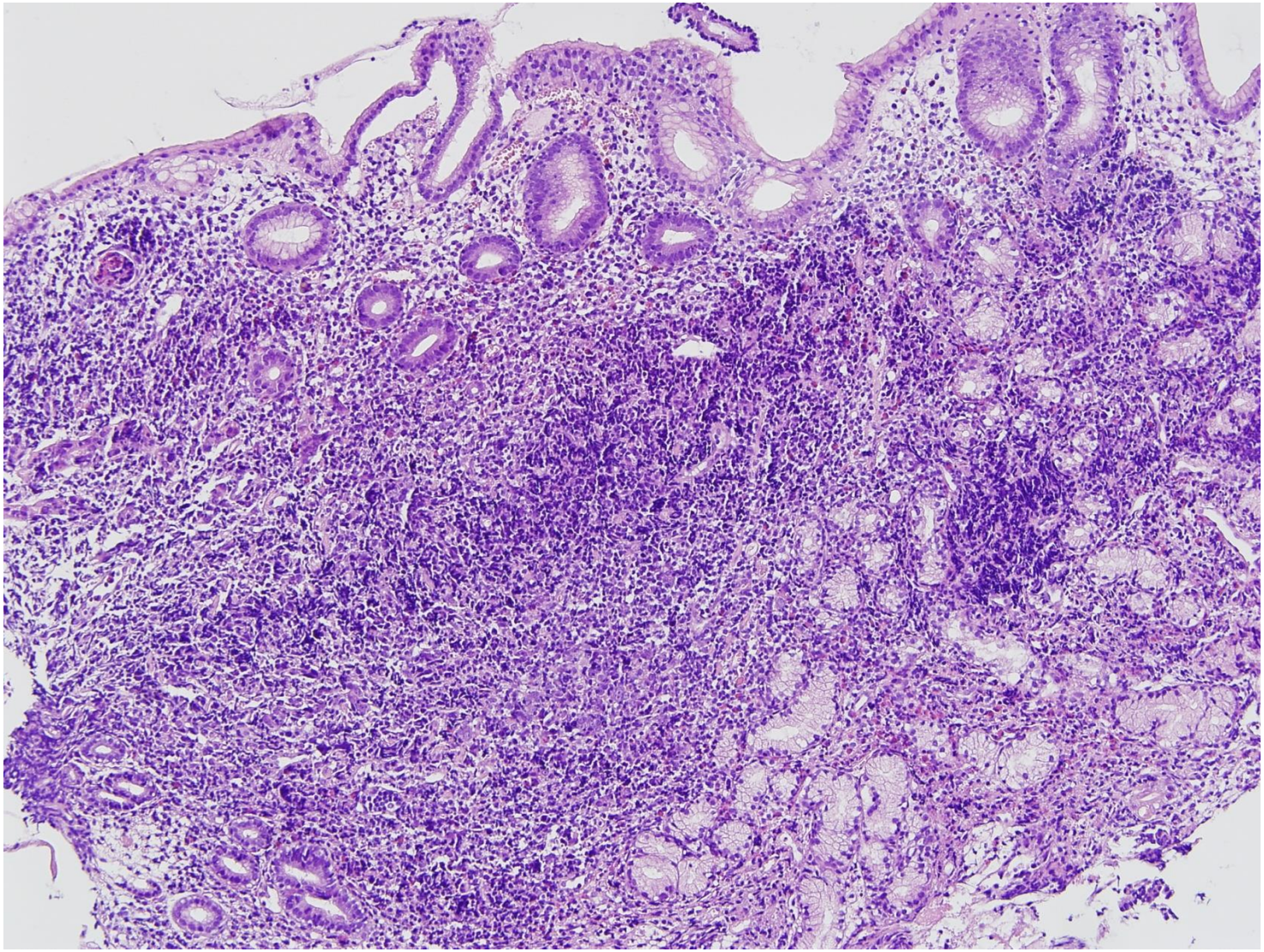
EBV-positive GC

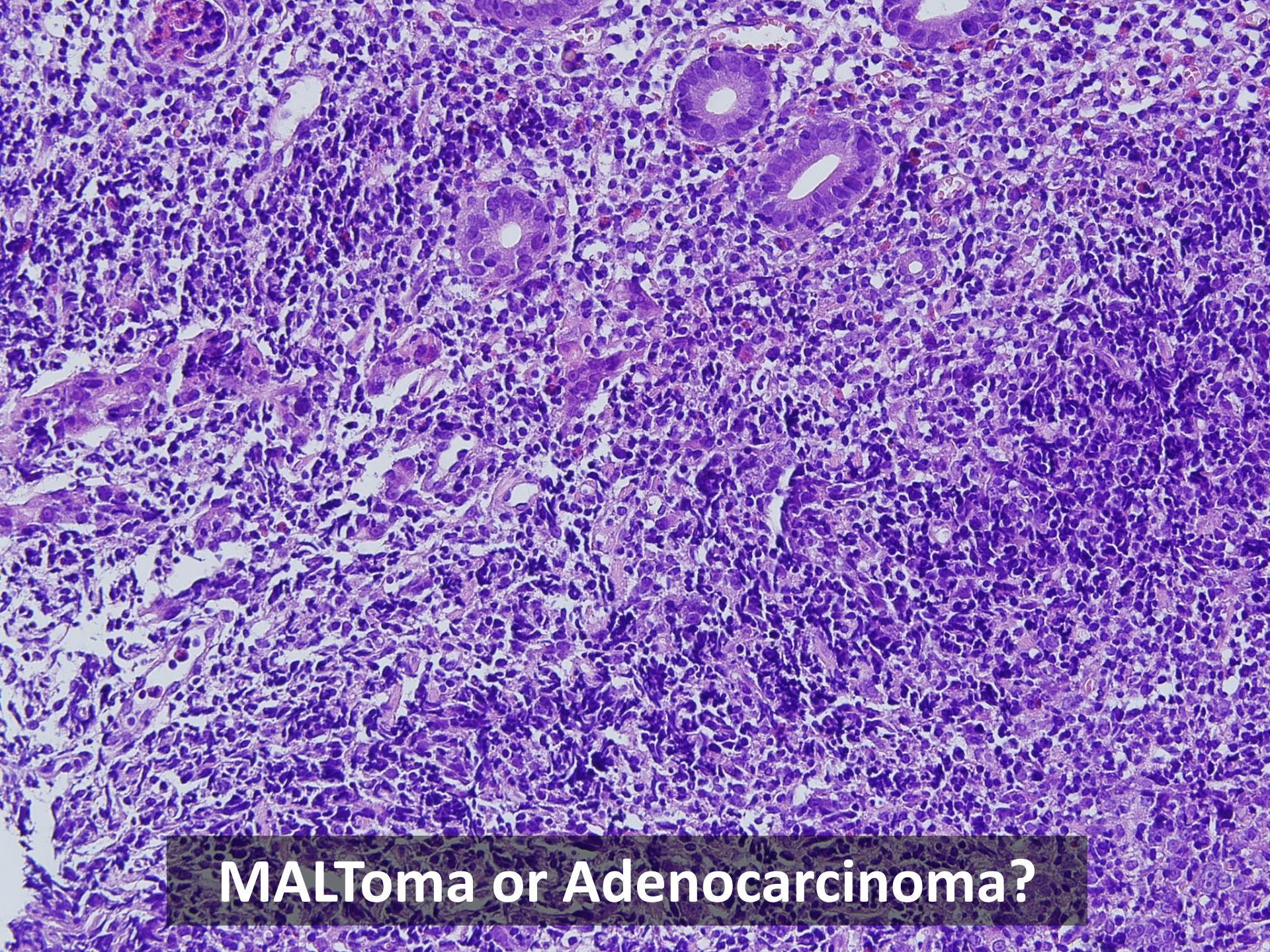
- ~9 % of gastric cancer patients

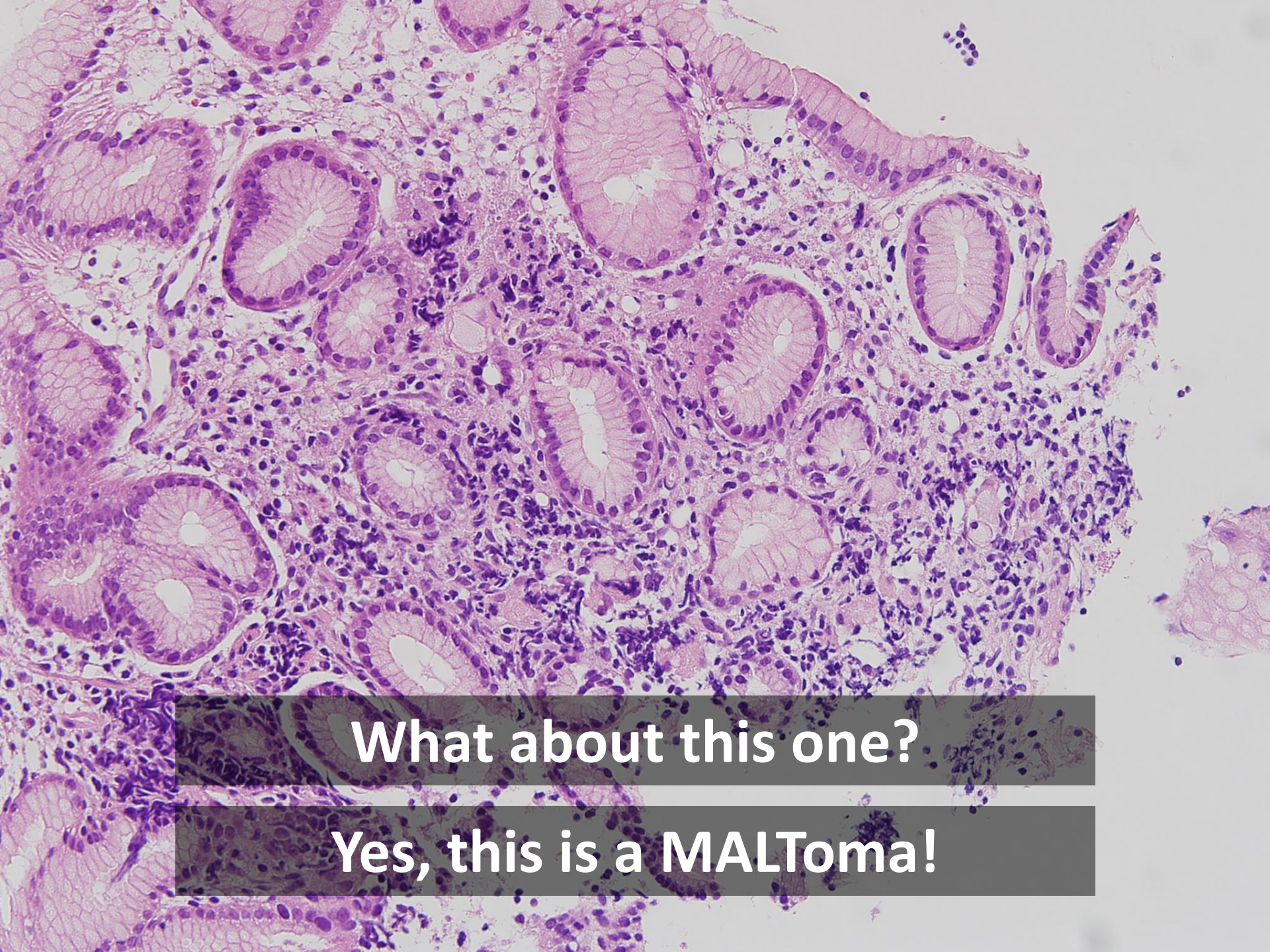
- Clinicopathologic characteristics:
  - Predominantly proximal location
  - Frequent *PIK3CA* mutations and **amplification of *CD273* (PD-L1) / *PDCD1LG2* (PD-L2) locus**
  - Heavy CpG island methylations
  - Prominent intra-tumoral and peri-tumoral inflammatory cell infiltrations

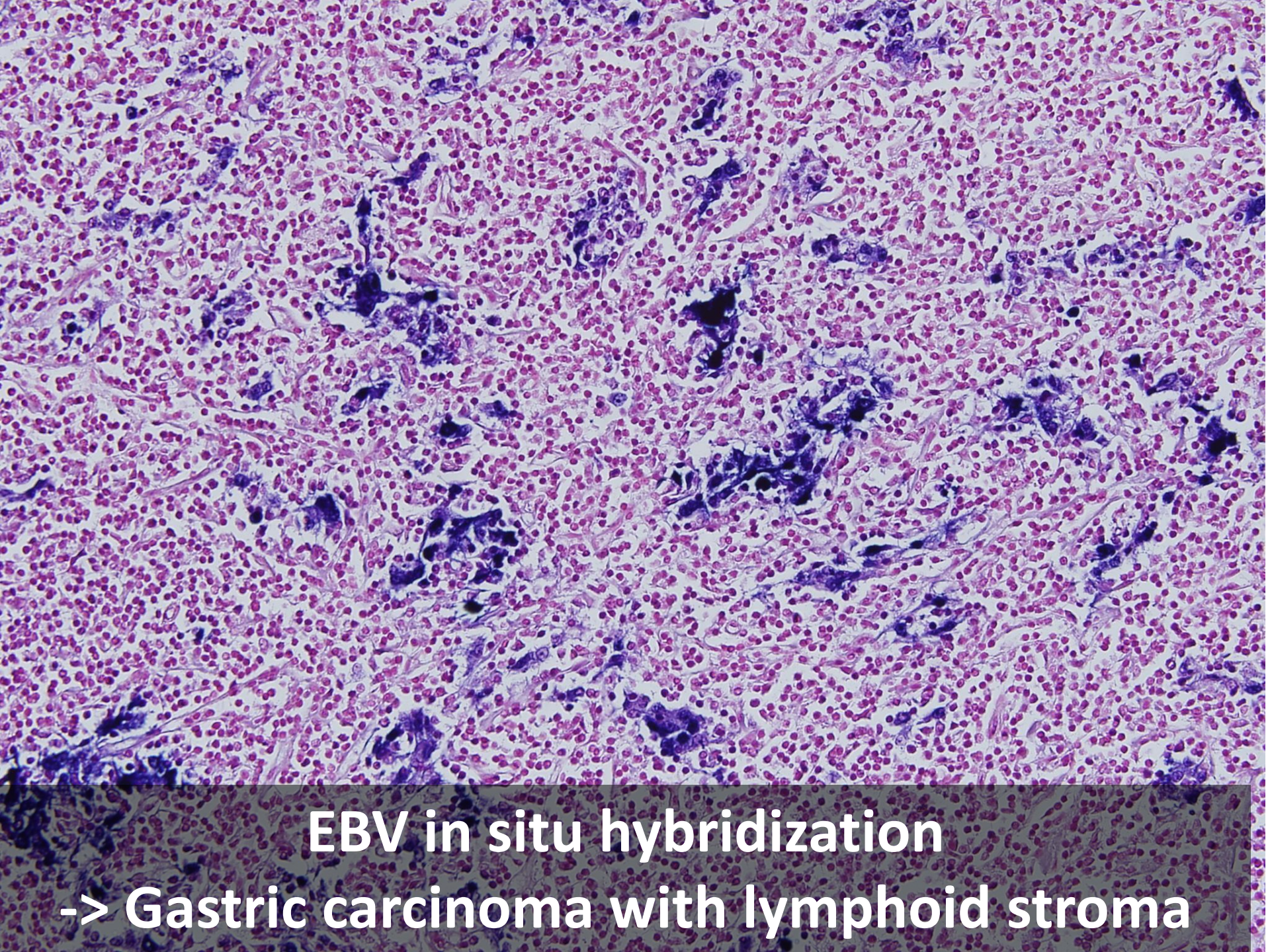
- Clinical implications:
  - **Response to pembrolizumab:** durable response achieved in all 6 patients with EBV+ GC (Kim ST et al., *Nat Med* 2018; 24:1449-1458)
  - Sometimes cause diagnostic difficulty in small biopsies: masked by inflammatory cell infiltration

Diagnostic utility of EBV in situ




MALToma or Adenocarcinoma?


What about this one?
Yes, this is a MALToma!


EBV in situ hybridization
-> Gastric carcinoma with lymphoid stroma

MMR deficient gastric cancer

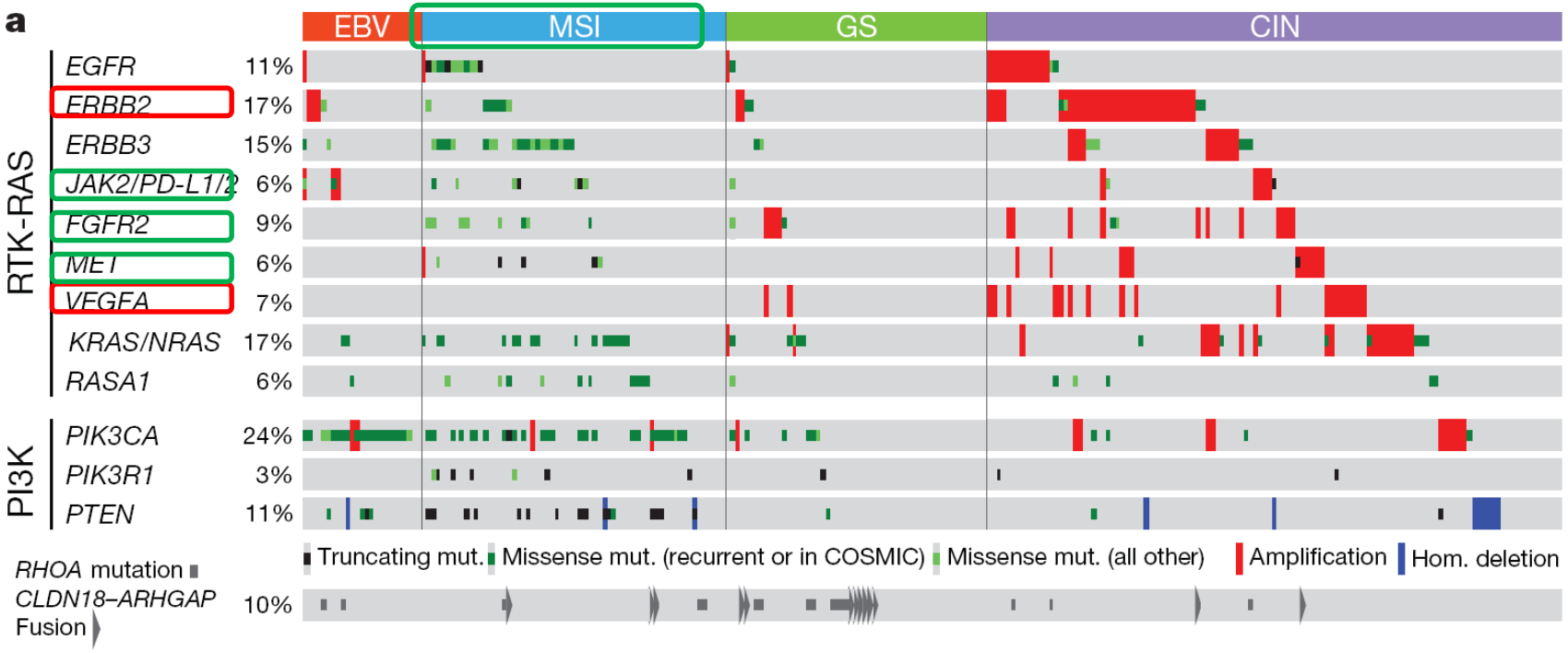
- ~8 % of gastric cancer patients

- Clinicopathologic characteristics:
  - Predominantly distal location
  - Intestinal type

(Pietrantonio F et al., *J Clin Oncol* 2019; 37(35):3392-3400)

- Clinical implications:
  - Response to immunotherapy: **pembrolizumab (6/7, 85.7%)** (Kim ST et al., *Nat Med* 2018; 24:1449-1458)
  - Better disease-free and overall survivals than MSS GCs
  - Less benefit from standard adjuvant chemotherapy

Other potentially druggable targets

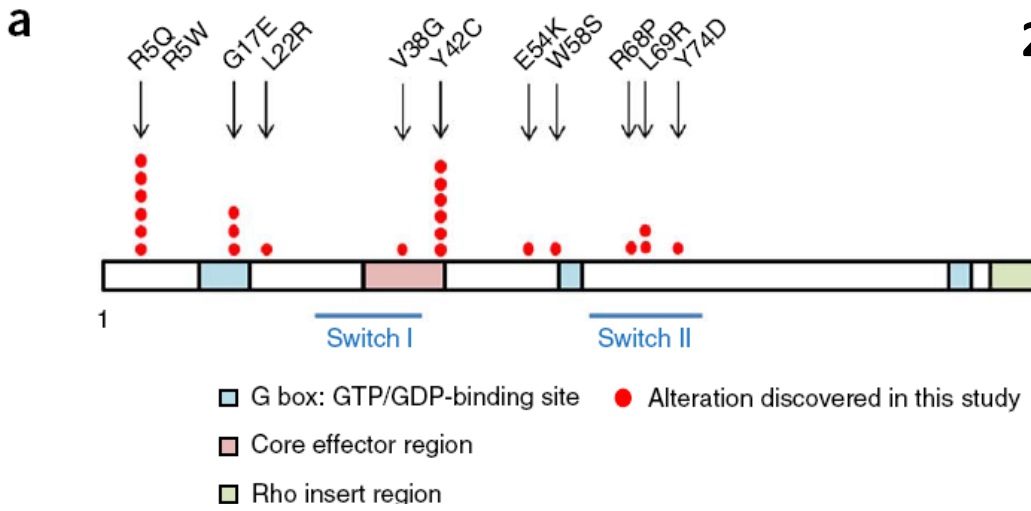


(The Cancer Genome Atlas Network, *Nature* 2012)

