Australasian Gastrointestinal Pathology Society
4th Annual Scientific Meeting
27 & 28 October 2018
Clinical Education Centre, Auckland Hospital, New Zealand
www.agps.org.nz

ANCILLARY STUDIES IN THE UPPER GIT NEOPLASMS

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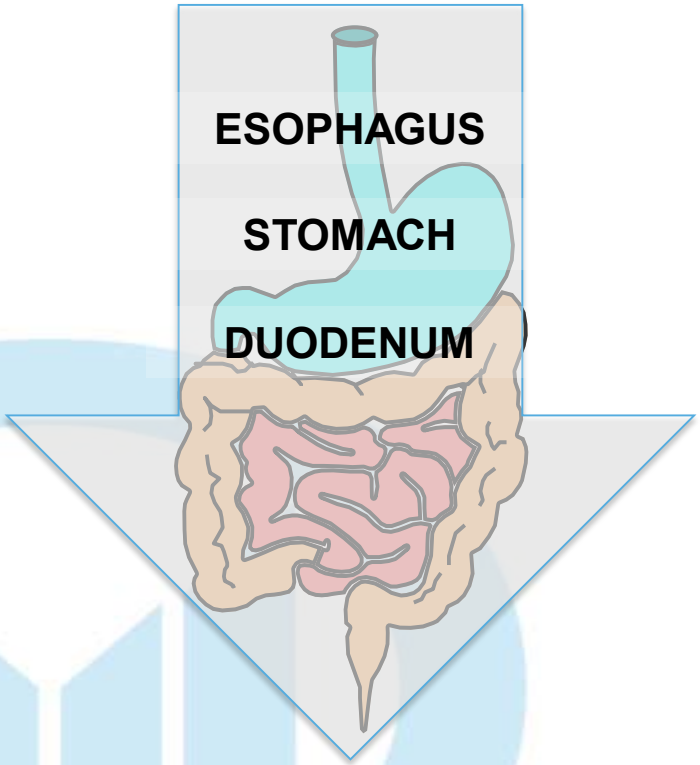
ANCILLARY STUDIES IN THE UPPER GIT NEOPLASMS

OUTLINE



- DIAGNOSIS
- CLASSIFICATION
- PROGNOSIS
- GUIDE TO THERAPY

MOFFITT CANCER CENTER

DIAGNOSIS	CLASSIFICATION	PROGNOSIS	GUIDE TO THERAPY
P53	MUC stains	EBV	HER2-NEU
CK19	CDX2	MMR	PDL-1
KI-67	KI-67		EBV
[IMP-3]	CDH1		MMR
[MUC2]	MMR		
[CDX2]			

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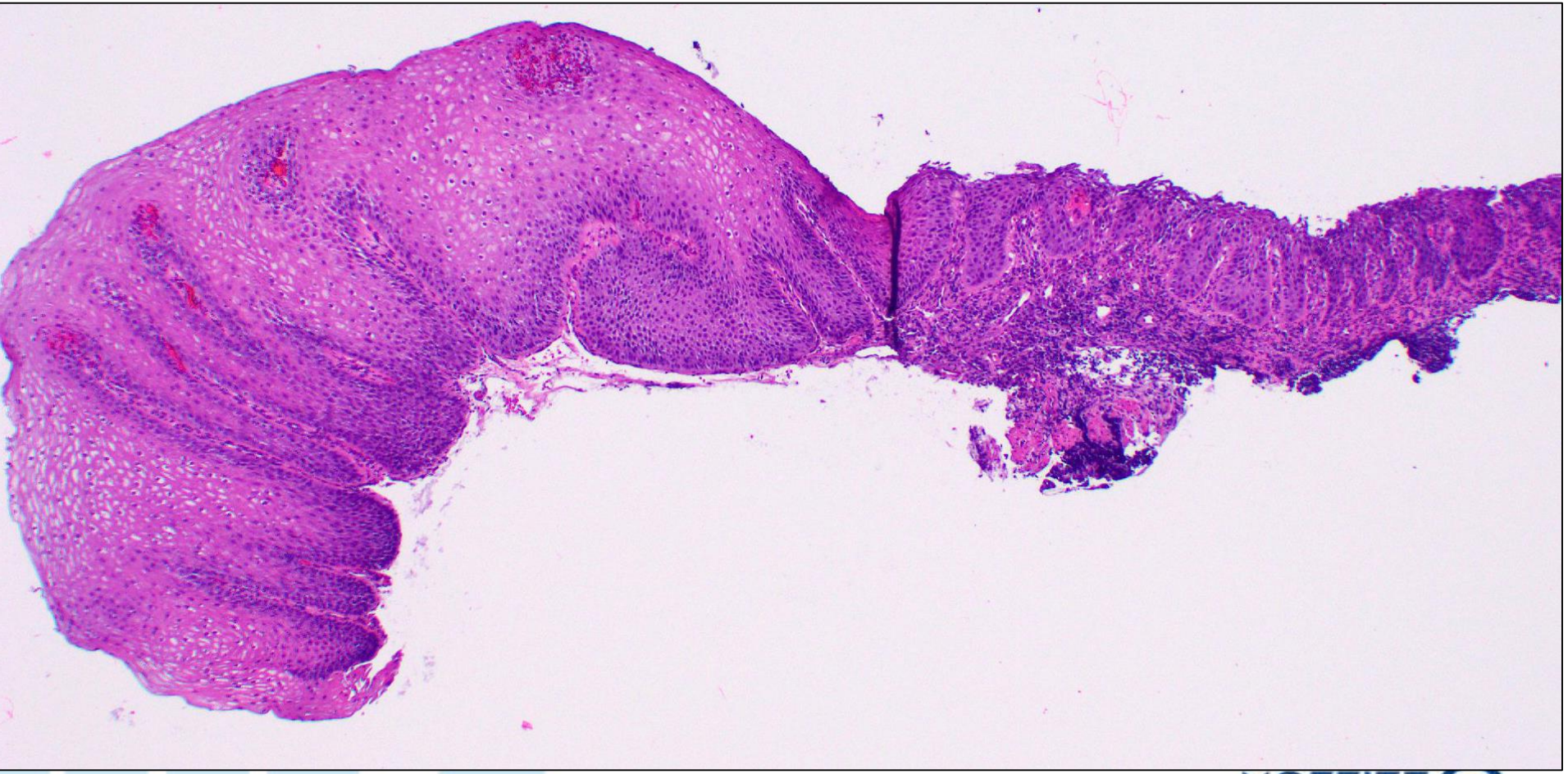
ESOPHAGUS - SCC/DYSPLASIA