Major molecular targets on clinical trials in gastric cancer

Molecular targets	Subtype	Suggested therapeutics	Clinical trials
JAK2, PD-L1/2 overexpression	EBV	Pembrolizumab	Phase II **(6 patients with promising results)**
ERBB2 amplification / overexpression	CIN	Pertuzumab, Trastuzumab emtansine	Phase III (JACOB), Phase II/III (GATSBY): **mixed results but basically HER2 is a valid therapeutic target**
MET amplification / overexpression	CIN	Onartuzumab Crizotinib AMG337	Phase III (METGASTRIC): Addition of Onartuzumab to Chemo was not effective [MET IHC 2+ or above] Phase II (with AMG337 drug): **some anti-tumor activity** ORR 29.6% (8/27) [9.9%, 11/111 for unselected patients]
VEGFR2/TIE2 overexpression	CIN	Regorafenib Ramucirumab	Phase II (**INTEGRATE, regorafenib, modest PFS gain**) and (**Ramucirumab, effective**) but **not associated with VEGFR2 overexpression**
MMR deficiency	MSI	Pembrolizumab	Phase II **(6 patients with promising results)**
FGFR2 amplification / overrexpression	CIN	Dovitinib AZD4547 (PR in 3/9 pts)	Phase II **(with some promising data)**

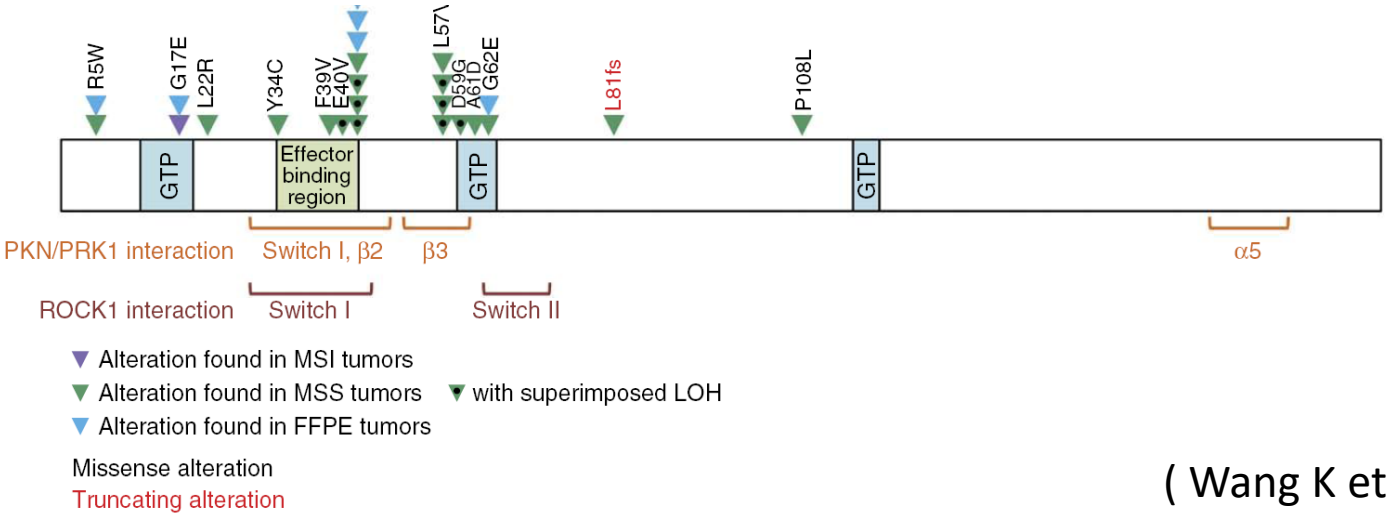
RHOA mutations: oncogene pattern



25.3% (22/87) of diffuse GC

(Kakiuchi M et al., *Nat Genet* 2014)

No clinical role has been documented.



(Wang K et al., *Nat Genet* 2014)

Role of *KRAS* in GC

- Mutations in 1.5~5.8 % of GC (van Grieken NC et al., *Br J Cancer* 2013)

- New drugs (sotorasib, adagrasib) showed promising efficacy in *KRAS* G12C-mutant NSCLC (NCT03600883; NCT03785249). Significance in GC is unknown.

- Frequent wild type *KRAS* amplifications (~14%): *KRAS*-amplified GC cell lines do not respond to MEK inhibitors (Laboratory data)

Summary of esophago-gastric adenocarcinoma part

- Standardization of HER2 testing is important. Intra-tumoral heterogeneity is a problem.
- EBV-associated gastric cancer: frequent PD-L1/PD-L2 amplification, response to immune checkpoint blockade
- MSI-H: good prognosis, response to immune checkpoint blockade
- A few amplified targets (under investigation): *FGFR2*

Molecular pathology of colorectal cancer

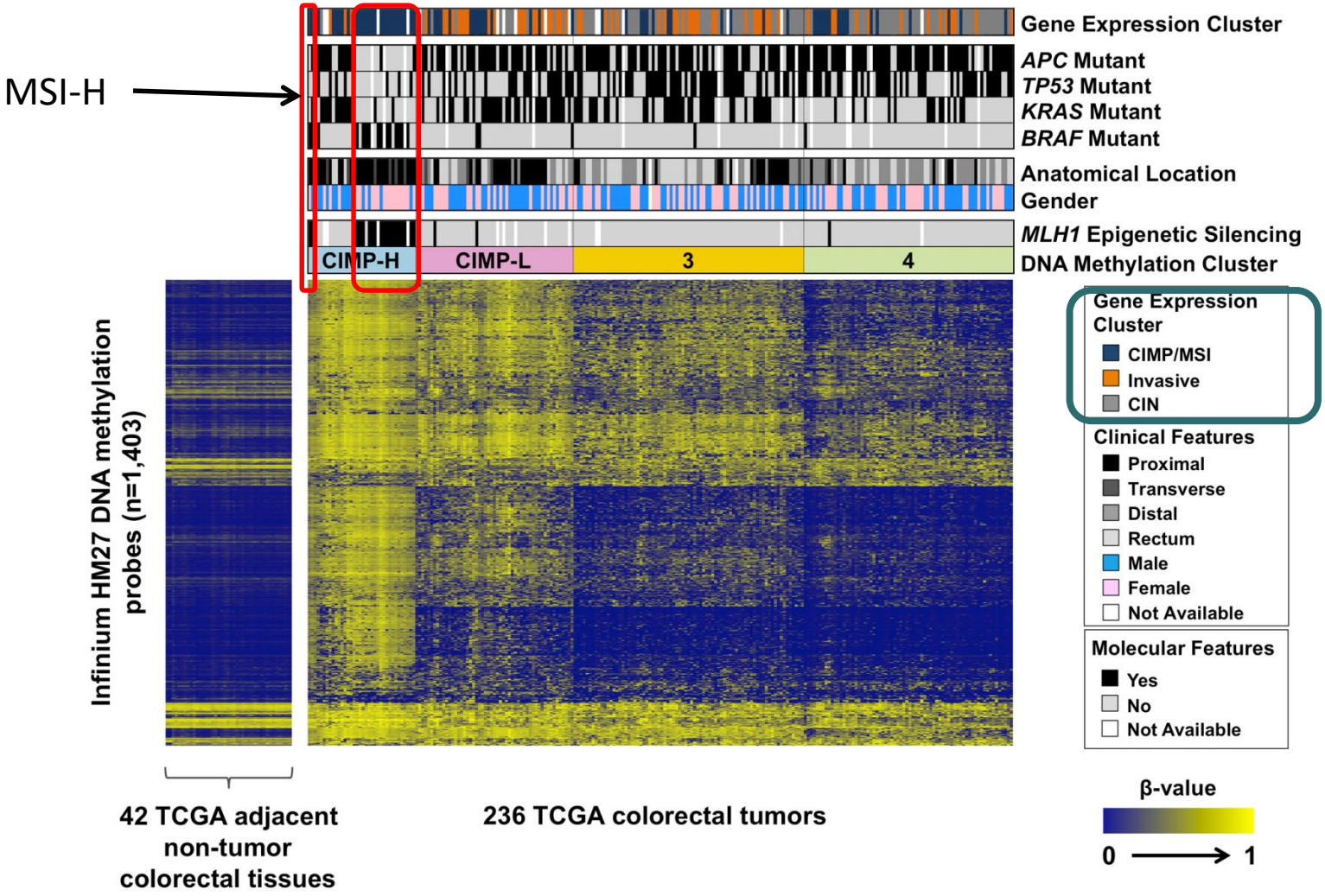
Traditional molecular classification

- **CIN**: “classical” type (~70-80%), canonical pathway

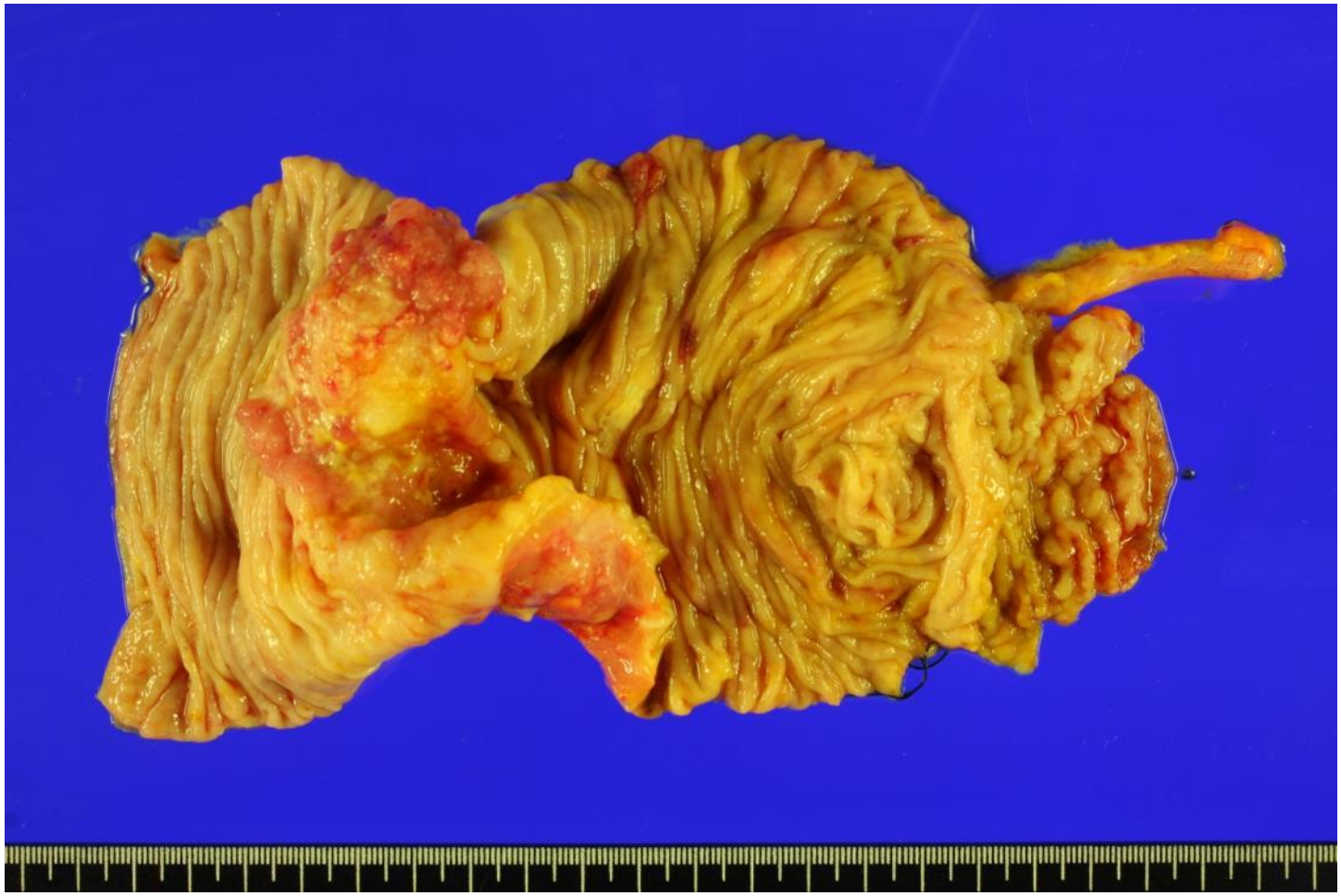
- **MSI** (microsatellite instability) (~14% in West, ~8% in Korea), serrated pathway:
  - Hypermutator phenotype -> **Immunotherapy**
  - **Lynch syndrome**: germline mutation of MMR genes, **no *BRAF* mutation**
  - **Sporadic cases**: promoter hypermethylation of *MLH1, BRAF* mutation (not always though), favorable prognosis.

- **CIMP**: high frequency of CpG island methylation (~10-20%), some cases overlap with MSI

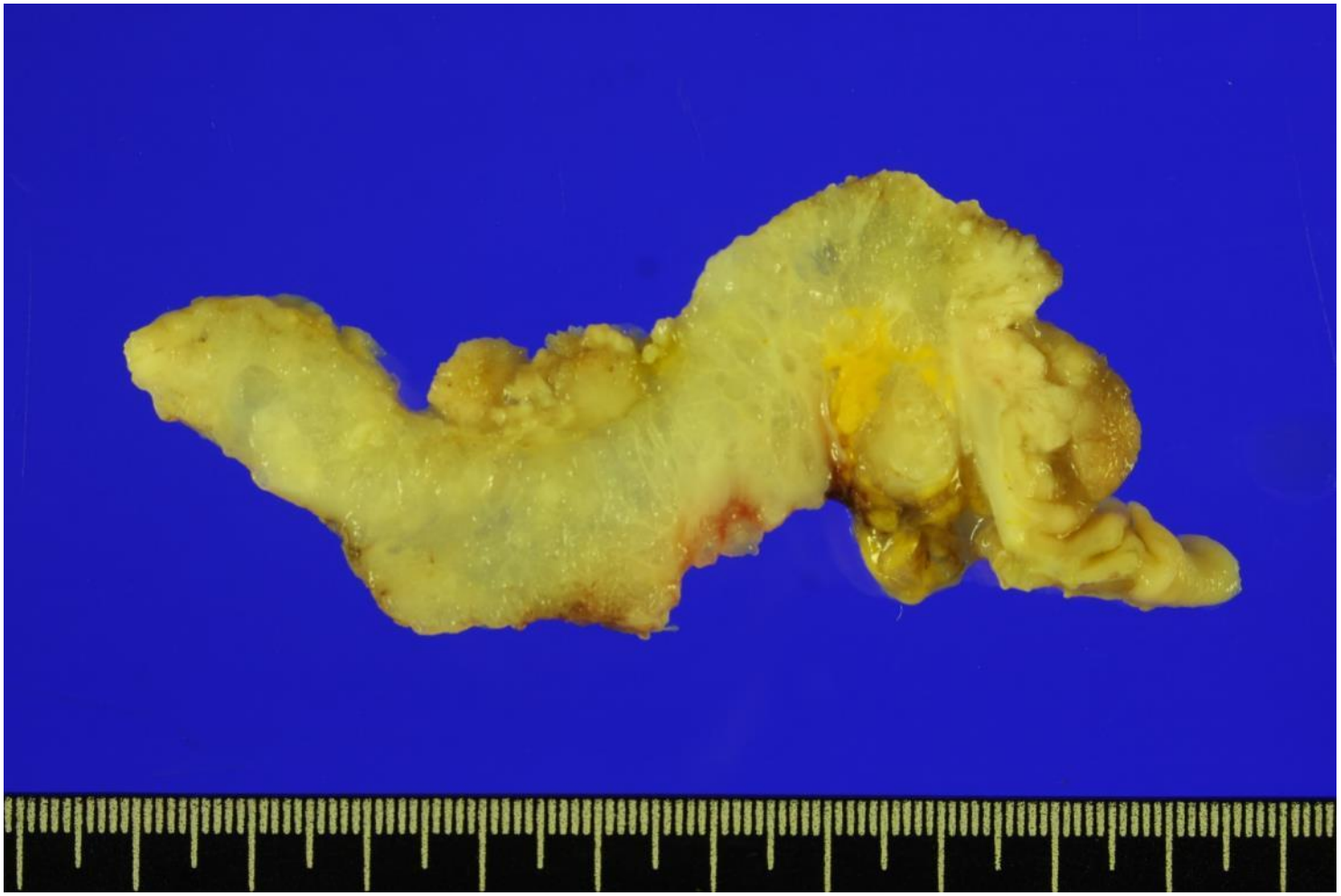
Molecular subtypes revealed by TCGA

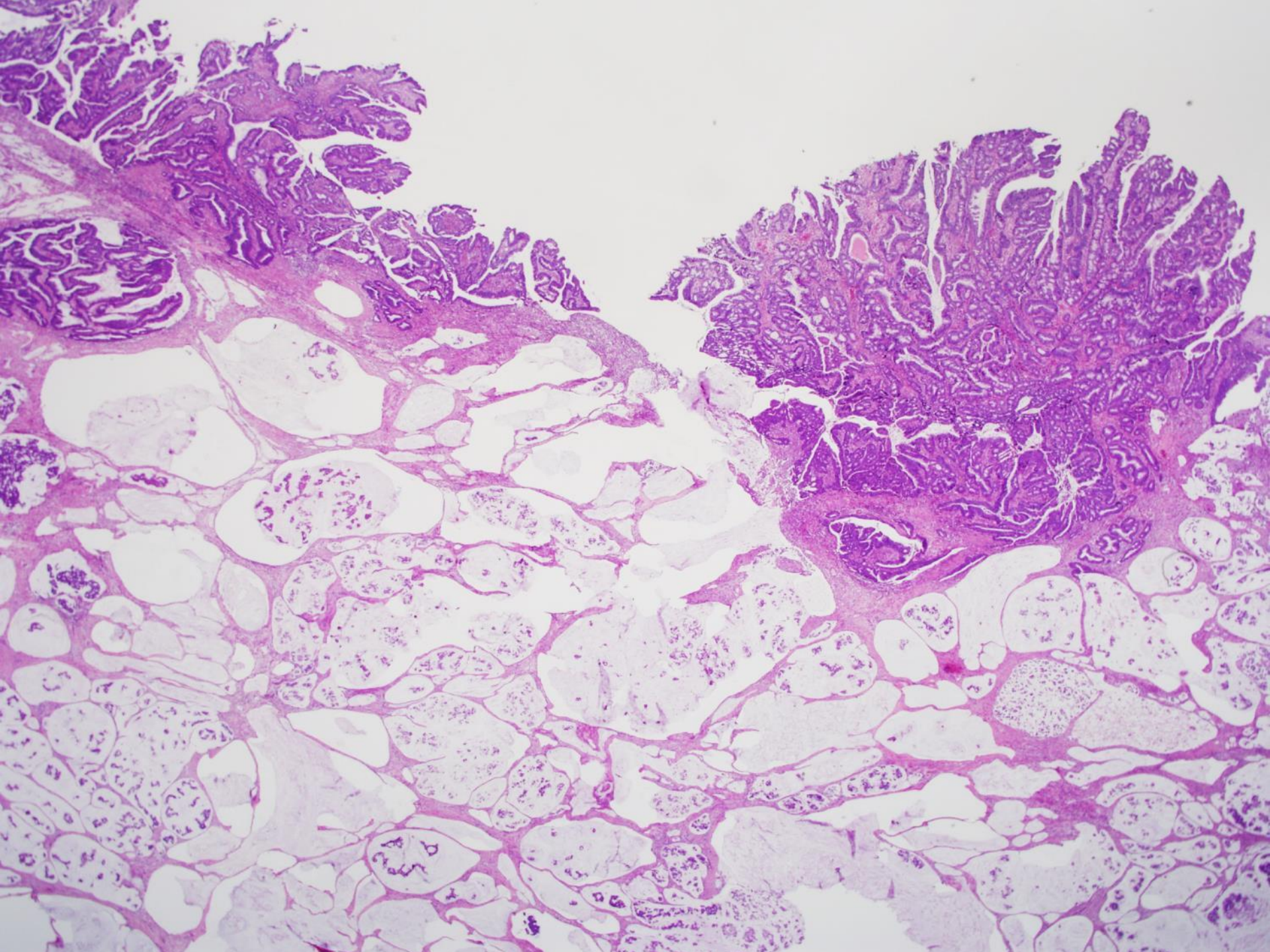


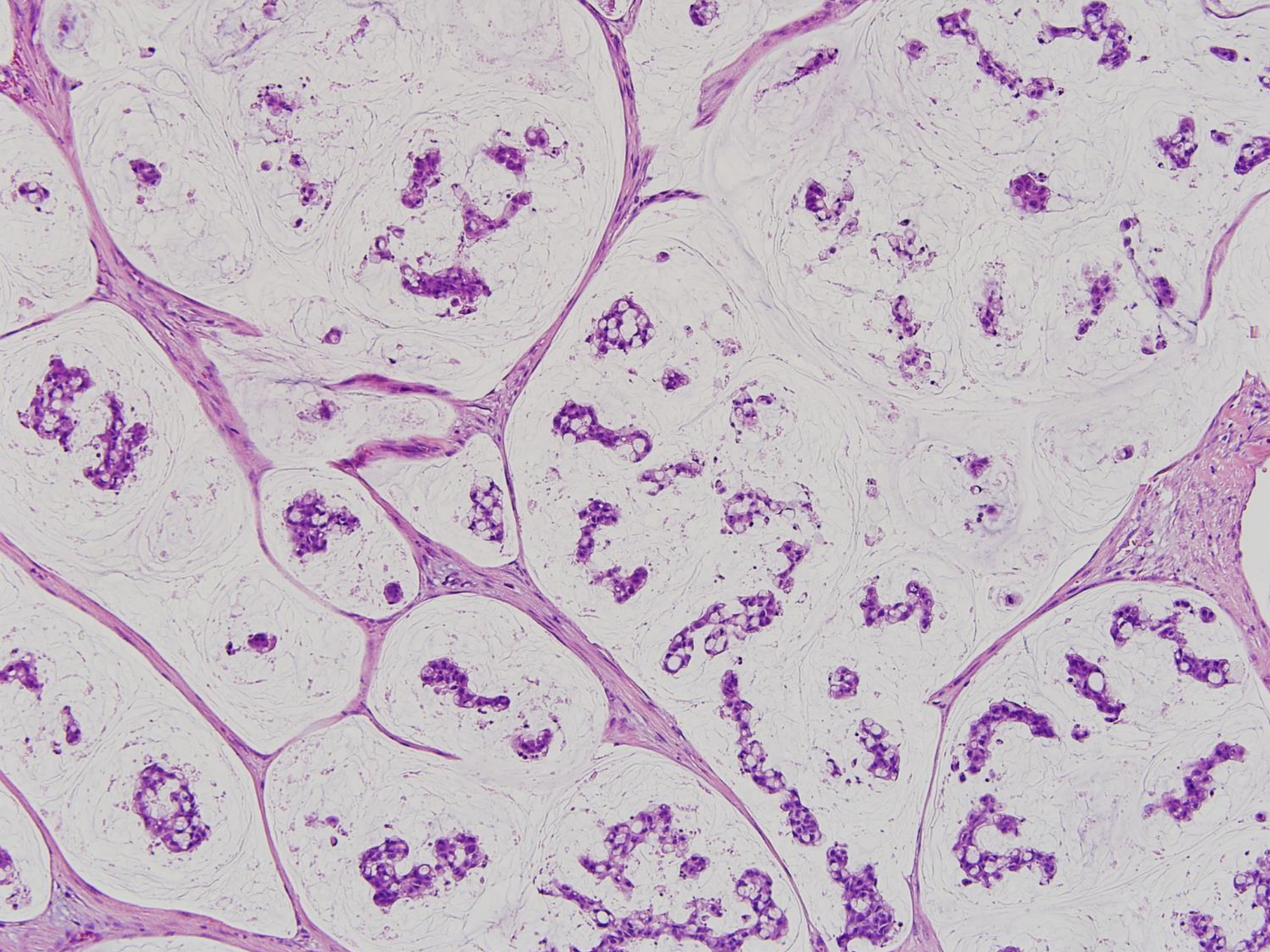
M/25, transverse colon cancer

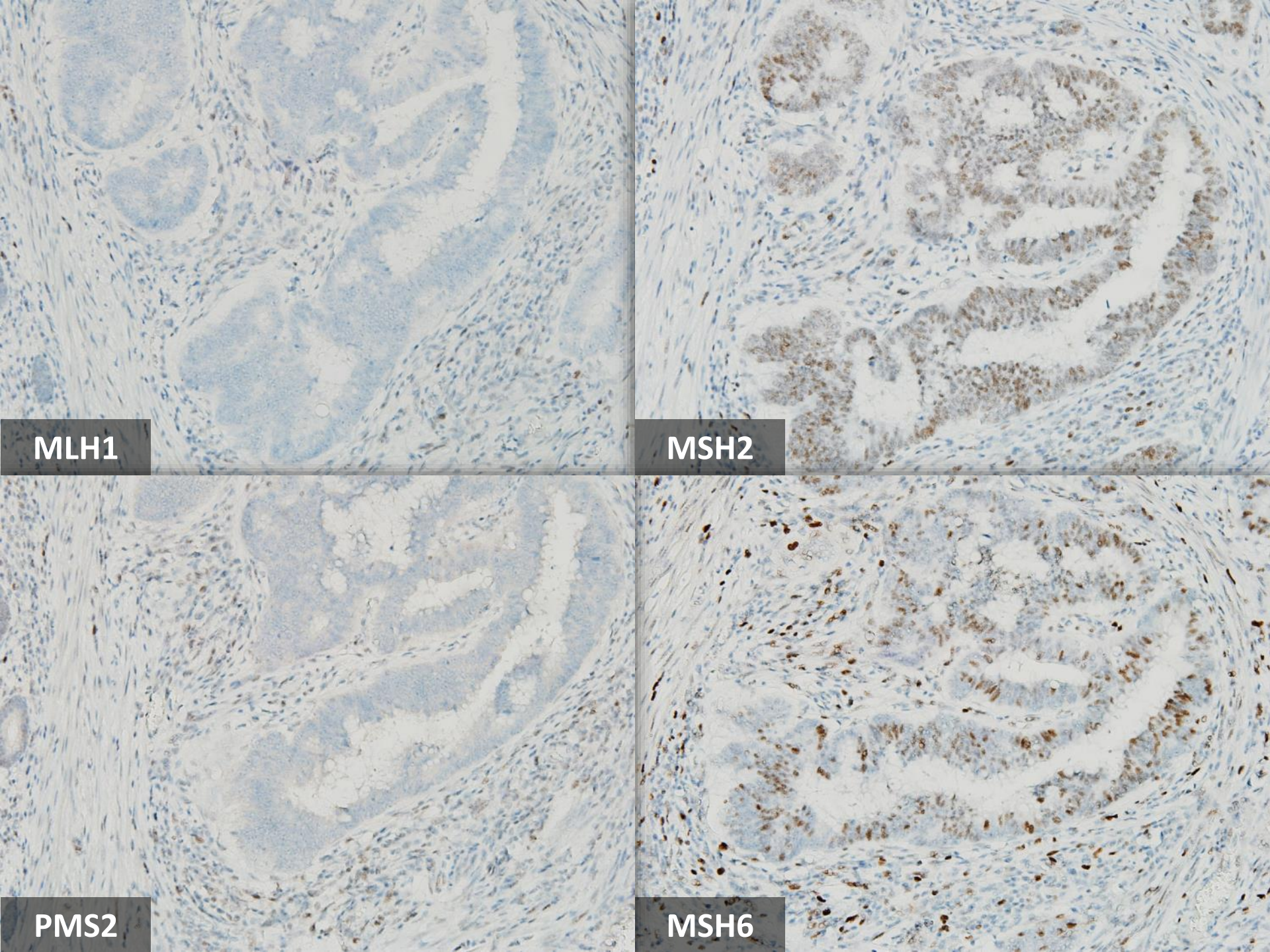


Gross








MLH1
MSH2
PMS2
MSH6

Genomic Alterations Detected

Gene	Type	Alteration	Allele frequency
ASH1L	DEL	T2895Qfs*44	0.29
ARV1	DEL	K173Sfs*8	0.33
ALK	SNV	R1061Q	0.28
ALK	SNV	T733A	0.04
ACVR2A	DEL	K437Rfs*5	0.29
COBLL1	INS	L907Ffs*25	0.18
BARD1	SNV	V604L	0.32
MLH1	SNV	Q391*	0.81
CTNNB1	SNV	T41A	0.64
DOCK3	DEL	P1852Qfs*45	0.30
ATR	SNV	Y1535H	0.35
PIK3CA	SNV	H1047R	0.31
ABCC5	DEL	L1090Cfs*26	0.30
RGS12	DEL	Q1292Rfs*49	0.25
CLOCK	DEL	L123Sfs*13	0.28
TET2	SNV	A347V	0.35
RAD50	DEL	M502Wfs*3	0.32
KIAA1919	DEL	C202Vfs*4	0.31
ROS1	SNV	V1005A	0.27
AKAP7	DEL	K79Rfs*21	0.53
PMS2	DEL	D414Tfs*34	0.28

KRAS	SNV	G12D	0.33
BRCA2	SNV	R1512C	0.04
CKAP2	DEL	K606Rfs*14	0.30
NFKBIA	SNV	S32G	0.32
OR4M2	DEL	G152Afs*23	0.14
AXIN1	SNV	G791W	0.33
CREBBP	DEL	I1084Sfs*15	0.33
RNF43	DEL	G659Vfs*41	0.25
RBBP8	DEL	K357Nfs*3	0.34
MADCAM1	INS	P230Qfs*69	0.05
DOT1L	DEL	S938del	0.33
NOTCH3	SNV	R1873H	0.28
WDR87	DEL	K2720Rfs*57	0.31
TEAD2	DEL	H299Mfs*12	0.29
SRC	SNV	A27T	0.33
ADNP	DEL	K1016Rfs*11	0.28
CDH26	DEL	-	0.33
OR4M2	CNV	Amplification	-

MMR-related genes

Mismatch Repair Genes

Gene	Type	Alteration	Allele frequency
MLH1	SNV	Q391*	0.81
PMS2	DEL	D414Tfs*34	0.28

Tumors harboring MMR gene mutation show microsatellite instability (MSI). OncoPanel AMC v3 includes 4 MMR genes (MLH1, MSH2, MSH6, and PMS2), and reports hypermutator phenotype based on mutation burden reflecting MSI status.

Tumor purity inferred from MAF of truncal mutations = ~60%
MLH1 Q391* mutation is highly likely a germline mutation!

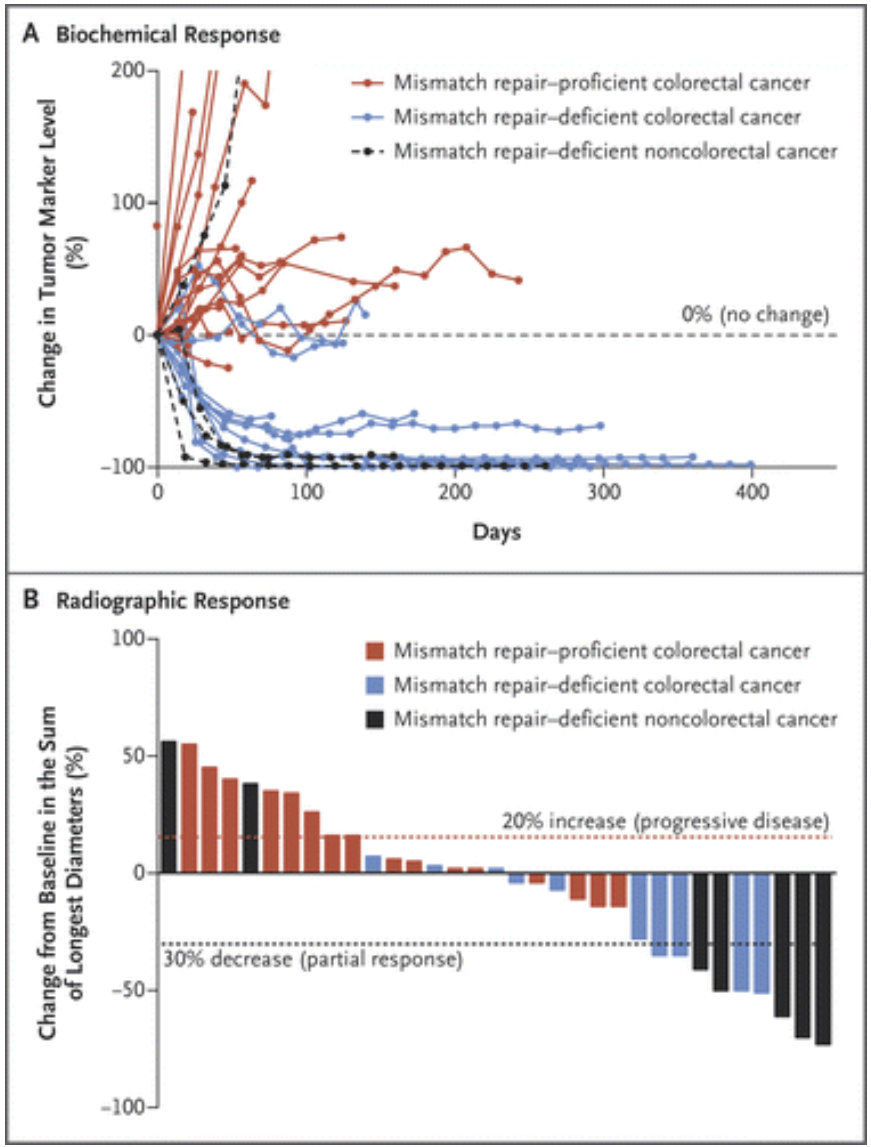
And this patient turned out to be Lynch syndrome.

MSI testing in colorectal cancer

- Strongly recommended in all colorectal cancer patients for identification of patients at risk for **Lynch syndrome** and/or **prognostic stratification.**

- Predict **responsiveness to immune checkpoint blockade therapy** in advanced disease setting

MMR deficiency and PD1 blockade



(Le DT et al. *N Engl J Med* 2015)

Diagnosis of MMR deficiency

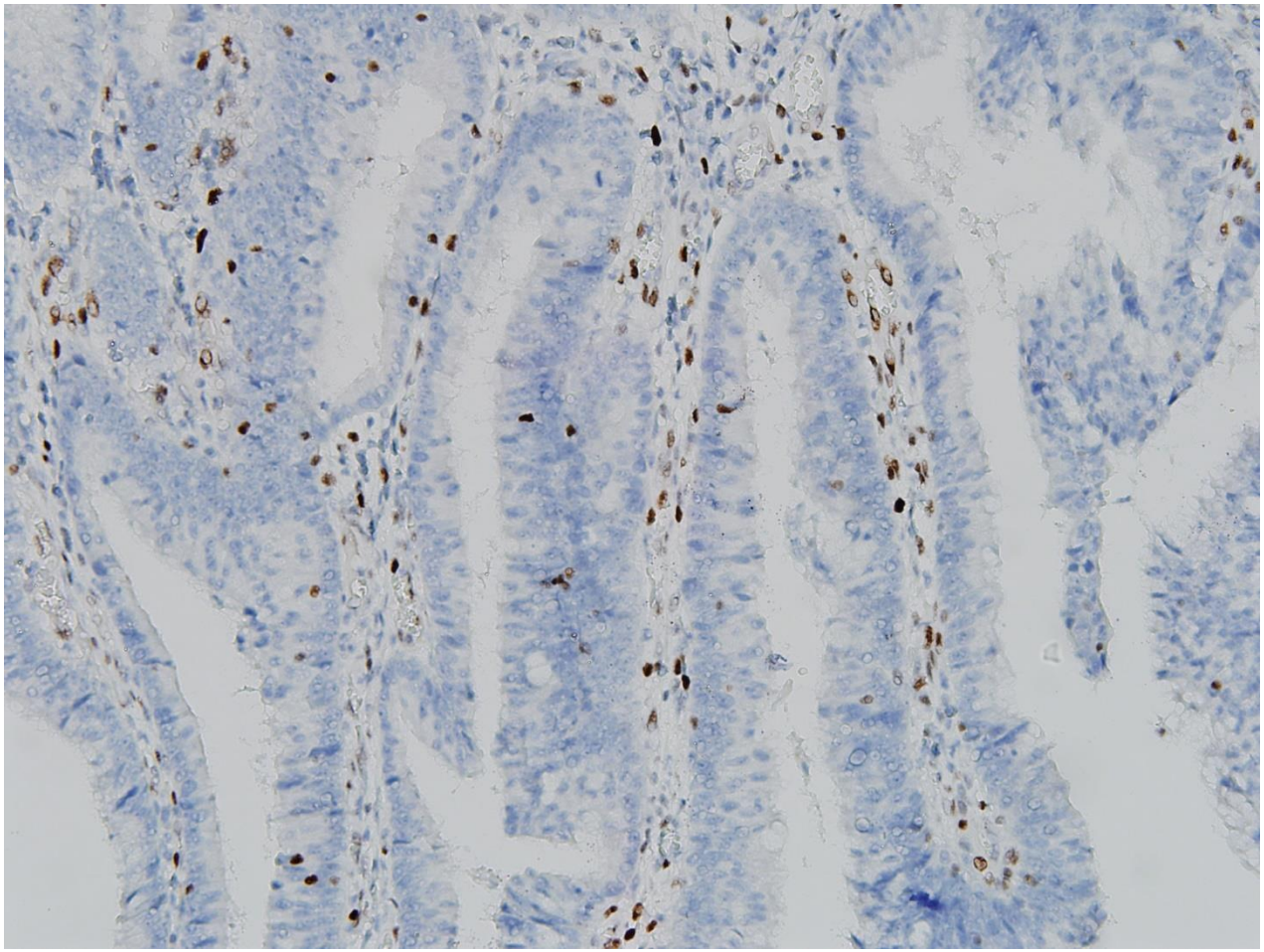
- Standard PCR fragment analysis:
  - NCI-5 markers: mixture of mono- (BAT25, BAT26) and dinucleotide repeats (D2S123, D17S250, D5S346)
  - PentaPlex mononucleotide markers: commercial kit (BAT25, BAT26, NR21, NR22, NR24)
  - 30% or more of the repeats are unstable: MSI-H
- MMR protein IHC:
  - Recommendation: panel of 4 MMR proteins: MLH1, PMS2, MSH2, MSH6
  - Robust quality control is essential.
  - A few caveats

Interpretation of MMR protein IHC

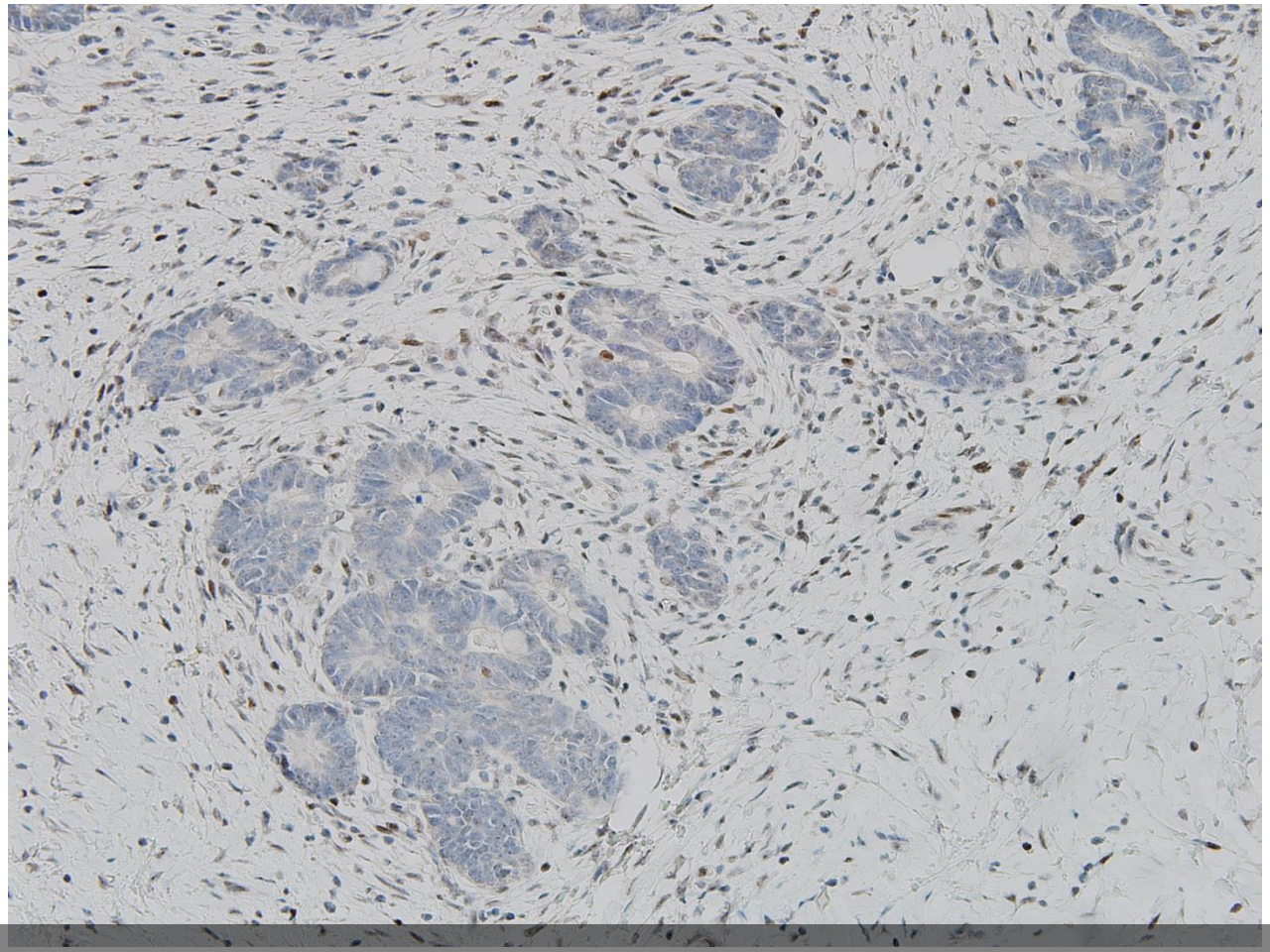
- Typical staining pattern:
  - Simultaneous loss of MLH1 & PMS2
  - Simultaneous loss of MSH2 & MSH6