- DIAGNOSIS
  - P53
  - Ki-67
  - CK19



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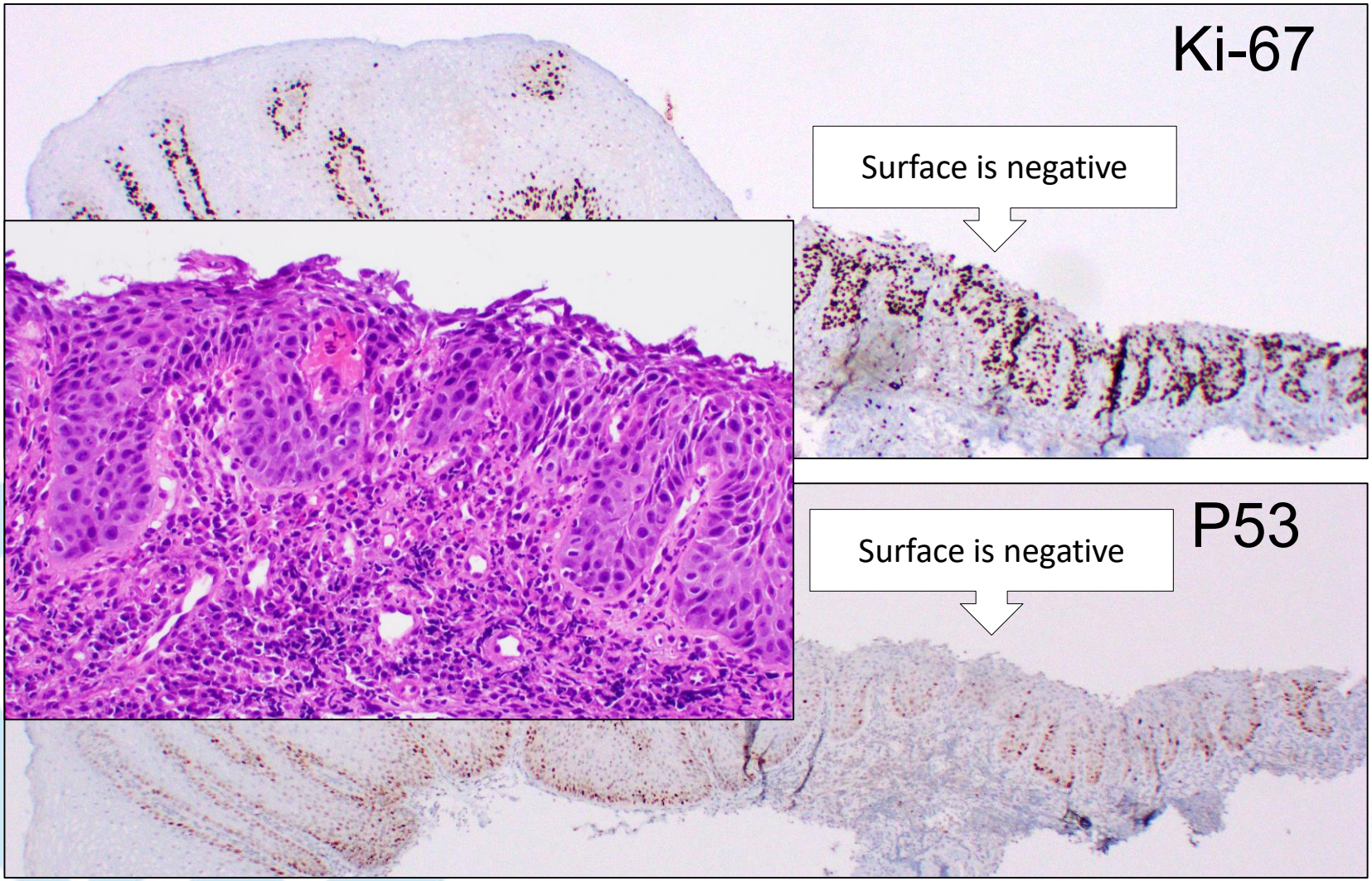
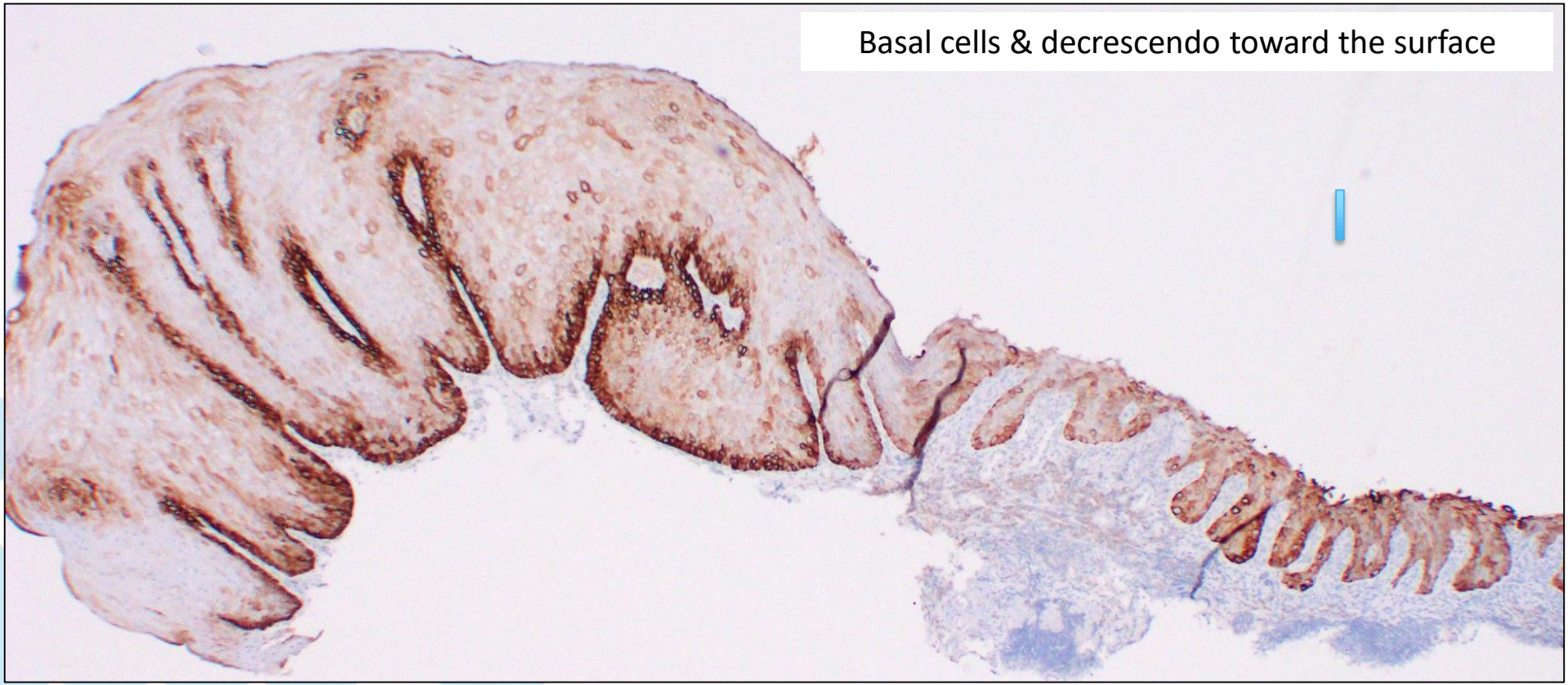