- Interpretation guide:
  - Positive: convincing nuclear staining (stronger than internal controls: normal crypts, lymphocytes, stromal cells) >5%
  - Negative: absence of nuclear staining in the presence of control staining

Typical expression loss (MSH6 example)

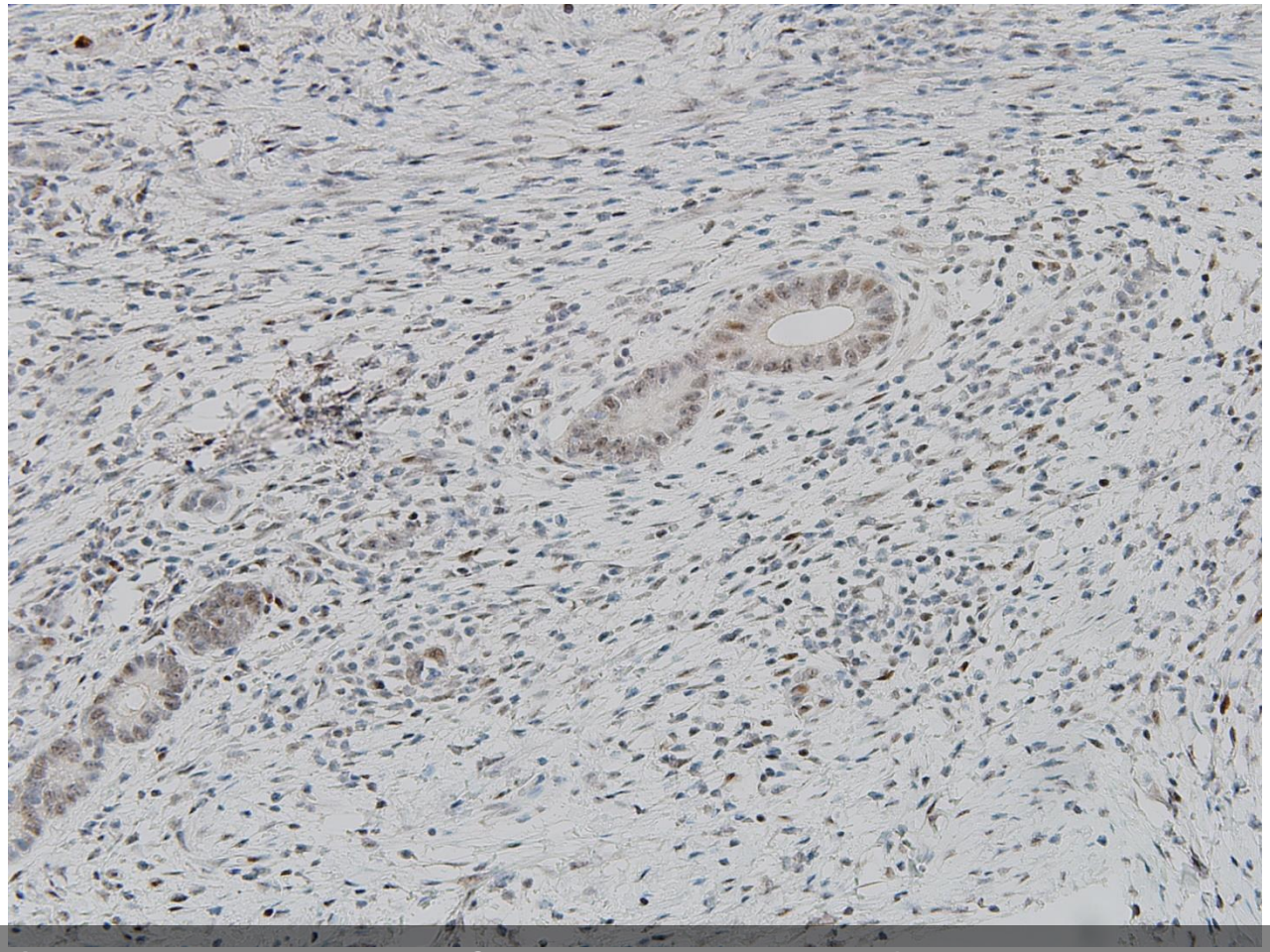


PMS2 staining



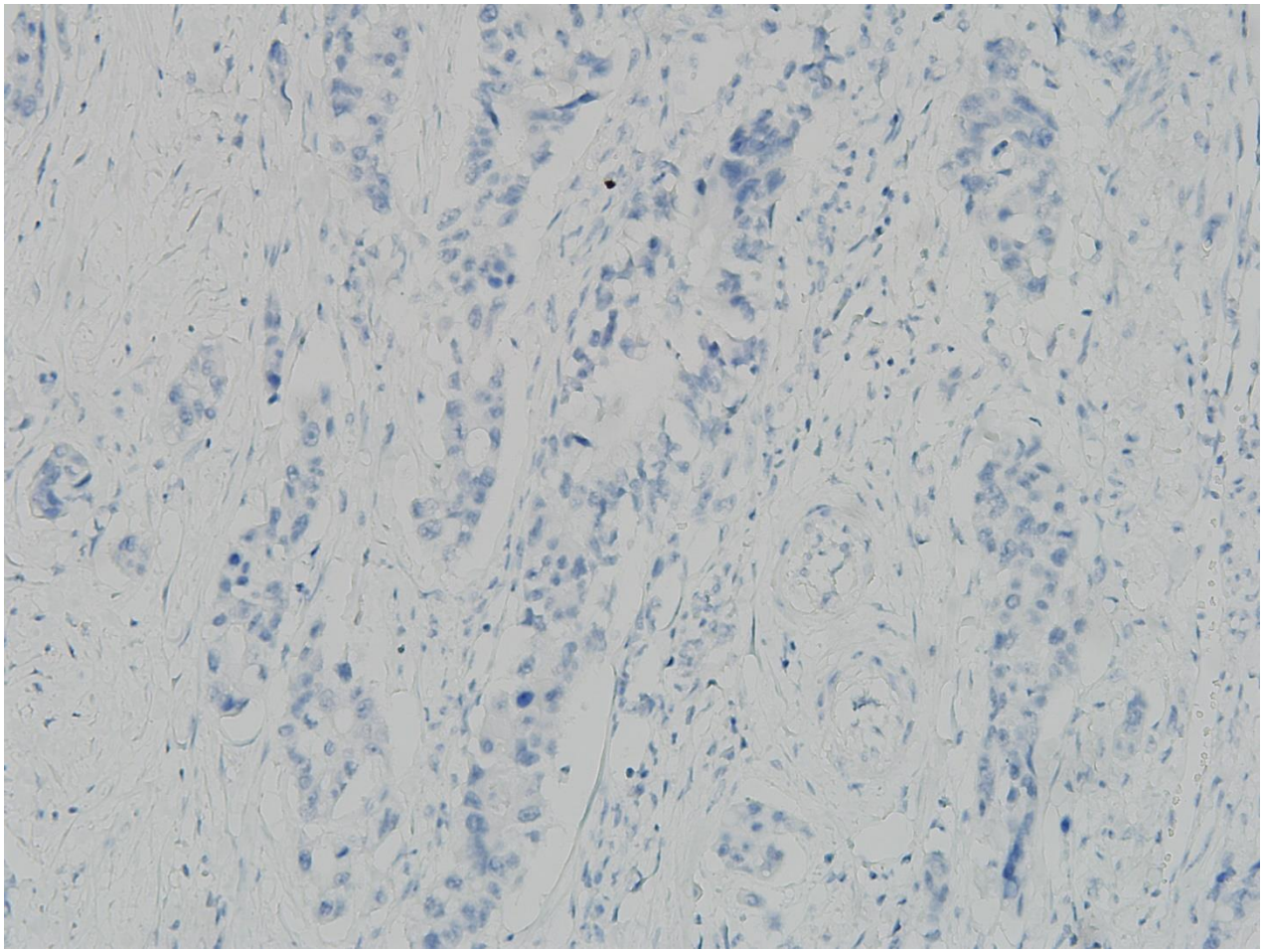
Loss of PMS2 expression?

Search for positive area…



In fact, this picture came from post-neoadjuvant surgical resection specimen and this tumor was MSS (judged by PCR fragment analysis)

MLH1



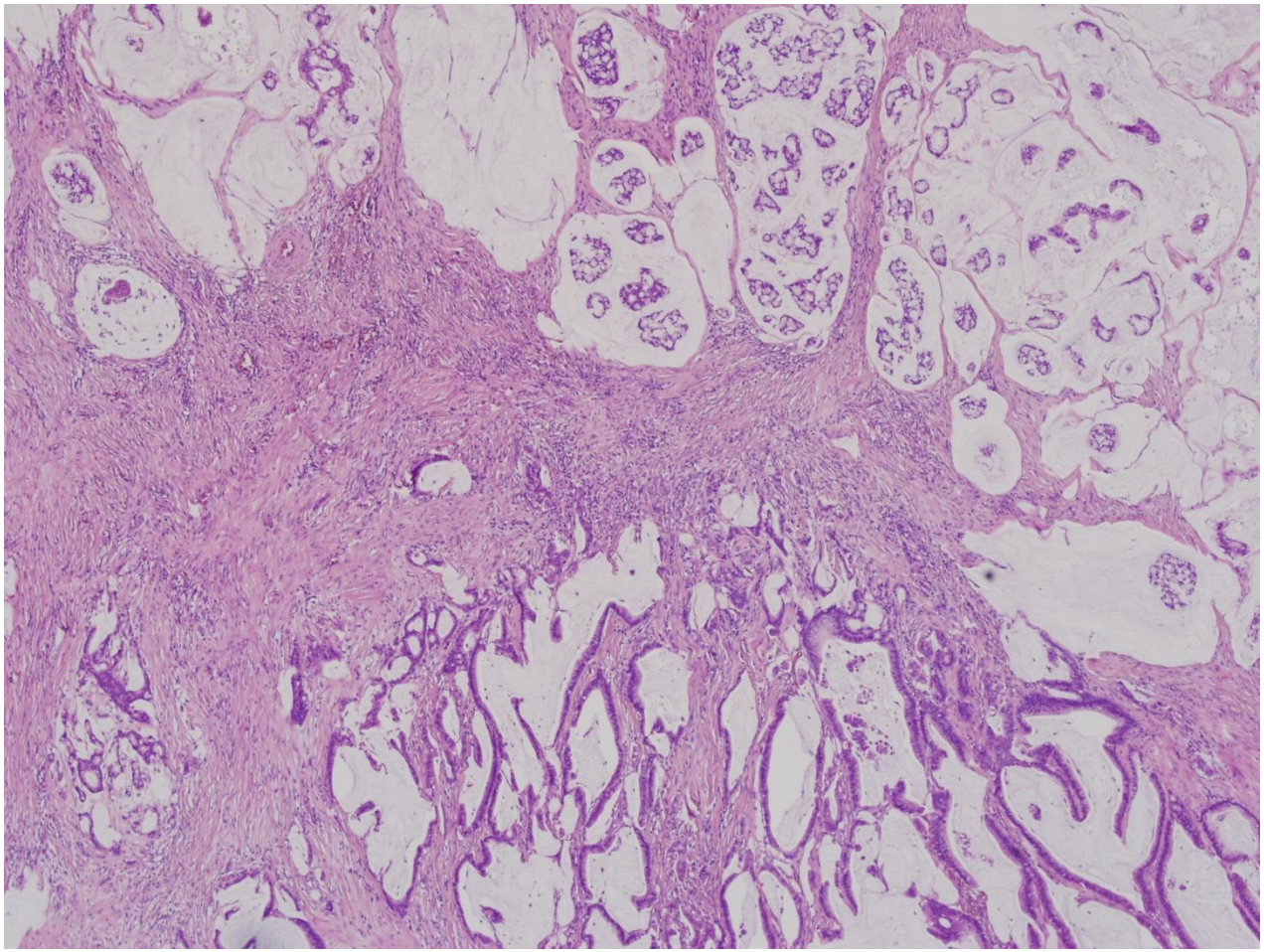
Please don’t call this staining as MLH1 loss!

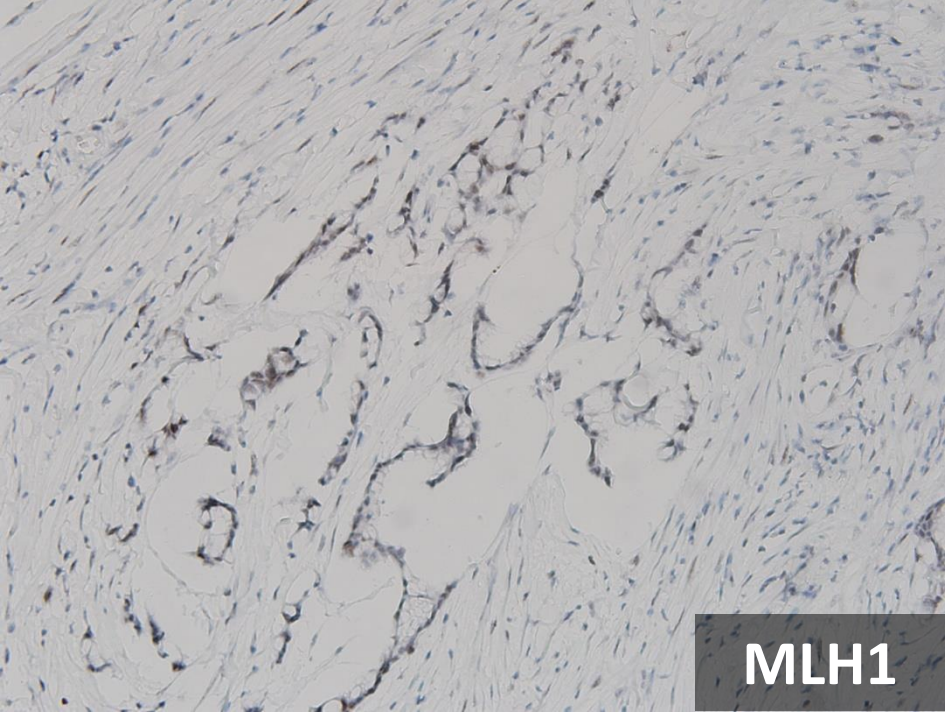
Challenging MMR IHC interpretation

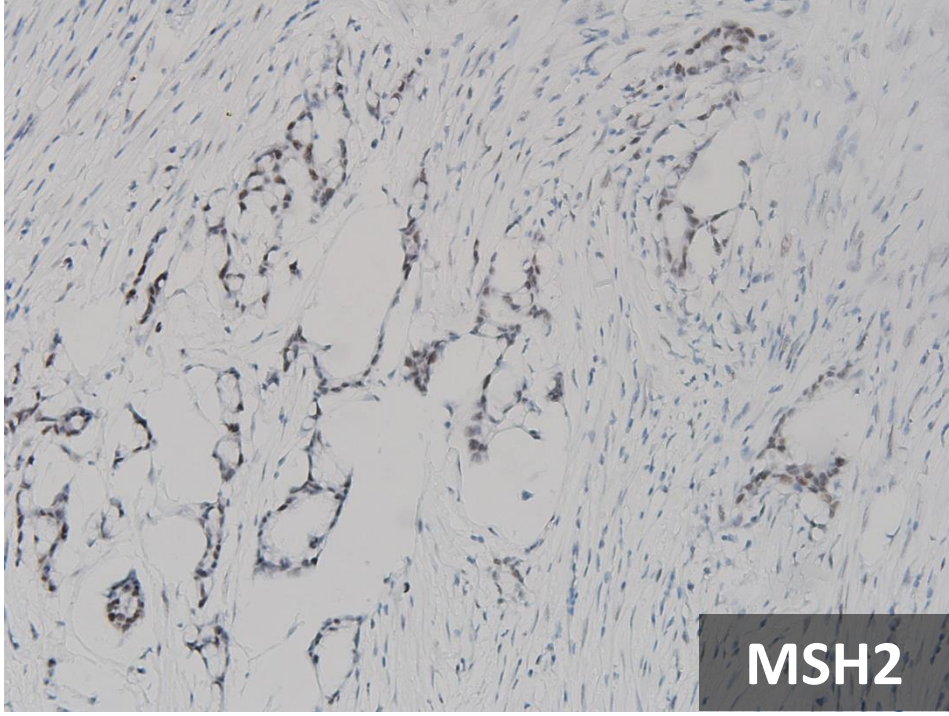
Setting	Action
Cytoplasmic staining	Repeat if nuclei are obscured. Compare with nuclear staining in control cells. -> Call loss.
Weaker tumor signals than controls	Check controls and repeat staining. -> Call loss.
Post-neoadjuvant, weak or focal positive	Test pre-treatment biopsy.
Heterogeneous tumor staining	Check controls, edge artifacts, or uneven antibody coverage.