Ki-67
Surface is negative
P53
Surface is negative

CK19



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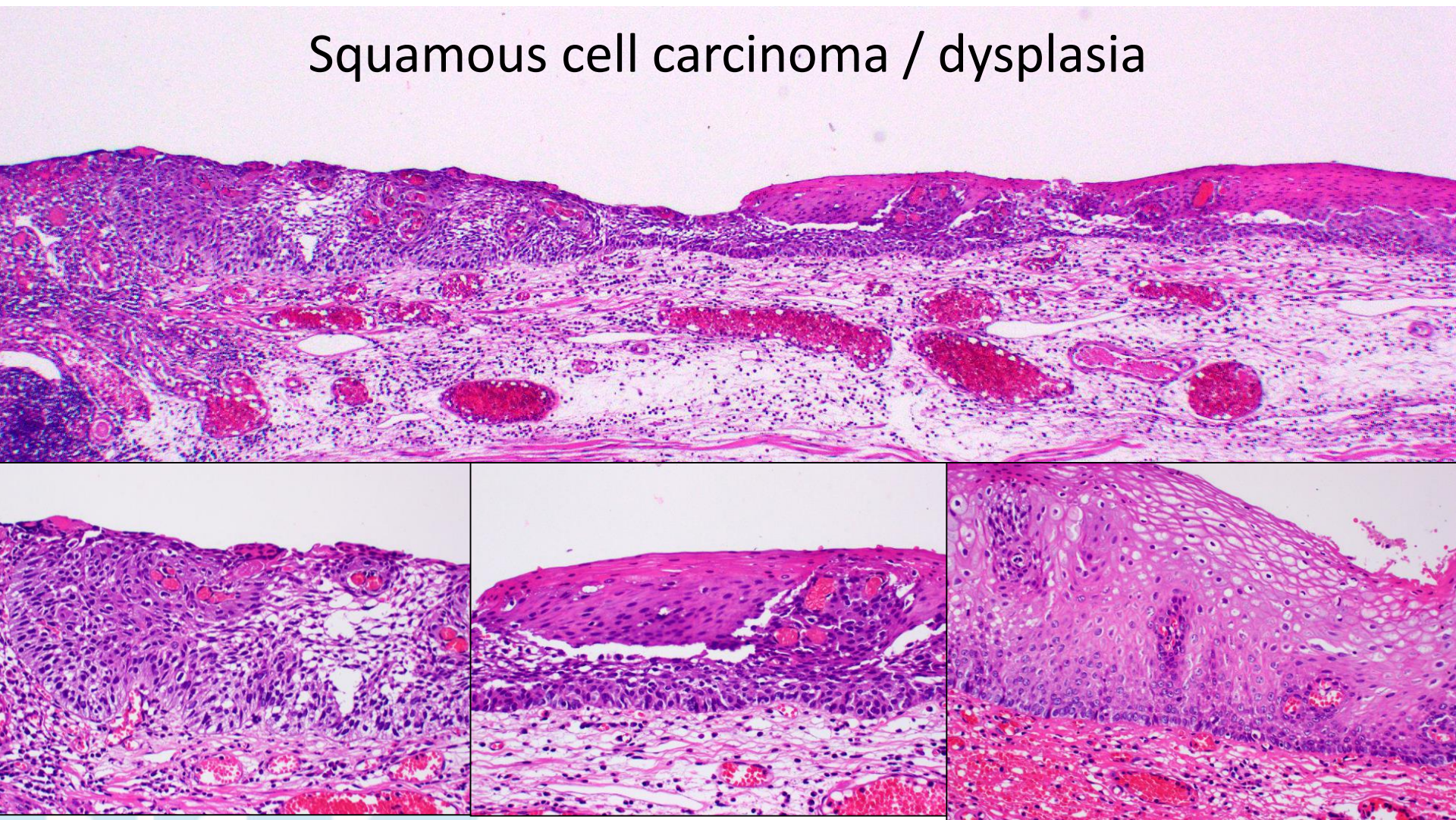
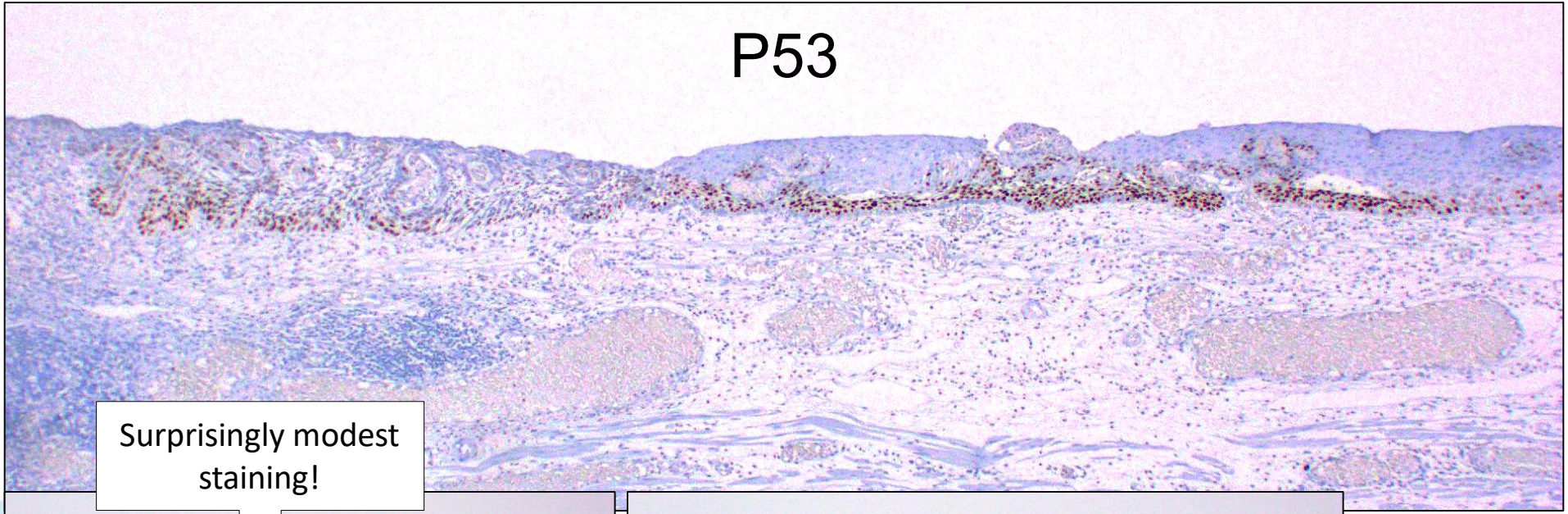
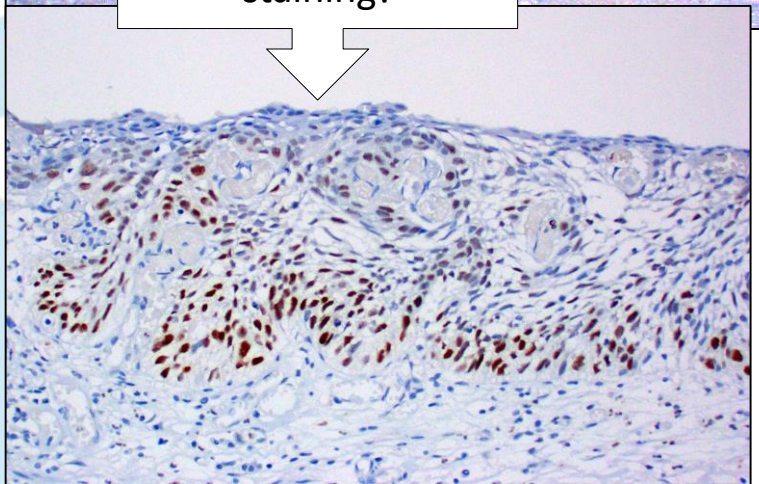
Squamous cell carcinoma / dysplasia

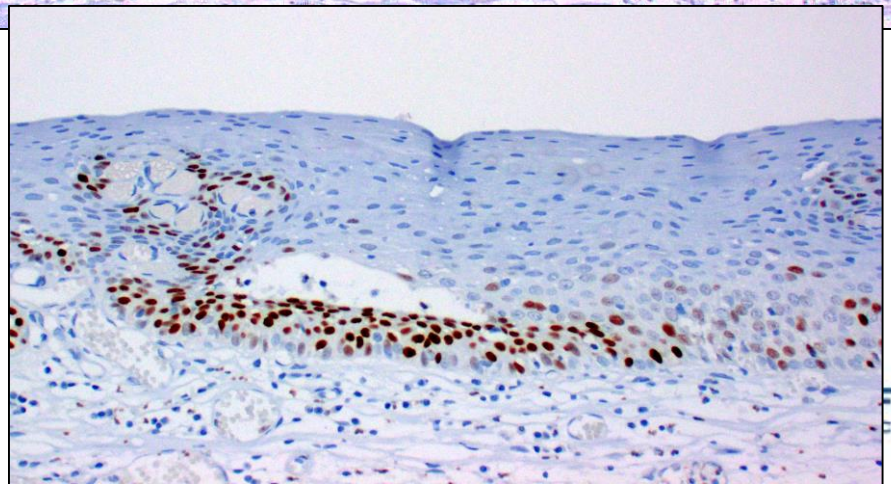


P53

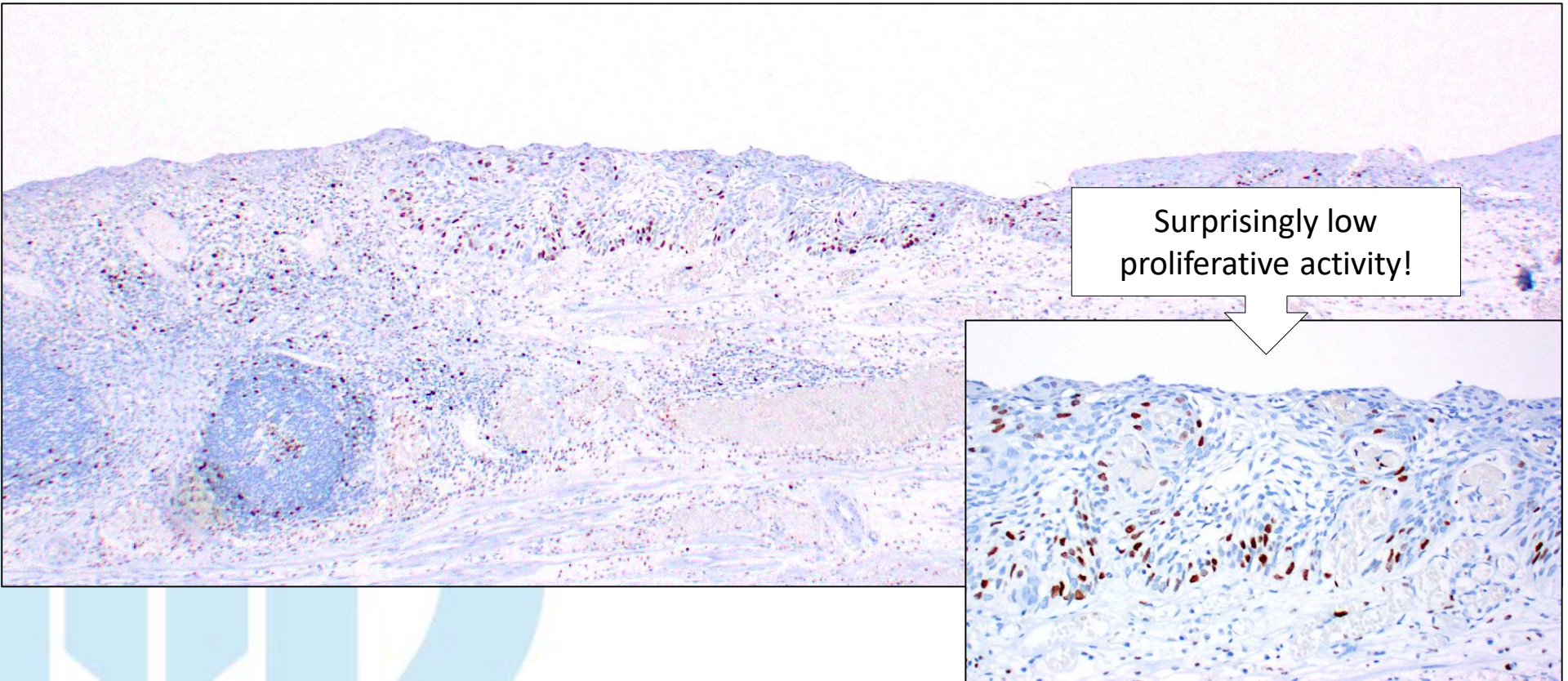


Surprisingly modest staining!



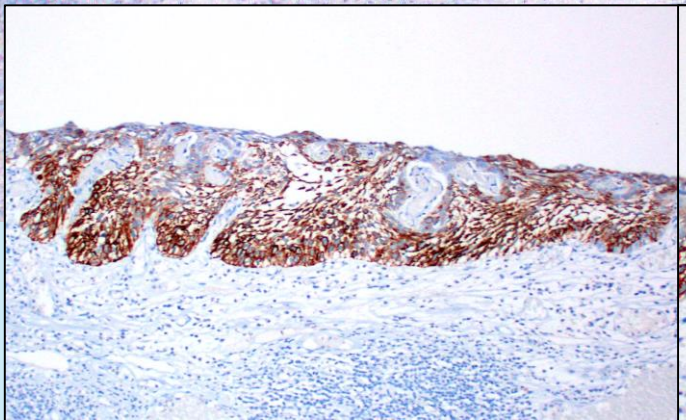


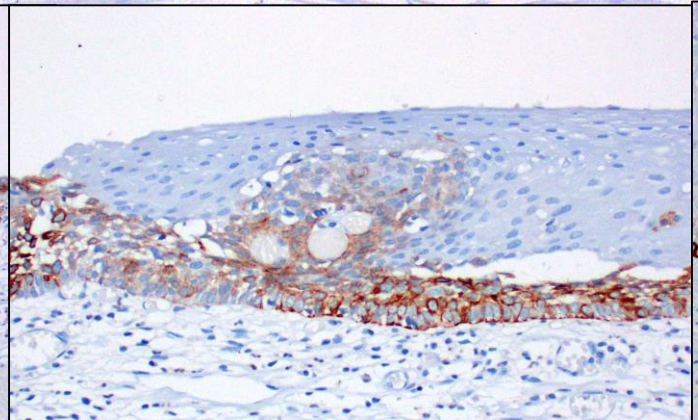
Ki-67

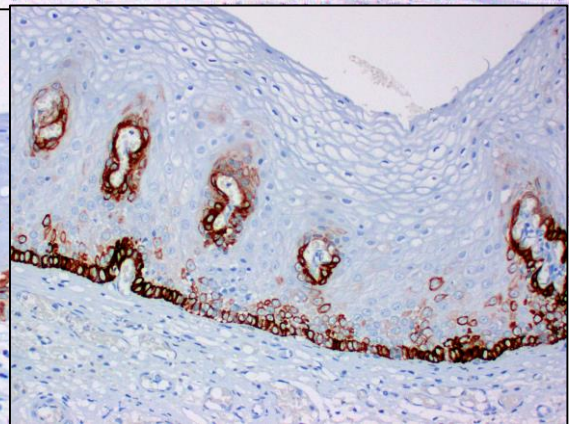


CK19

The intensity of CK19 expression increases w/ higher grade. ~70% of low-grade SCCs stain in less <50% of cells, whereas almost all high-grade SCC are diffusely & strongly reactive.

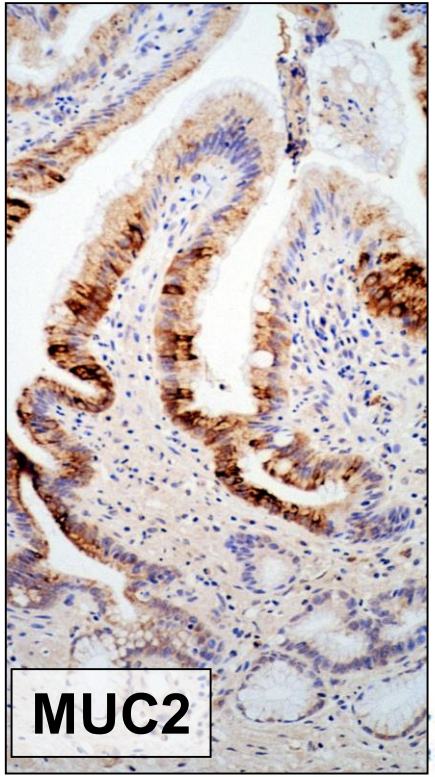






BARRETT ESOPHAGUS + DYSPLASIA

- DIAGNOSIS of BE
  - Alcian blue / MUC2 / CDX2
- DIAGNOSIS of DYSPLASIA
  - P53 / [IMP3]
- IMMUNOPHENOTYPE
  - MUCs
- PROGNOSIS
  - P53
- GUIDE TO THERAPY
  - Her2 neu



P53 Immunohistochemistry

- Use in the diagnosis of dysplasia
- Use in predicting disease progression

The Use of Ancillary Stains in the Diagnosis of Barrett Esophagus and Barrett Esophagus–associated Dysplasia

Recommendations From the Rodger C. Haggitt Gastrointestinal Pathology Society

Amitabh Srivastava, MD, Henry Appelman, MD,† Jeffrey D. Goldsmith, MD,‡ Jon M. Davison, MD,§ John Hart, MD,‖ and Alyssa M. Krasinskas, MD¶*

Despite the current widespread use of p53 IHC, we believe that additional studies are needed to develop and validate precise criteria before p53 staining can be fully endorsed and incorporated into the morphologic dysplasia diagnosis algorithm.

Additional studies are needed to determine the best definition of "abnormal" p53 staining and to show how integrating p53 testing into routine practice could improve patient care.