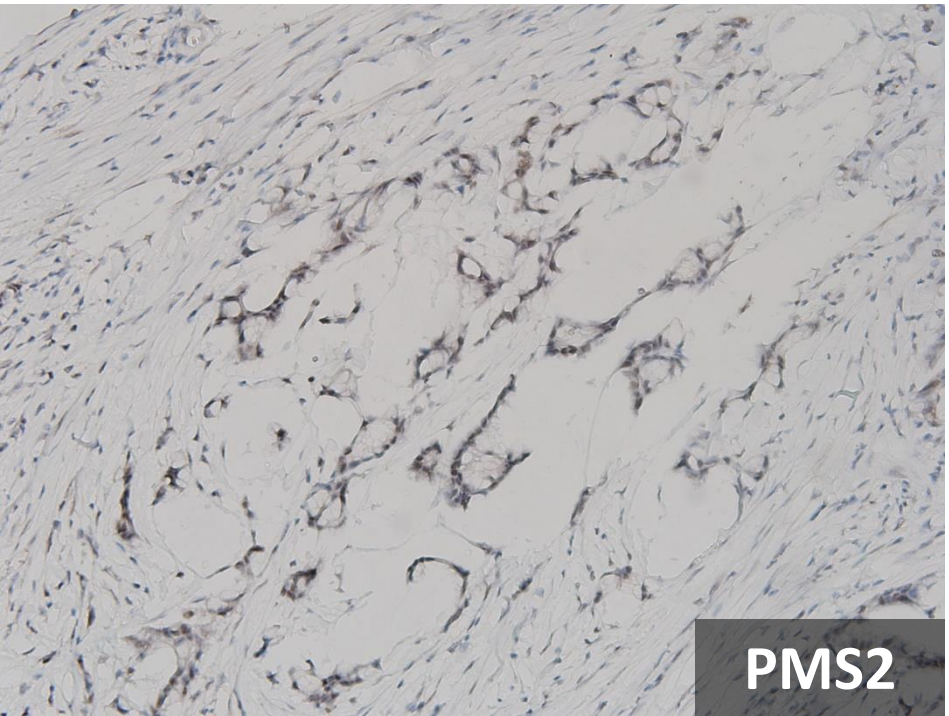
(Shia et al., *Mod Pathol* 2013; Graham et al., *Am J Surg Pathol* 2015; Pai et al., *Am J Surg Pathol* 2016; Pearlman et al., *Mod Pathol* 2018)

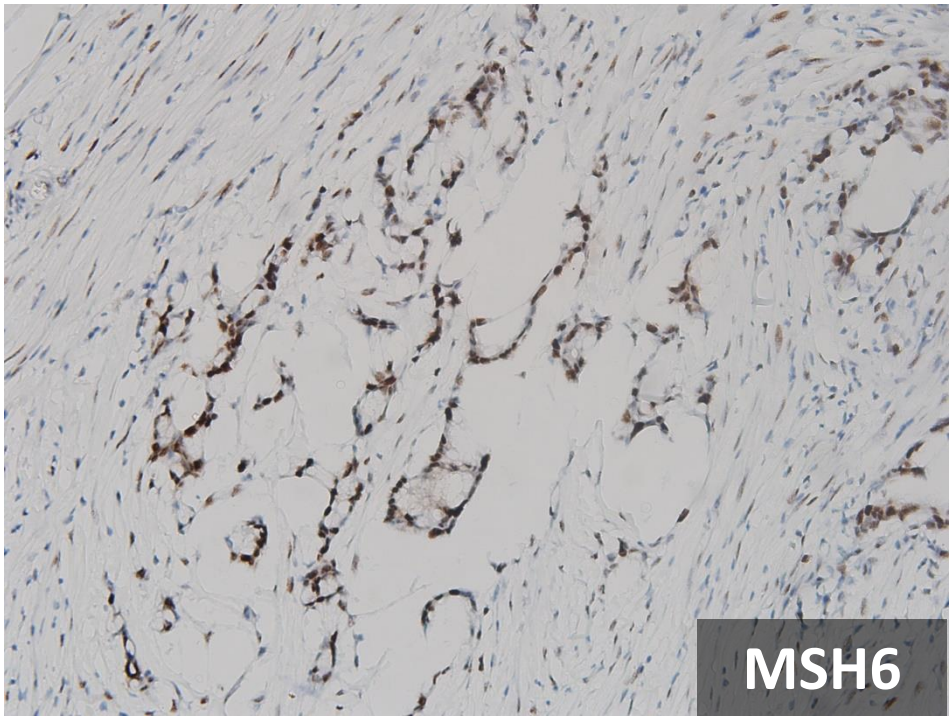
M/55, descending colon cancer



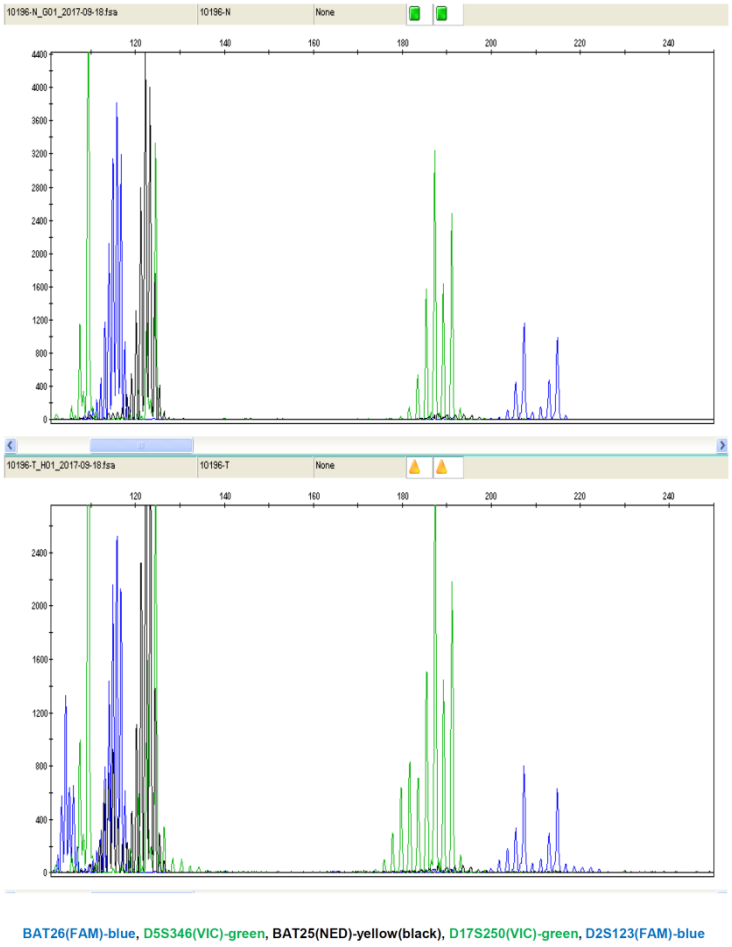

MLH1


MSH2


PMS2


MSH6

MSI test and NGS results



LIPT1	DEL	K123Sfs*8	0.09
ACVR2A	DEL	K437Rfs*5	0.18
COBLL1	DEL	L907Cfs*12	0.08
DYNC1I2	DEL	R57Gfs*13	0.08
PPARG	DEL	L5Gfs*6	0.09
CTNNB1	SNV	Q203*	0.07
DOCK3	DEL	P1852Qfs*45	0.14
PBRM1	SNV	R595W	0.08
MITF	SNV	V421I	0.09
EPHA3	DEL	K365Nfs*6	0.11
PIK3CA	SNV	H1047R	0.09
ABCC5	DEL	L1090Cfs*26	0.08
FGFBP1	DEL	V27*	0.13
CLOCK	DEL	L123*	0.08
FBXW7	SNV	R441Q	0.12
TNPO1	DEL	C844Lfs*45	0.11
APC	SNV	R876*	0.12
APC	DEL	S1465Wfs*3	0.08
APC	SNV	Q2701H	0.08
WDR55	DEL	K341Rfs*8	0.05
KCTD16	DEL	A384Lfs*20	0.05
KIAA1919	DEL	C202Vfs*4	0.20
ARID1B	DEL	P174Rfs*6	0.09
NOS3	DEL	G440Afs*64	0.15
XRCC2	DEL	L117Wfs*17	0.08
EPPK1	SNV	R2239C	0.15

MSH2 S900* (likely pathogenic, VAF 0.5), *MSH2* N671Y (VUS, VAF 0.1), *MSH6* L909S (VUS, VAF 0.1), estimated tumor purity ~20%: MSI-H associated with probable germline *MSH2* S900* mutation [IHC may be positive if the Ab detects N-terminal side (to codon 671) of MSH2 protein]

Diagnosis of MSI status with NGS

Table 2 Mutation Load in MSS and MSI-H Tumors

Variable	Test set (n = 79)			Validation set (n = 128)		
	MSS (n = 41)	MSI-H (n = 38)	P	MSS (n = 120)	MSI-H (n = 8)	P
Mutation load (total SNV and indel), median (range)	19 (11–180)	70 (28–110)	<0.0001	16 (5–388)	52 (46–139)	0.007
Indel, median (range)	1 (0–8)	23 (2–37)	<0.0001	1 (0–5)	26 (14–29)	<0.0001
I index (indel–total mutation ratio) (%)	7.0 (0.0–22.7)	30.9 (7.0–41.0)	<0.0001	5.9 (0.0–30.0)	29.0 (19.0–36.0)	0.008

Indel, insertions and deletions; MSI-H, microsatellite instability–high; MSS, microsatellite stable; SNV, single-nucleotide variant.

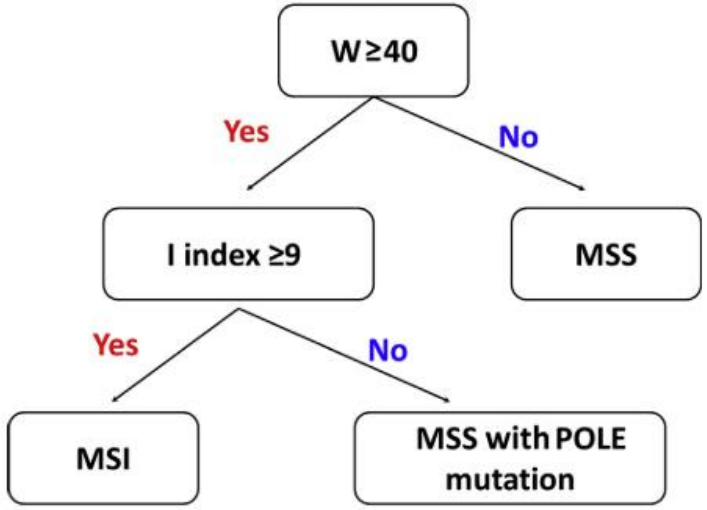


Figure 3 Decision tree for microsatellite instability (MSI) detection using values of mutation burden and indel index (I index) from targeted nest-generation sequencing panel. MSS, microsatellite stable.

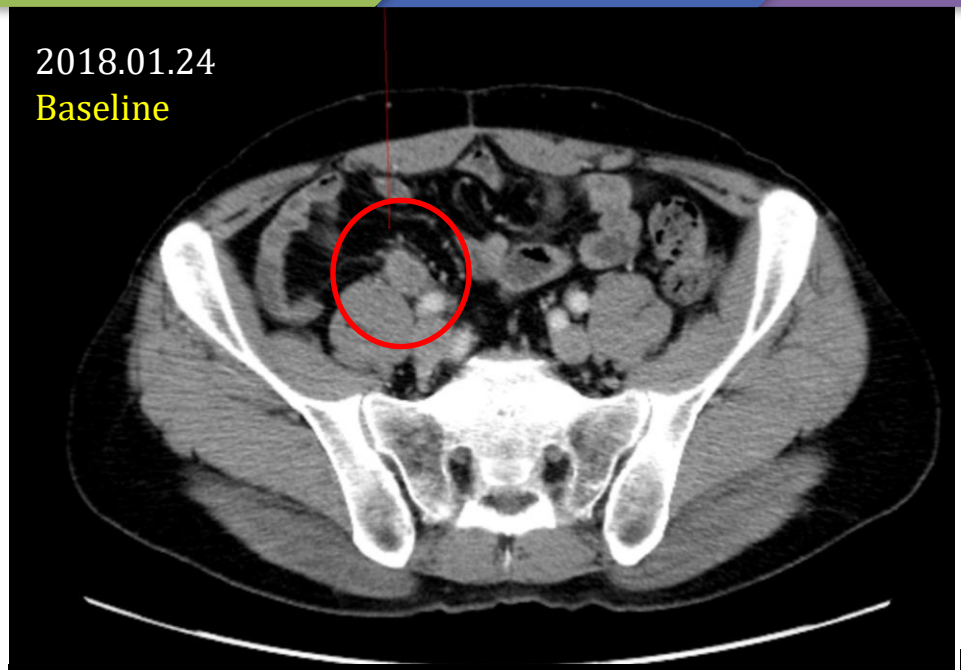
These criteria achieved **97.4% sensitivity** (95% CI, 90.8% to 100%) and **100% specificity** (95% CI, 91.4% to 100%) in the test set.

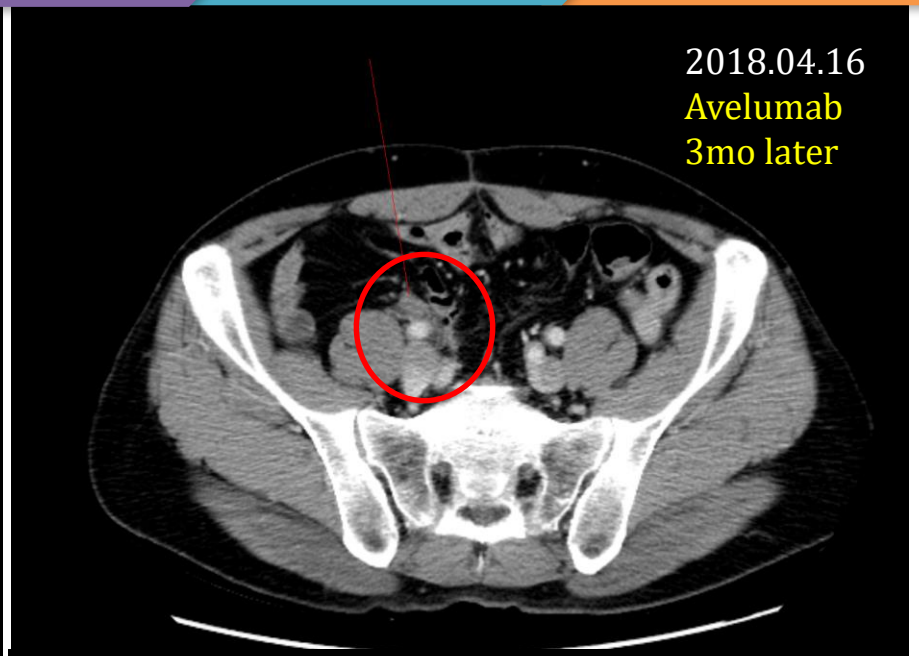
In the validation set, both sensitivity and specificity were 1.0 (95% CI, 0.91 to 1.0) for detecting MSI-H CRC.

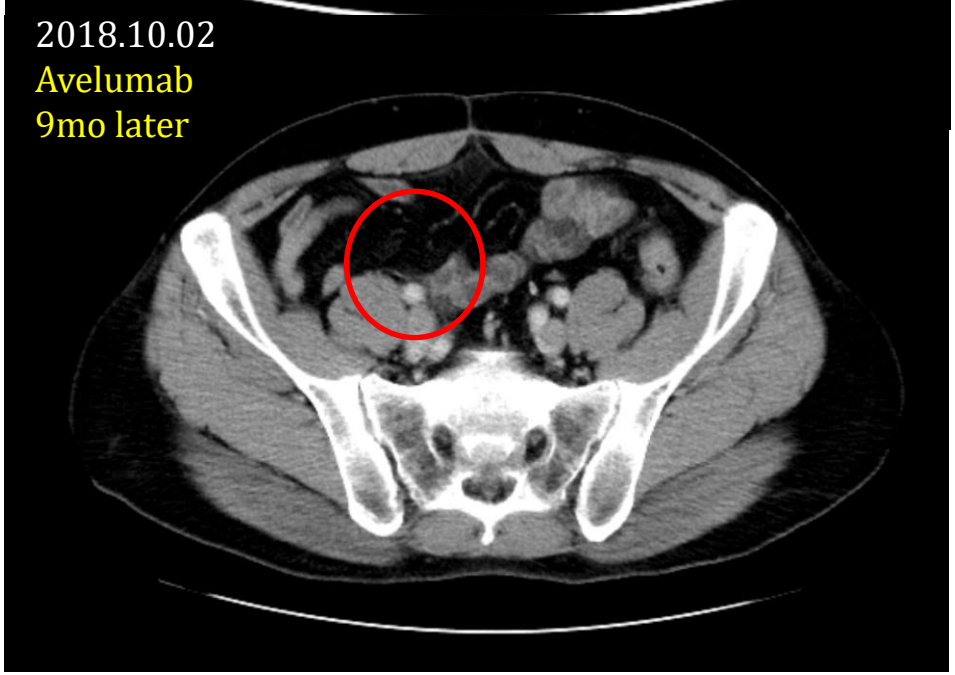
(Chun SM et al. *J Mol Diagn*. 2019 Mar;21(2):241-250.)

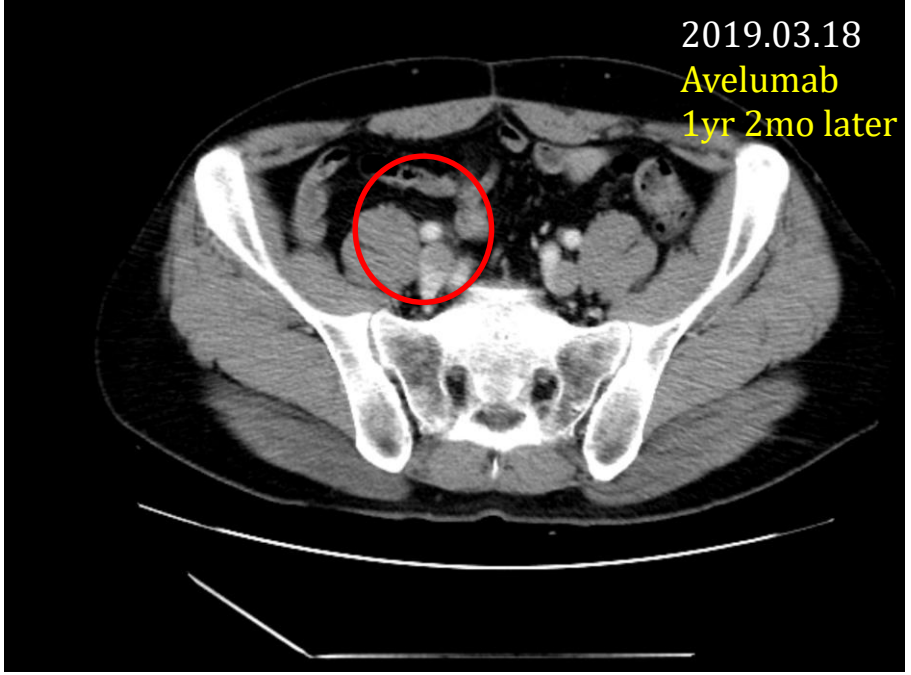
M/52

- Right hemicolectomy for ascending colon cancer (5 years ago): pStage IIIc adjuvant XELOX #8
- Abdominal wall localized seeding (3 years ago): surgical excision + Avastin/FOLFIRI #12
- Recur in right 2nd mammary station (2 year ago): excision + Xeloda #8
- Recur in RLQ (1 year ago): NGS -> MSI-H


2018.01.24
Baseline


2018.04.16
Avelumab
3mo later


2018.10.02
Avelumab
9mo later


2019.03.18
Avelumab
1yr 2mo later

Mutation-based markers: CAP recommendations

- **Extended RAS (*KRAS, NRAS, BRAF*) oncogenic mutations:**
  - Contraindication of anti-EGFR mAb therapy
  - Should include codons 12, 13 [Exon 2], 59, 61 [Exon 3], 117, 146 [Exon 4] (for *KRAS, NRAS*), V600 (for *BRAF*)

- ***BRAF* V600E mutation:**
  - Prognostic: poor prognosis, especially in MSS tumors
  - Predictive of sporadic MSI-H CRC: *BRAF* V600E mutation in MMR deficient tumors with loss of MLH1 strongly favors sporadic tumors. The absence of *BRAF* V600E mutation does not guarantee Lynch syndrome.

Considerations in extended *RAS* testing

- In order to use Sanger sequencing…:
  - Sensitivity issue: high tumor purity (greater than 40%) is required for clinical use

- CAP guidelines for selection of mutation analysis in CRC:
  - Should use testing methods that are able to detect mutations with **at least 5% variant allele frequency (VAF)**: Sanger sequencing is inadequate, consider realtime PCR (like Cobas) or NGS
  - Limit of detection of 5% VAF requires **at least 20% tumor purity**: tumor enrichment through macrodissection

Extended RAS alterations
- NGS experiences at AMC -

- Total: 911 cases (as of 2019.04.10)

- Extended *RAS* alterations:
  - *KRAS* activating (n=425, **46.7%**): G12X(290), G13X(76), **D33E(4)**, **A59T(1)**, Q61X(19), **K117N(3)**, A146X(32)
  - *NRAS* activating (n=31, **3.4%**): G12X(12), G13X(3), Q61X(16)

- Contraindication for anti-EGFR therapy: *KRAS* or *NRAS* activating = **456 cases (50.1%)**: ~53% in a meta-analysis (Sorich MJ et al. 2015)

Mutation-based markers: no recommendations by CAP or others

- ***PIK3CA* mutation:**
  - No recommendation for therapy selection
  - Retrospective studies have suggested improved survivals with post-operative aspirin use in patient with *PIK3CA*-mutant CRC.

- ***PTEN* analysis (expression by IHC or deletion by FISH):**
  - No recommendation for therapy selection
  - Prognostic value: unknown due to discordant results

- ***ERBB2* amplification (~3.4% at AMC):**
  - Putative marker for unresponsiveness to anti-EGFR therapy (Jeong JH et al., *Clin Colorectal Cancer* 2017)
  - Anti-ERBB2 mAb trial is ongoing.