AJSP 2017;41:e8-e21

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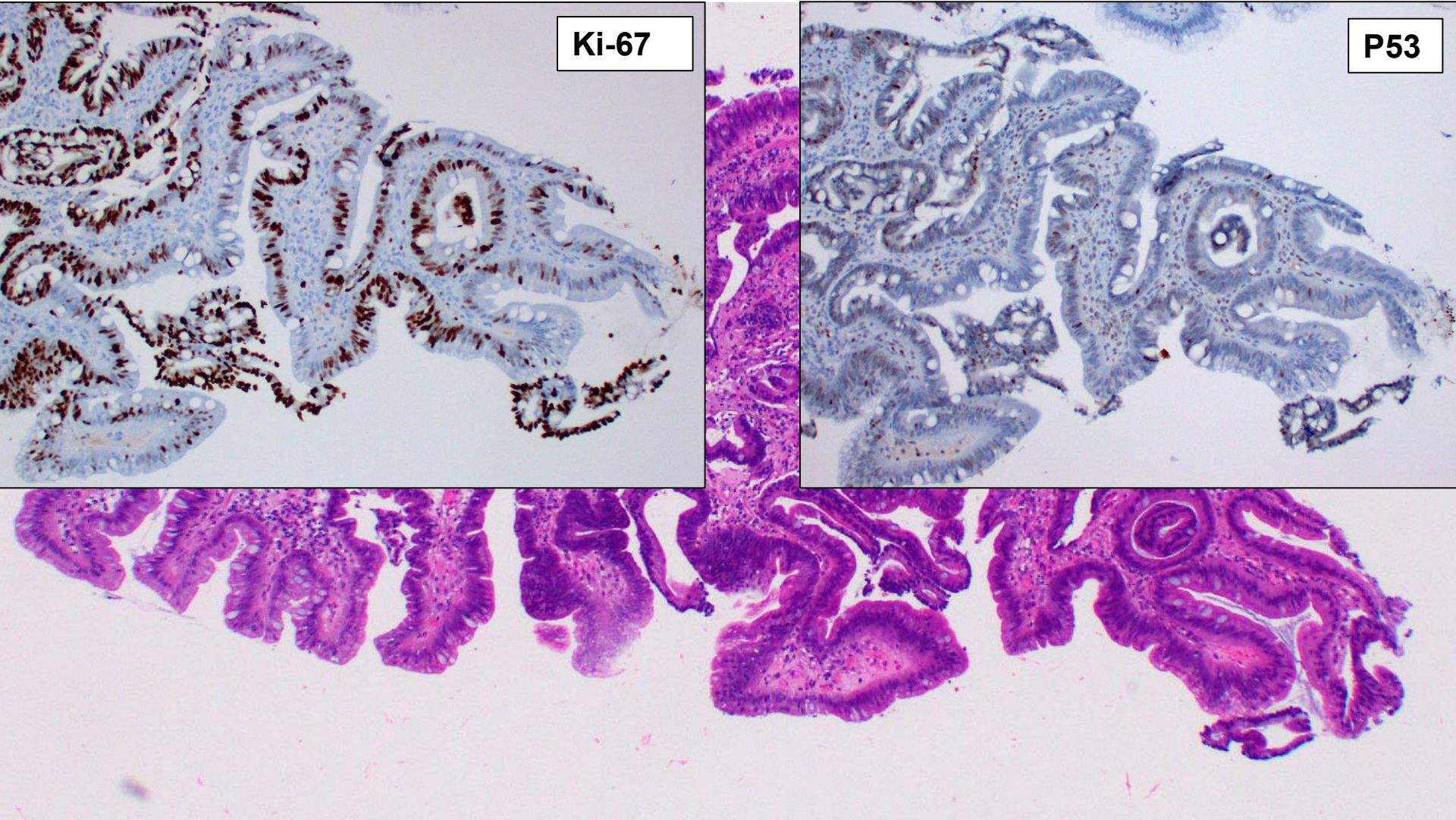
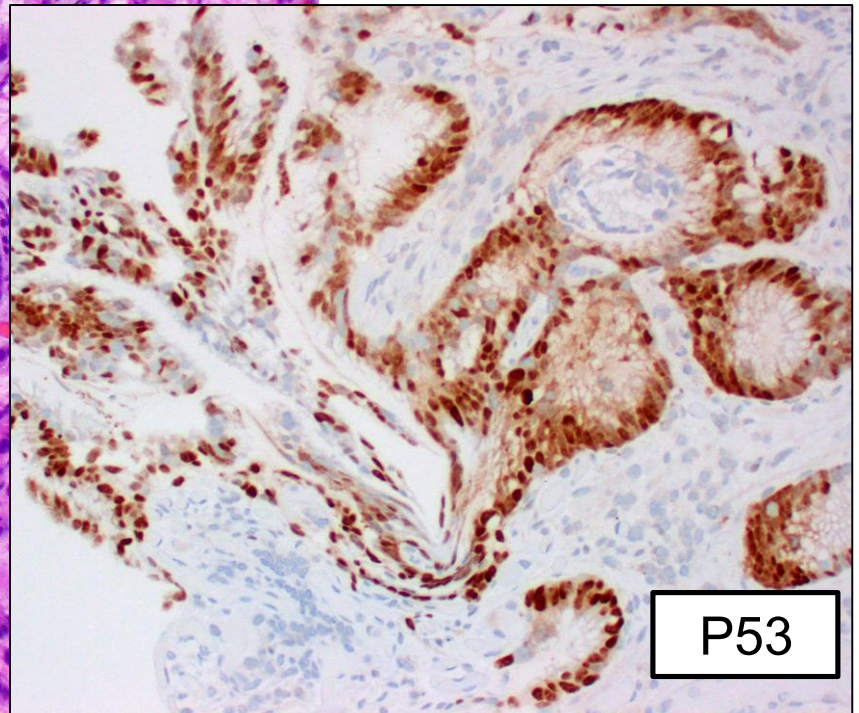

Ki-67
P53

FOVEOLAR DYSPLASIA



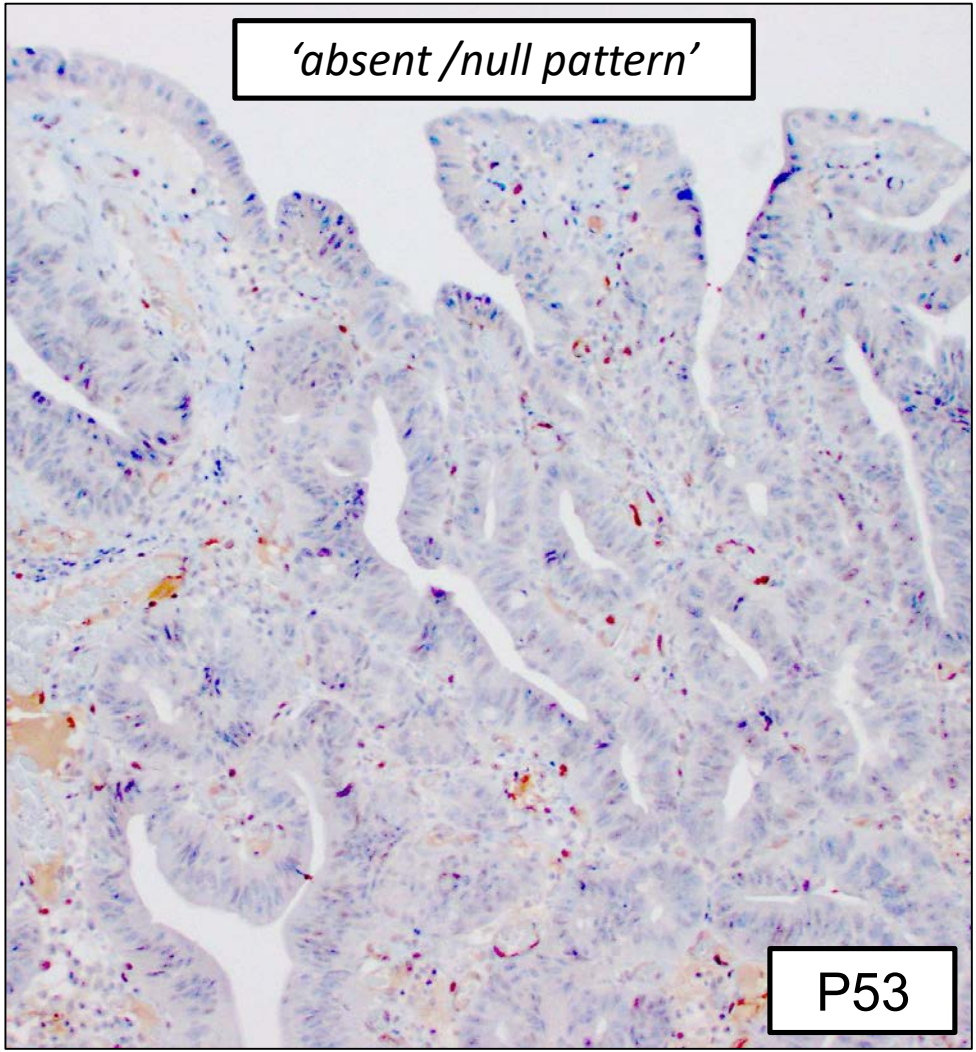
Histopathology 2010, 57, 933–940

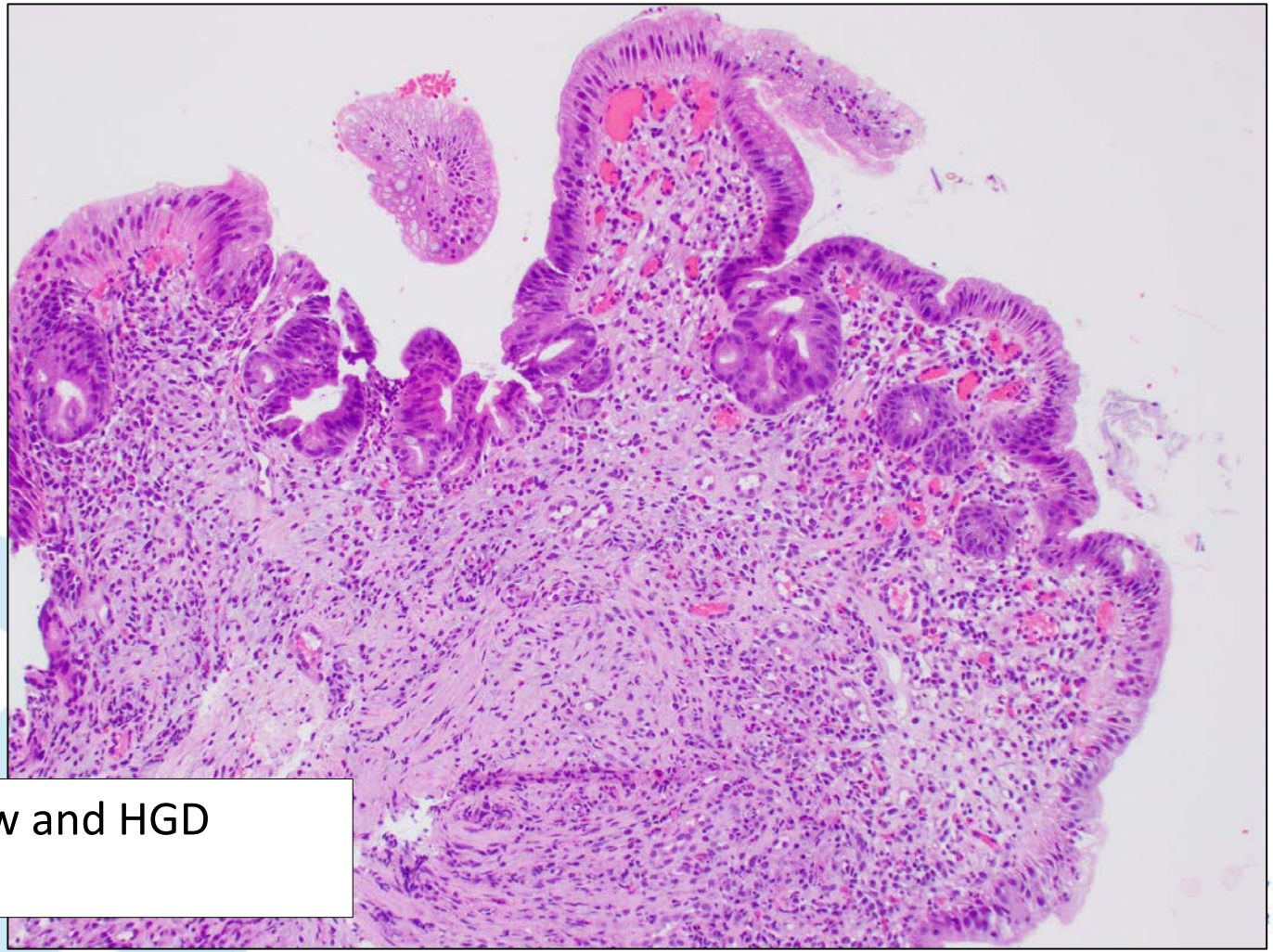
Correspondence

Novel staining pattern of p53 in Barrett's dysplasia – the absent pattern

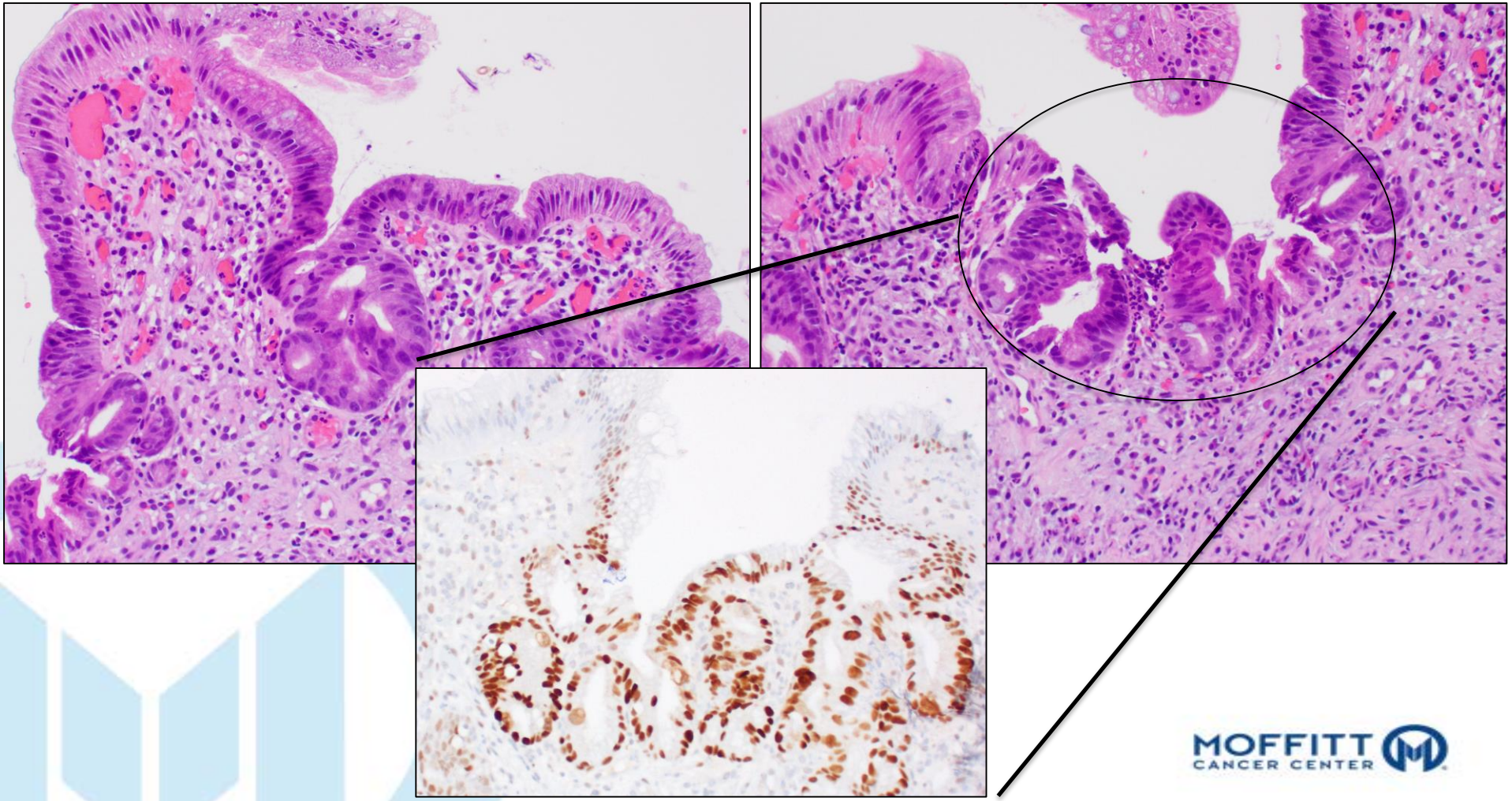
DOI: 10.1111/j.1365-2559.2010.03715.x

Sir: Barrett's oesophagus (BO) is conversion of oesophageal squamous mucosa to a glandular phenotype, and is a consequence of gastro-oesophageal reflux. This is a precursor to oesophageal adenocarcinoma (OA), which is rising rapidly in western countries and carries a poor prognosis.[1] This pathway is characterized by intestinal metaplasia and increasing grades of dysplasia before cancer supervenes. Recognizing dysplasia early allows close monitoring as well as treatment preventing OA or cure at an early stage. The recog'nition of dysplasia by pathologists is critical, and while pathologists can recognize dysplasia reproducibly[2,3] this may sometimes be difficult. Therefore additional prognostic markers would be helpful.