Transcriptome-based classification

- Like breast cancer

- Several versions:
  - Schlicker et al, 2012
  - Marisa et al, 2013
  - Sadanandam et al, 2013: CRCA subtype
  - De Sousa E Melo et al, 2013: CCS subtype
  - Budinska et al, 2013
  - Roepman et al, 2014

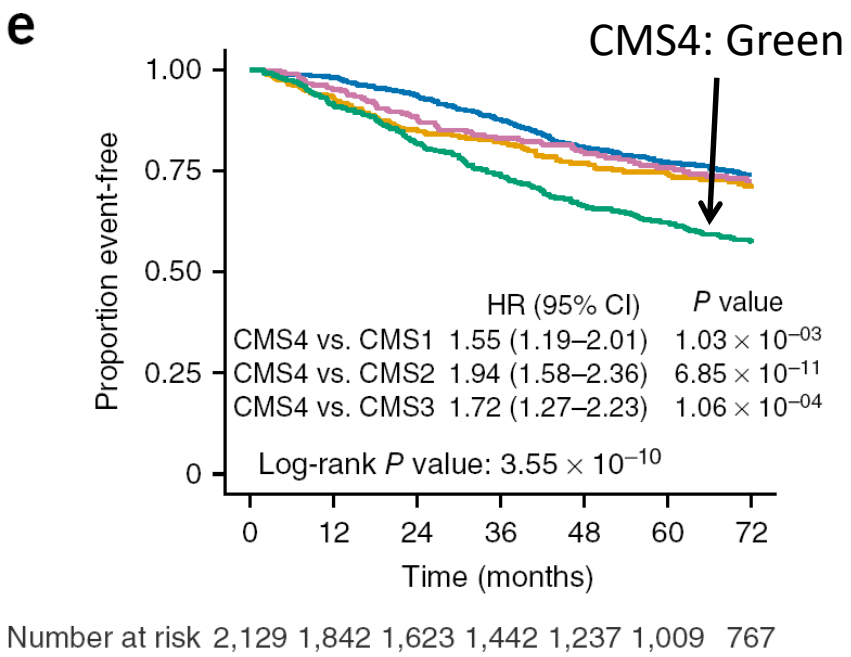
CMS (Consensus molecular subtype)

- **CMS1 (MSI immune, 14%)**: hypermutated, MSI, frequent *BRAF* mutation, CpG island methylation, SCNA-low
- **CMS2 (canonical, 38%)**: Wnt/Myc activation,,SCNA-high, conventional adenoma-carcinoma sequence
- **CMS3 (metabolic, 13%)**: prominent Warburg effect, SCNA-medium, frequent *KRAS* mutations
- **CMS4 (mesenchymal, 23%):** EMT gene upregulation, MSS, aggressive behavior

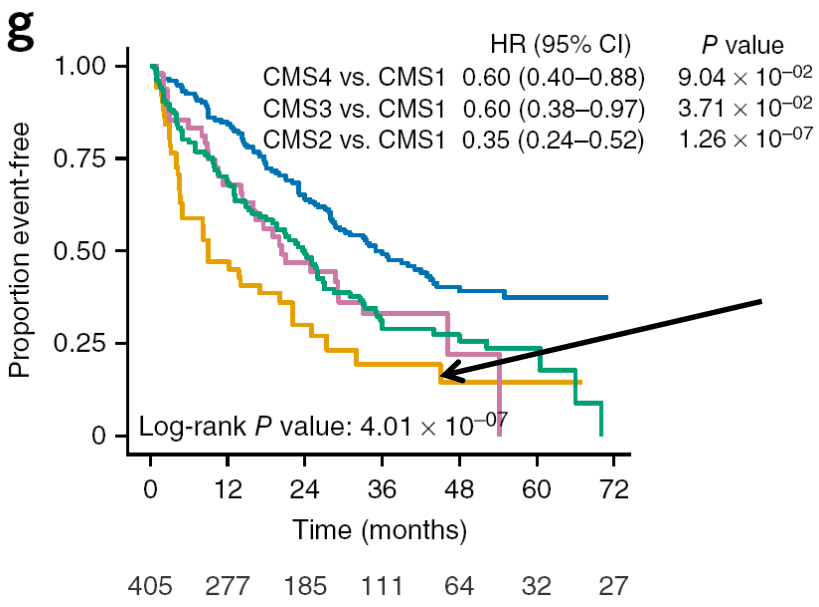
(Guinney J et al., *Nat Med* 2016)

CMS

-Clinical correlates-



(Overall survival)



(Survival after relapse)

CMS1, Yellow; CMS2, Blue; CMS3, Magenta; CMS4, Green

Limitations of expression-based subtypes

- Standardization and generalization is difficult: batch effect, normalization problem (for new i ndividual samples)

- Sample processing and experimental procedur e: clinical implementation is difficult.

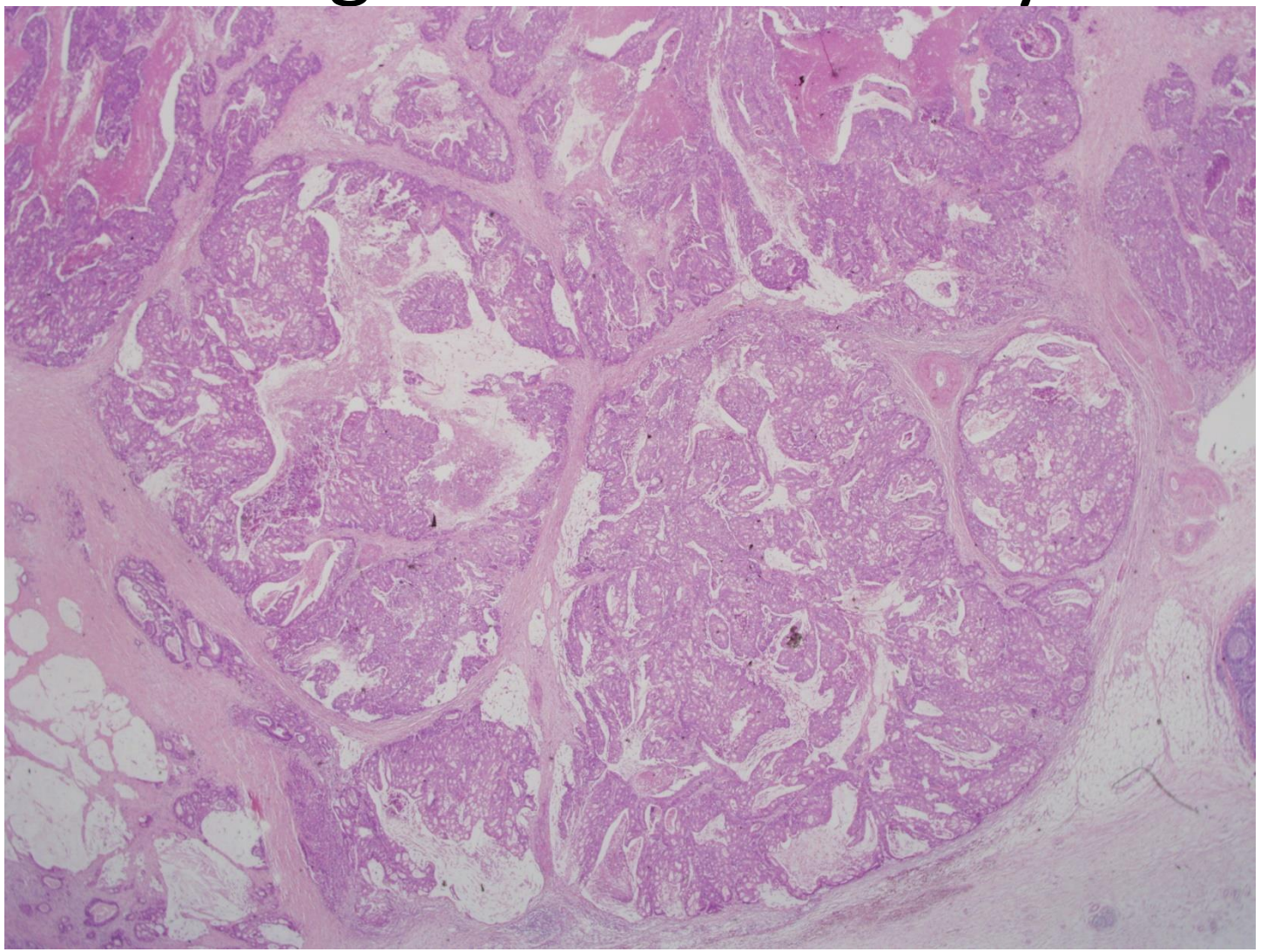
Other rare but potentially druggable alterations

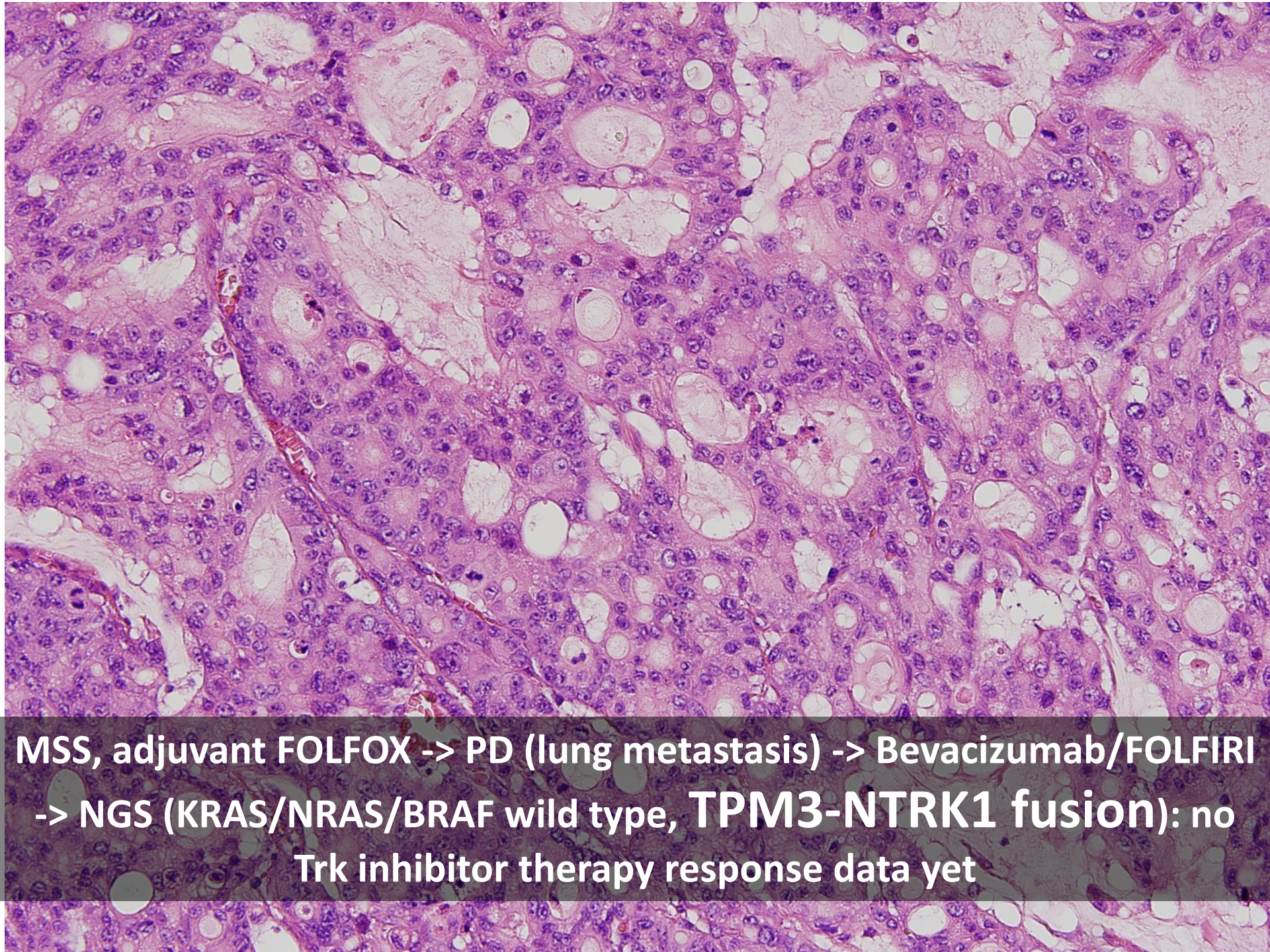
- ***NTRK1* fusion:** 3 cases (0.3%)

- ***POLE* hotspot mutations** (proofreading deficiency): 4 cases (0.4%), some promising data about immunotherapy.

- **Pathogenic/Likely pathogenic *BRCA* mutations**: 4 cases (0.4%)

F/69 colon cancer in hepatic flexure: right hemicolectomy




MSS, adjuvant FOLFOX -> PD (lung metastasis) -> Bevacizumab/FOLFIRI
-> NGS (KRAS/NRAS/BRAF wild type, TPM3-NTRK1 fusion): no
Trk inhibitor therapy response data yet

POLE-mutant CRC
(at AMC, out of 911 cases)

- V411L (2 cases)
- P286R (1 case), A456P (1 case)

- All men
- Age: 54, 58, 62, 47

- Extremely high tumor mutation burden (>100 mutations/Mb) for all 4 cases

M/54, sigmoid colon cancer

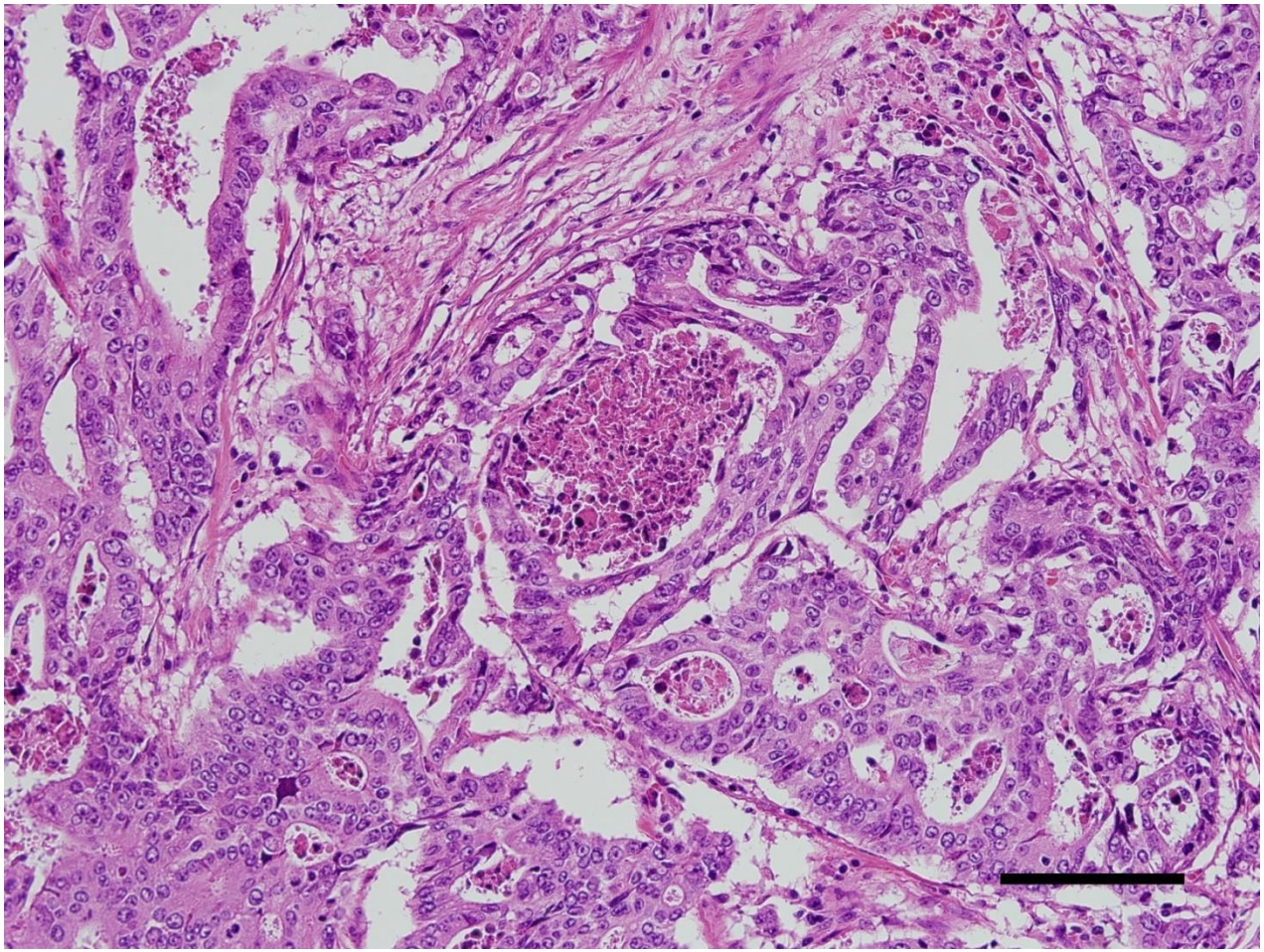
Genomic Alterations Detected

Gene	Type	Alteration	
ERRFI1	SNV	A161S	0.11
MTOR	SNV	K1993T	0.14
ARID1A	SNV	S1544A	0.24
MPL	SNV	R426*	0.13
JAK1	SNV	K452T	0.22
ASH1L	SNV	P2879S	0.23
NTRK1	SNV	K441T	0.23
DDR2	SNV	F275C	0.21
DDR2	SNV	A414T	0.24

Detected alterations (continued)

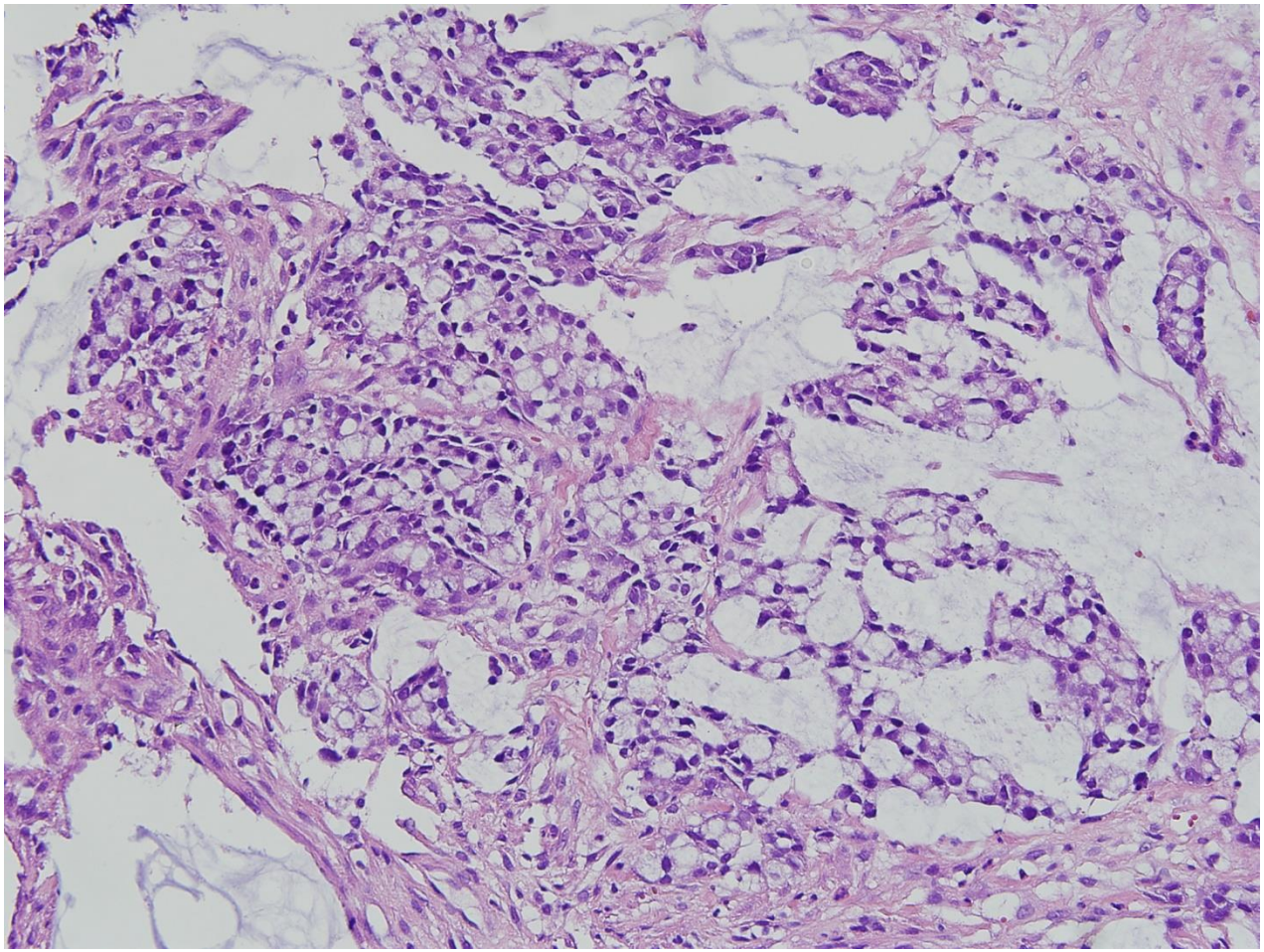
DDR2	SNV	R489Q	0.24
GEN1	SNV	T859A	0.19
CEBPZ	SNV	D501G	0.23
MSH2	SNV	D864Y	0.16
MSH2	SNV	K918N	0.20
MSH6	SNV	E206*	0.22
MSH6	SNV	R1005Q	0.22
LIPT1	SNV	K122T	0.21
LRP1B	SNV	D3061N	0.22
LRP1B	SNV	R2075H	0.20
LRP1B	SNV	L1576I	0.17
LRP1B	SNV	R441Q	0.17
ERBB4	SNV	S303Y	0.21
ERBB4	SNV	R103C	0.19
CTNNB1	SNV	D299N	0.22
PBRM1	SNV	R710*	0.21
EPHA3	SNV	V195A	0.12
EPHA3	SNV	E249K	0.18
PIK3CB	SNV	R321Q	0.24
ATR	SNV	K1600E	0.06
PIK3CA	SNV	R88Q	0.21
ETV5	SNV	R30S	0.47
CPEB2	SNV	R1009C	0.24
KDR	SNV	Y938C	0.17
KDR	SNV	E361K	0.07
TET2	SNV	K780N	0.24
TET2	SNV	K1887N	0.20
FBXW7	SNV	K164*	0.19
TERT	SNV	L548P	0.24
TERT	SNV	R194Q	0.25
RICTOR	SNV	R1340I	0.20
RICTOR	SNV	R1130Q	0.22
APC	SNV	R1114*	0.22
APC	SNV	S1503*	0.21
APC	SNV	R2085I	0.19
APC	SNV	L2115*	0.10
RAD50	SNV	R807I	0.23
RAD50	SNV	M1053I	0.24
BRD2	SNV	E413D	0.25
ROS1	SNV	R1035*	0.21
ROS1	SNV	G140V	0.26
MAP3K4	SNV	K333N	0.05
HDAC9	SNV	P524S	0.13
HDAC9	SNV	R663Q	0.06
BRAF	SNV	I666M	0.13
EZH2	SNV	S410P	0.24
EZH2	SNV	R362Q	0.24
JAK2	SNV	X622_splice	0.17
RET	SNV	S65G	0.18
PTEN	SNV	F154L	0.22
TCF7L2	SNV	R471C	0.26
ATM	SNV	R1086C	0.14
ATM	SNV	D1246E	0.20
ATM	SNV	A2626D	0.21
ATM	SNV	L2680R	0.13
KMT2A	SNV	D251Y	0.22
KMT2A	SNV	R608Q	0.38
CBL	SNV	D458Y	0.16
ETV6	SNV	N382D	0.22
ARID2	SNV	R314C	0.25
ERBB3	SNV	R1077W	0.19
MDM2	SNV	K387Q	0.23
PTPN11	SNV	E541K	0.22
POLE	SNV	T1791I	0.19
POLE	SNV	V411L	0.21
[illegible]	[illegible]	[illegible]	[illegible]
BRCA2	SNV	K121T	0.21
BRCA2	SNV	S1242R	0.14
BRCA2	SNV	R2318*	0.06
RB1	SNV	F535C	0.16
NUTM1	SNV	S116L	0.24
MAP2K1	SNV	G61E	0.22
IGF1R	SNV	K194N	0.13
CREBBP	SNV	E664*	0.18
CBFB	SNV	E152K	0.20
CDH1	SNV	S829F	0.23
TP53	SNV	R213*	0.45
MAP2K4	SNV	X39_splice	0.11
MAP2K4	SNV	E203*	0.26
NF1	SNV	S302G	0.18
NF1	SNV	E1423*	0.21
NF1	SNV	R1769*	0.16
CDK12	SNV	S601Y	0.21
TOP2A	SNV	K1516N	0.19
TOP2A	SNV	E925*	0.25
BRCA1	SNV	R823I	0.19
SMAD4	SNV	V348A	0.15
NOTCH3	SNV	A564T	0.25
BRD4	SNV	H635R	0.24
ASXL1	SNV	D1265Y	0.23
ASXL1	SNV	E1383D	0.24
TOP1	SNV	M247V	0.20
ZNRF3	SNV	R245*	0.23
ZNRF3	SNV	C279R	0.23
AR	SNV	D696N	0.42
ATRX	SNV	E162D	0.33
BARD1	SNV	S241C	0.46
UBXN4-LRP1B	Rearrangement	Fusion	-

Microscopic morphology



Response to immunotherapy: no experience

Another *POLE*-mutant CRC with ultra-high mutation rate



M/56, *POLE* P286R, Tumor mutation burden: 151.6 mutations/Mb, *BRCA2* E2229* (VAF 0.11, heterozygous, estimated tumor purity ~22%)

BRCA mutations in tubular gastrointestinal tract

- Cbioportal data (colorectal and esophagogastric cancer N=6,815): Pathogenic or Likely pathogenic *BRCA1* (57, **0.8%**), *BRCA2* (185, **2.7%**) mutations

- AMC experience: 4/911 sequenced colorectal cancer (0.4%)
  - Probable germline or homozygous (N=3): colorectal cancer with pathogenic *BRCA2* K467* (VAF 0.65) mutation
  - In the settings of hypermutated tumor (N=1): heterozygous *BRCA2* E2229* mutation in *POLE*-mutant ultra-high mutated colorectal cancer

- Therapeutic implication:
  - PARPi approved for ovarian, prostate, breast, and pancreatic cancer
  - Role of other tumor types: not established

BRCA mutations, zygosity, and responsiveness to PARPi

LETTER

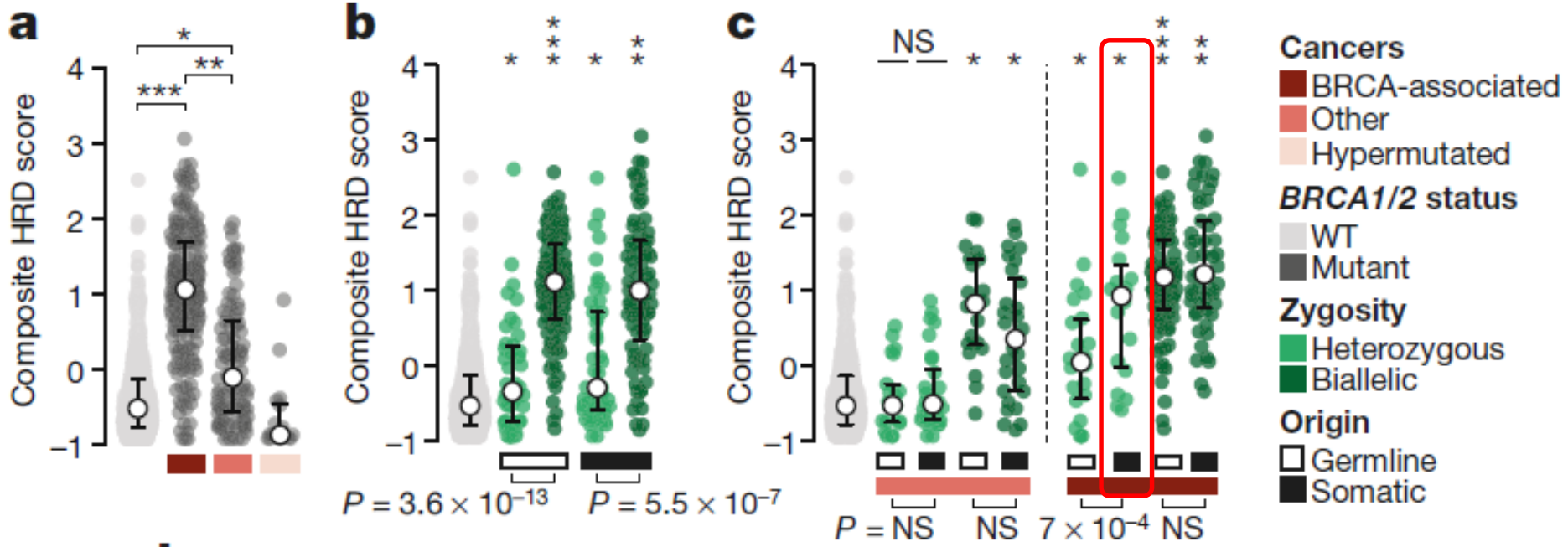
https://doi.org/10.1038/s41586-019-1382-1

Tumour lineage shapes BRCA-mediated phenotypes

Philip Jonsson[1,2,3], Chaitanya Bandlamudi[1], Michael L. Cheng[4,7], Preethi Srinivasan[5], Shweta S. Chavan[1], Noah D. Friedman[2,3], Ezra Y. Rosen[4], Allison L. Richards[1], Nancy Bouvier[1], S. Duygu Selcuklu[1], Craig M. Bielski[1,2,3], Wassim Abida[4], Diana Mandelker[5], Ozge Birsoy[5], Liying Zhang[5], Ahmet Zehir[5], Mark T. A. Donoghue[1], José Baselga[4,8], Kenneth Offit[4], Howard I. Scher[4], Eileen M. O'Reilly[4], Zsofia K. Stadler[4], Nikolaus Schultz[1,3], Nicholas D. Socci[1], Agnes Viale[1], Marc Ladanyi[2,5], Mark E. Robson[4], David M. Hyman[4,6], Michael F. Berger[1,5,6*], David B. Solit[1,2,4,6*] & Barry S. Taylor[1,2,3,6*]

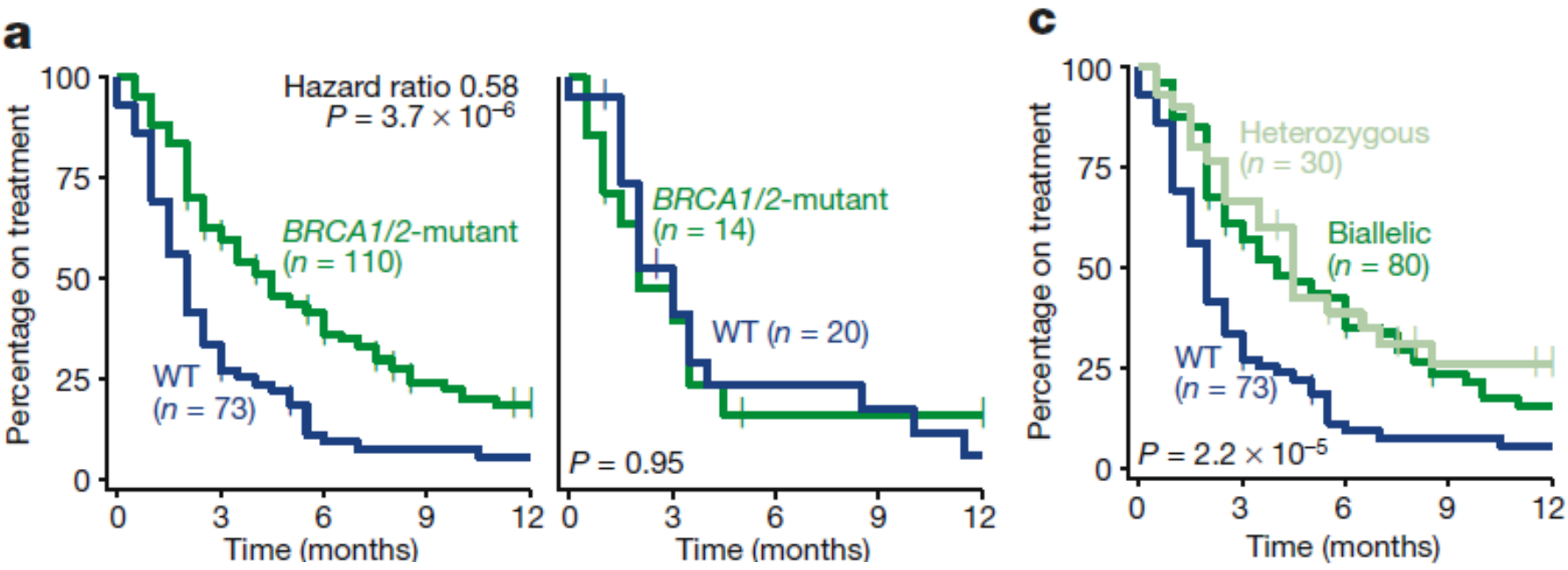
(*Nature* 2019; 571:576-583)

BRCA-associated cancer types and pathogenic *BRCA* mutation zygosity



- BRCA mutation leads to high HRD scores (genomic signature of HRD) in BRCA-associated tumor types
- BRCA mutations do not have any phenotypic relevance in hypermutated tumors
- Zygosity matters: Biallelic mutations have phenotypic relevance.
- Significance of BRCA mutations differs depending on tumor types.

Progression free survivals on PARPi



a. (Right) *BRCA*-associated cancer types (Left) Others (Right)
c. *BRCA*-associated cancer types

Predicted clinical significance of *BRCA* mutations

- In *BRCA*-associated tumor types (**ovary, breast, prostate, pancreas**): HRD phenotype and responsiveness to PARPi **regardless of germline/somatic and zygosity**

- In *BRCA*-unrelated tumor types: HRD phenotype and responsiveness to PARPi is expected **only in the context of biallelic mutations**

- Mutations in other HRD-related genes (*RAD51C, PALB2, ATM, BRIP1,* etc.): unknown at this time, probably actionable in *BRCA*-associated tumor types

Summary

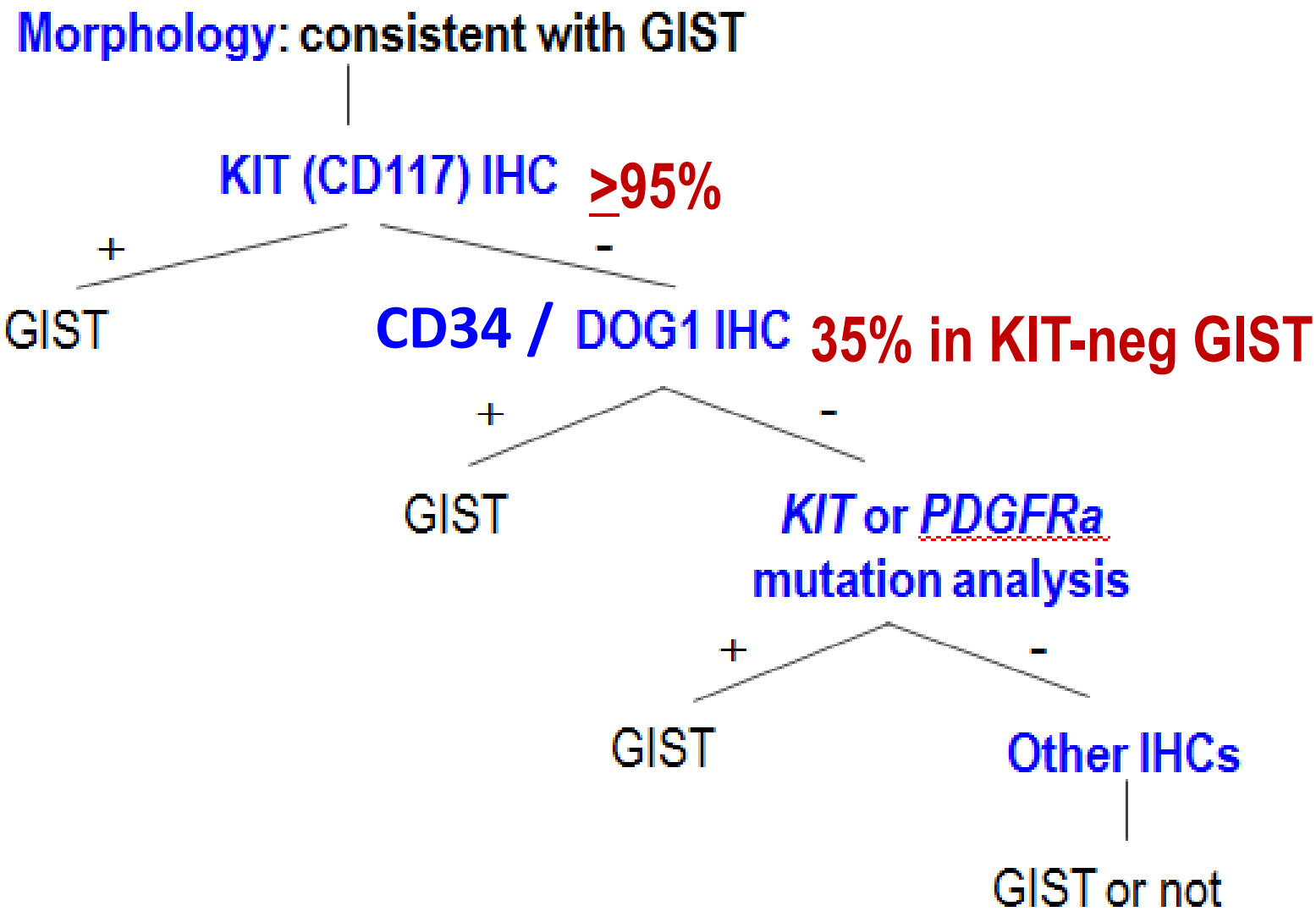
- Utility of clinical NGS in colorectal cancer:
  - **Extended *RAS*** testing: insensitivity to anti-EGFR Tx, includes activating mutations other than traditional hotspots (codon 33, 59, 117)
  - ***BRAF* V600E** mutation: prognostic implication
  - **Microsatellite instability**: immune checkpoint blockade
  - Homologous recombination defect: biallelic **pathogenic *BRCA1/2*** mutations
  - Rare druggable alterations: ***NTRK* fusions**
  - Genetic counseling: suspicious germline mutations in *MMR genes*

- Emerging biomarkers: *ERBB2* amplifications (insensitivity to anti-EGFR Tx, ERBB2-directed trials), Hotspot *POLE* mutations with ultra-high mutator phenotypes (Immunotherapy?)

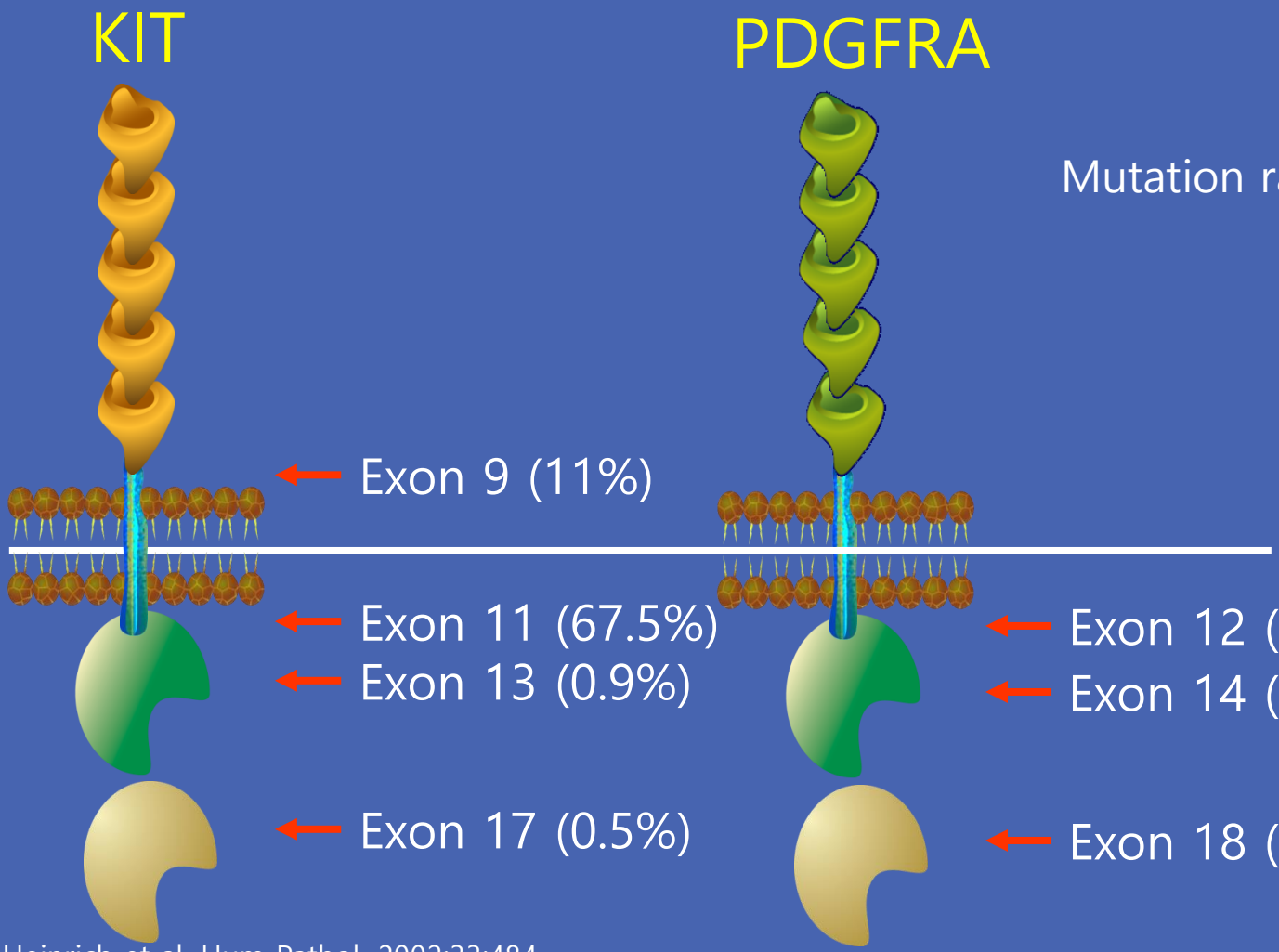
- Future biomarkers of interest: *PIK3CA* hotspot mutations, oncogenic *ERBB2* mutations, non-*V600* BRAF mutations, NGS with liquid biopsy samples (genomic tracking, finding resistant mutations)

Molecular pathology of gastrointestinal mesenchymal tumor

Diagnosis of GIST



KIT and PDGFRA Mutations



Heinrich et al. Hum Pathol. 2002;33:484.
Corless et al. Proc Am Assoc Cancer Res. 2003;44. Abstract R4447.