Male h/o of low and HGD
Treated by RFA



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Histopathology

Histopathology 2016, **69**, 431–440. DOI: 10.1111/his.12956

Dysplasia in Barrett's oesophagus: p53 immunostaining is more reproducible than haematoxylin and eosin diagnosis and improves overall reliability, while grading is poorly reproducible

Philip V Kaye,[1] Mohammad Ilyas,[1] Irshad Soomro,[1] Syeda A Haider,[1] Gurprit Atwal,[2]

Histopathology

Histopathology 2018, 72, 1015–1023. DOI: 10.1111/his.13462

Improved diagnostic stratification of digitised Barrett's oesophagus biopsies by p53 immunohistochemical staining

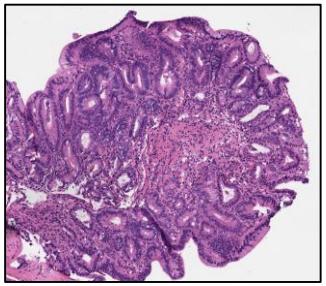
Myrtle J van der Wel,[1,2] Lucas C Duits,[2] Roos E Pouw,[2] Cornelis A Seldenrijk,[3]

Histopathology

Histopathology. 2017 Jul;71(1):27-33. doi: 10.1111/his.13193.

p53 protein accumulation predicts malignant progression in Barrett's metaplasia: a prospective study of 275 patients

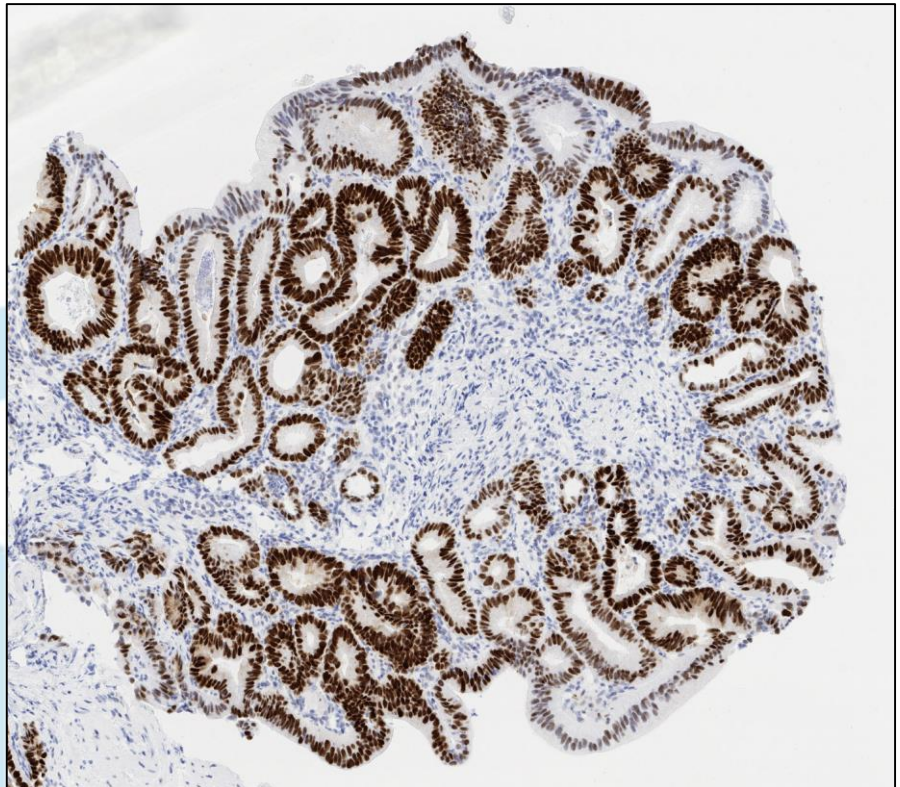
Mamoun Younes, Keith Brown, Gregory Y Lauwers, Gulchin Ergun, Frank Meriano, A Carl Schmulen, Alberto Barroso, Atilla Ertan

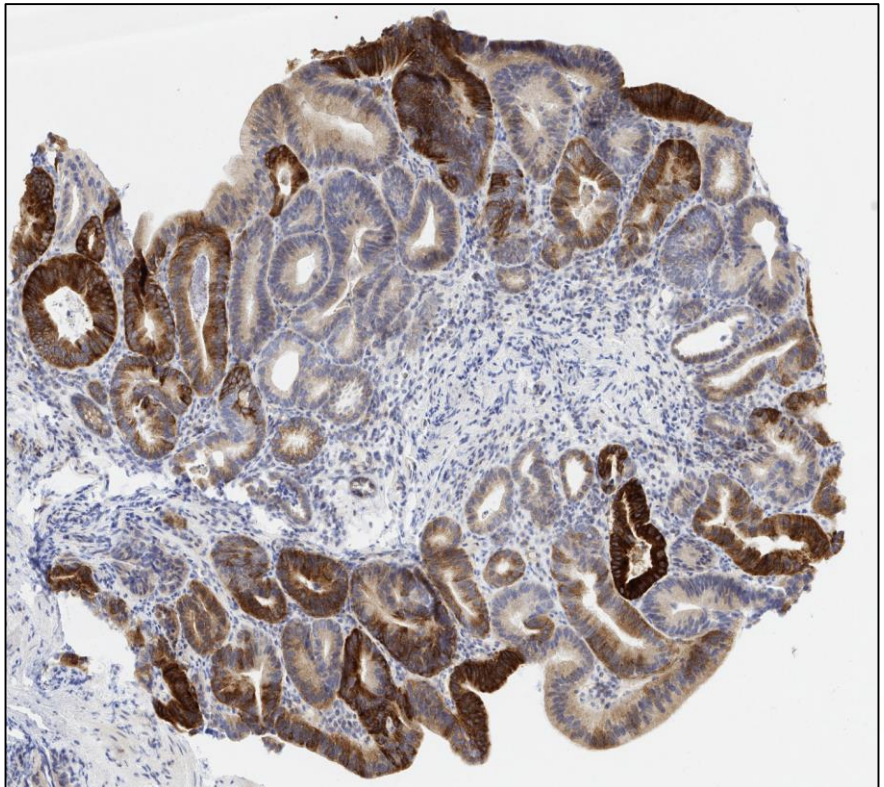


IMP3 Immunoreactivity is More Sensitive Than AMACR in Detecting Dysplastic Epithelium and Early Adenocarcinoma in Barrett Esophagus

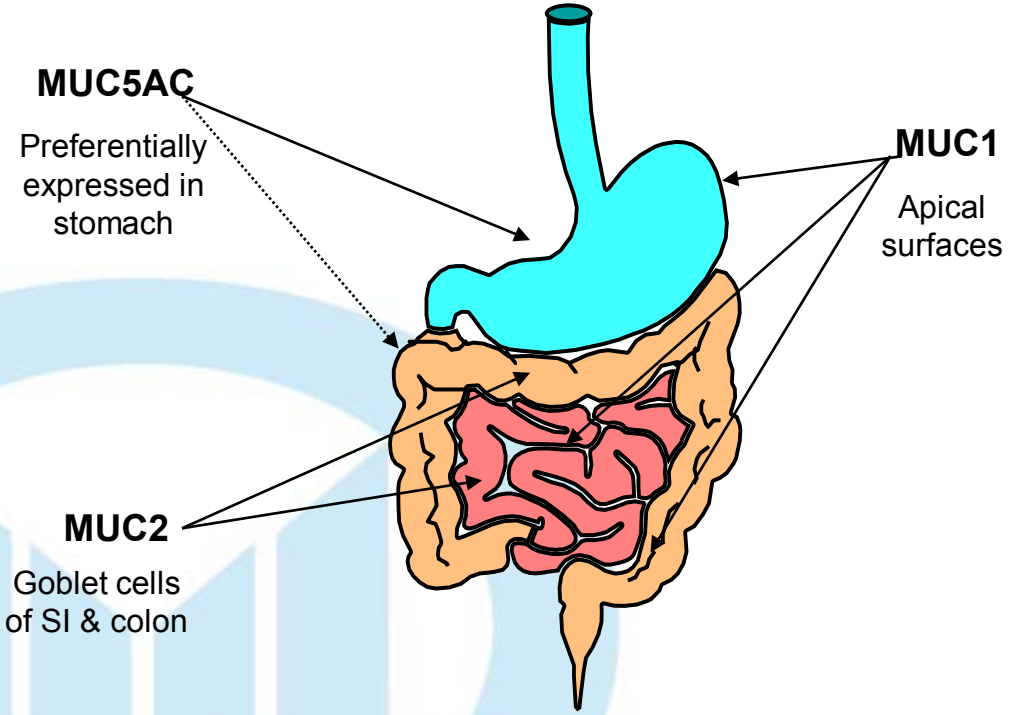
Manoj R. Gadara, MD, Maria Gonzalez, MD, Richard W. Cartun, PhD, and Saverio Ligato, MD