KIT / PDGFRA mutation

- *KIT* exon 9, 11 mutations:
  - **Exon 11: most sensitive to Imatinib (400mg per day)**
  - Exon 9: less sensitive to Imatinib (800mg per day)

- *KIT* exon 13, 14, 17 mutations (V654A, D820E, N822K, etc.):
  - Secondary *KIT* mutatons in Imatinib-resistant GIST

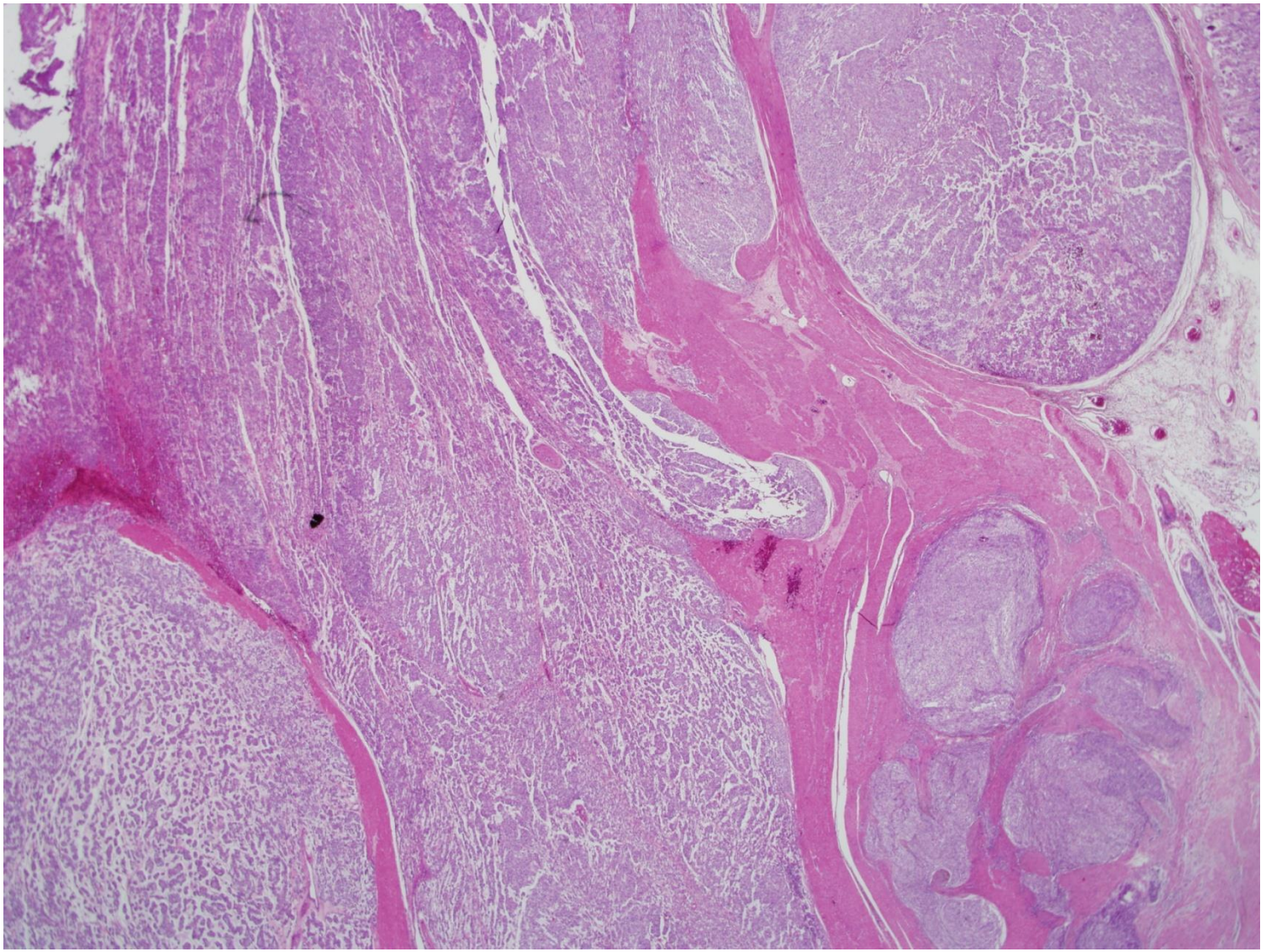
- *PDGFRA* mutation:
  - Diagnostic: confirm GISTs that are negative for KIT or DOG-1 IHC.
  - Predictive: D842V (primary resistance to Imatinib), several Imatinib-sensitive mutations

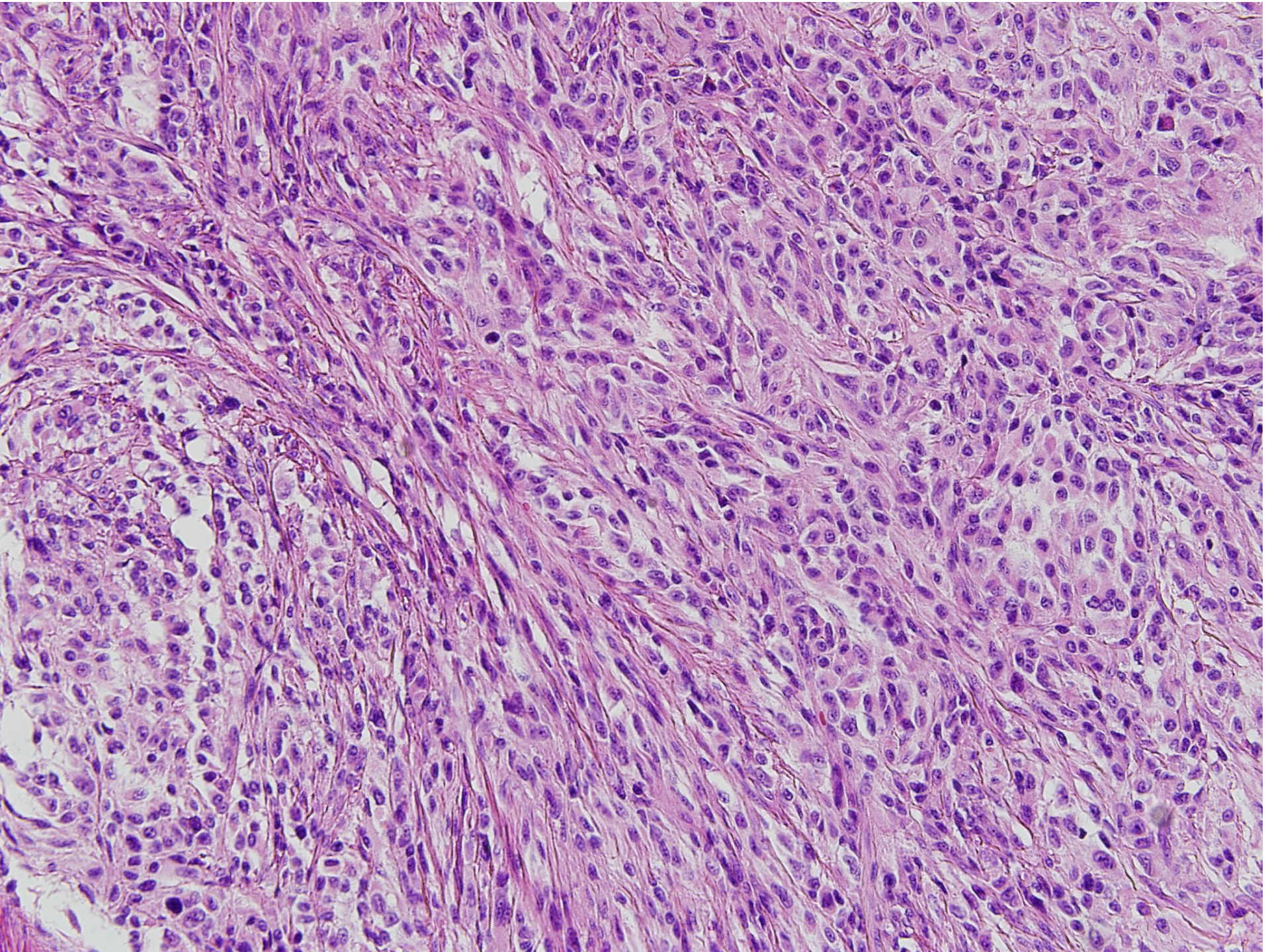
Diagnosis of KIT/PDGFRA-negative GISTs

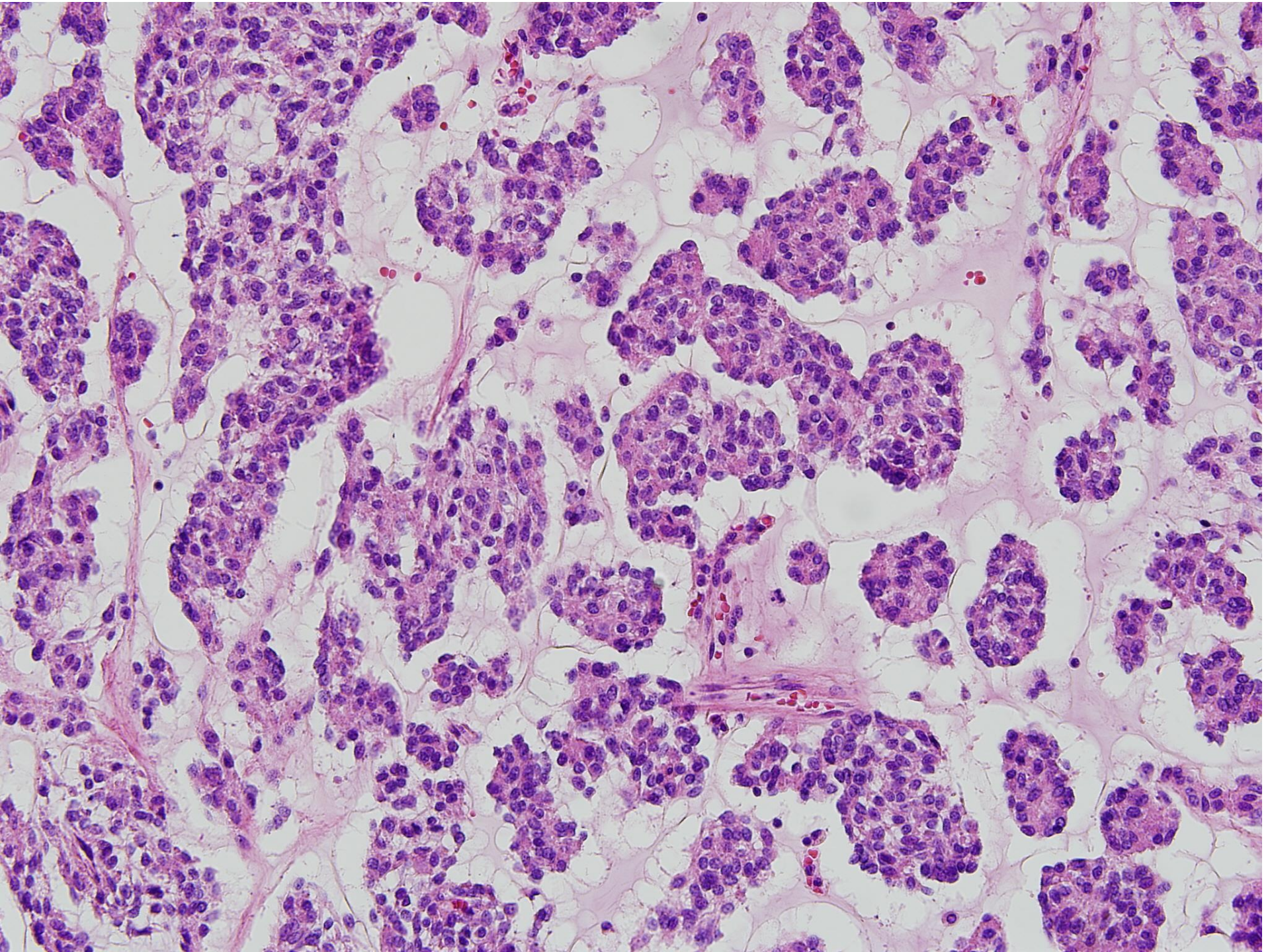
- SDH-deficient GIST:
  - Dx: **immunohistochemistry for SDHB**
  - Female, Young, Multinodular, Epithelioid, Lymph node metastasis, Indolent
  - Carney-Stratakis syndrome: multiple GISTs and paragangliomas, germline *SDHB, SDHC, or SDHD* mutations

- Other rare mutations found in GISTs:
  - *BRAF* V600E: rarely found in NGS test
  - *NF1* loss of function mutations: multiple GI masses, sometimes associated with neurofibromatosis, type 1

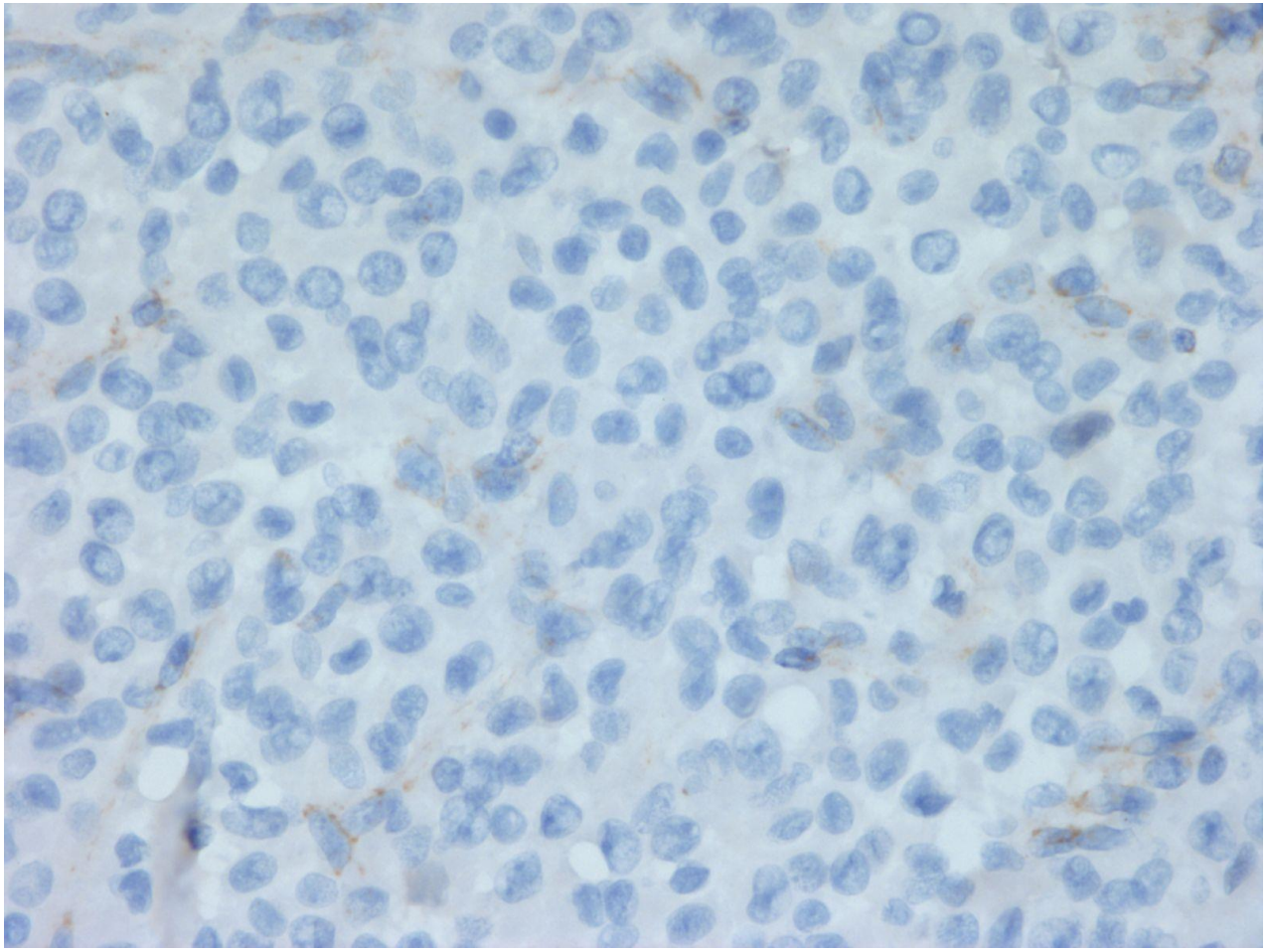
F/19, gastric mass



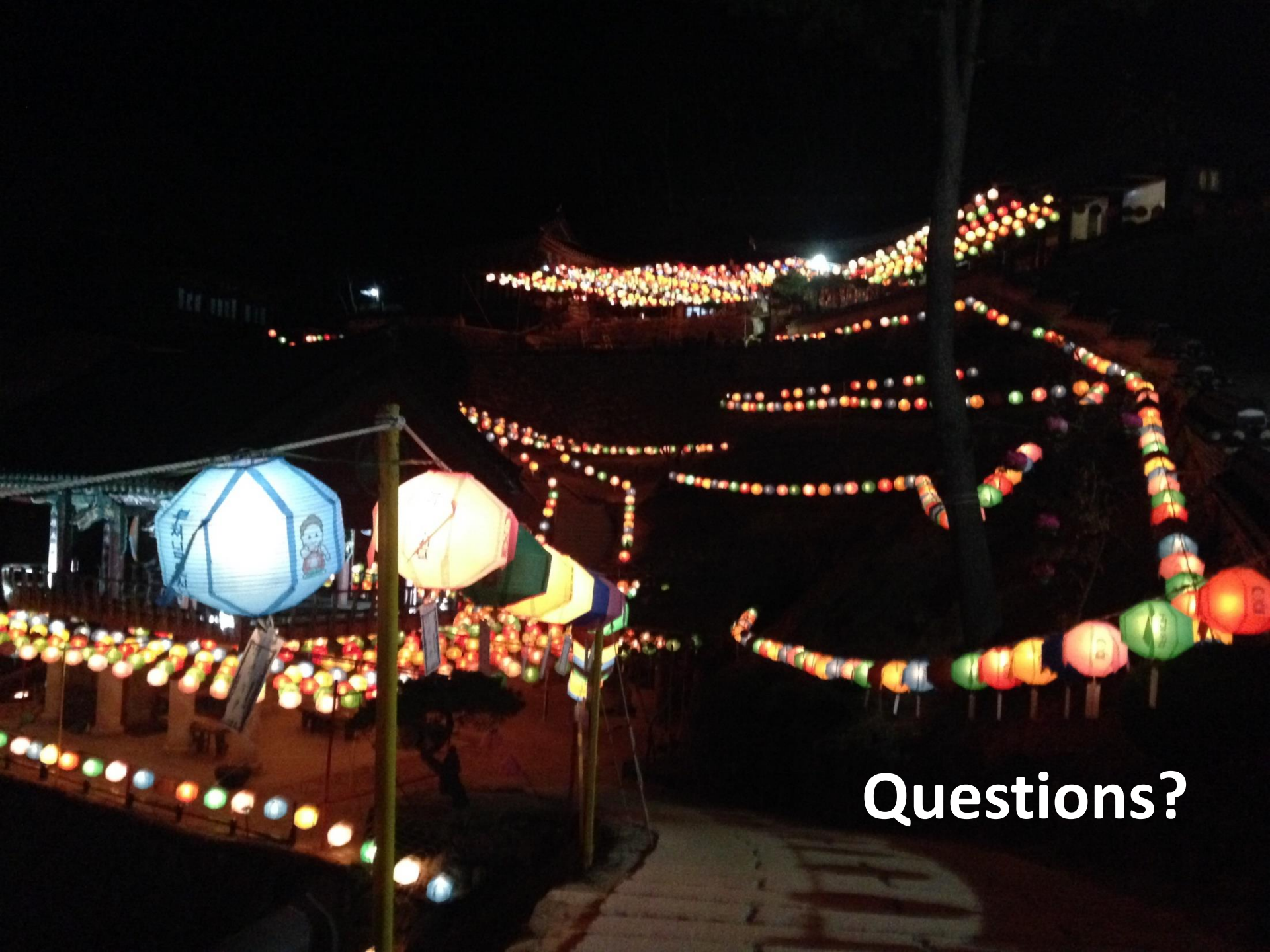




SDHB immunohistochemistry



NGS: *SDHA* D125N mutation (VUS until now: probably activating driver mutation)


Questions?