AIMM; 2017:6





MUC Expression Upper GI Neoplasms



- Maybe similar to normal tissues or altered.
- Relative tissue specificity used to discriminate between CAs of various sites
- *? Role in pathogenesis & prognosis?*

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MUC Analysis

Markers of differentiation:

- Classification of neoplasms

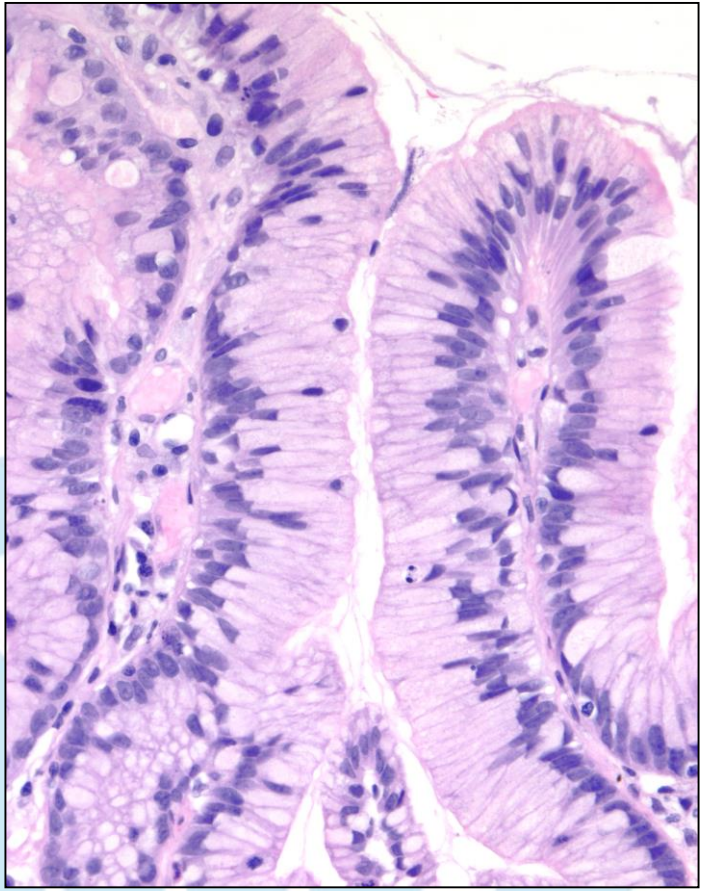
Biomarkers of cancer risk:

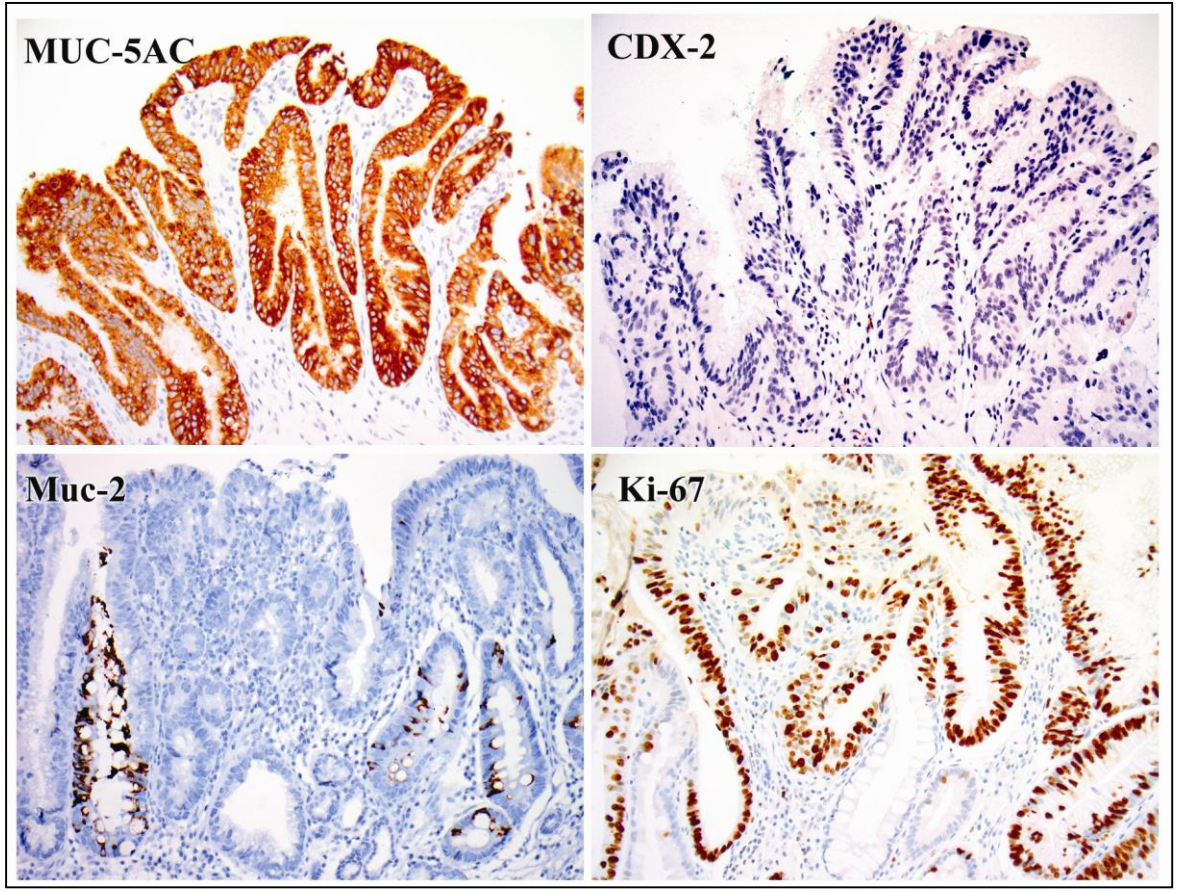
- Precancerous lesions

Prognosis:

- To be evaluated in BE neoplasms
- Likely in stomach
- Yes, in Ampullary CA

BE-foveolar dysplasia





BE-Foveolar dysplasia

- **Prevalence:46%.** (41 resections w/ dysplasia w or w/o associated inv. ACA)
  - HGD:58% -- Adjacent IM: 53%
- **Adenomatous (27%) & hybrid types (27%) of cases**
  - HGD in 91% / 100% of the cases
  - Adjacent IM: 100% / 82% (p<0.0001).

Brown IS . Mod Pathol 2010

Dysplasia	Association with		Progression to cancer
	Conventional LGD	Conventional HGD	
Conventional LGD (N=22)			1(5%)
Conventional HGD (N=16)			12(75%)
Foveolar Dysplasia (N=17)	4(24%)	13(76%)	8(47%)
Serrated Dysplasia (N=6)	3(50%)	3(50%)	3(50%)

Srivastava et al, USCAP 2010

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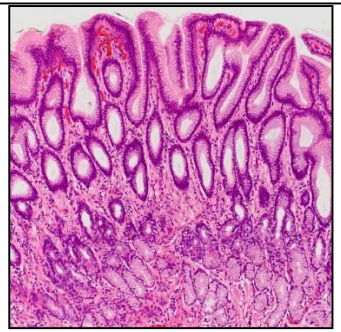
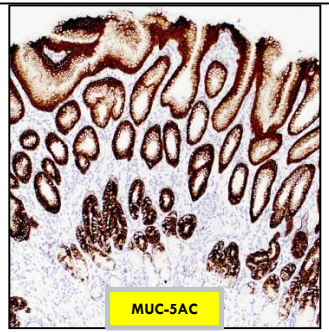
GASTRIC NEOPLASIA

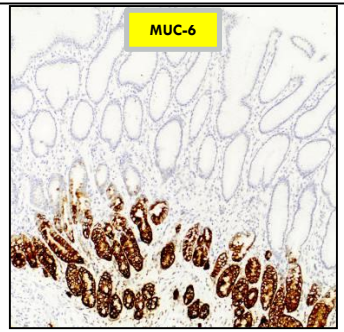
- DIAGNOSIS
  - MUC stains
- IMMUNOPHENOTYPE
  - MUC stains
- PROGNOSIS
  - MUC stains (?)
  - HER2-neu but also, CDH1, MMR and EBV
- GUIDE TO THERAPY
  - HER2-neu, MMR and EBV

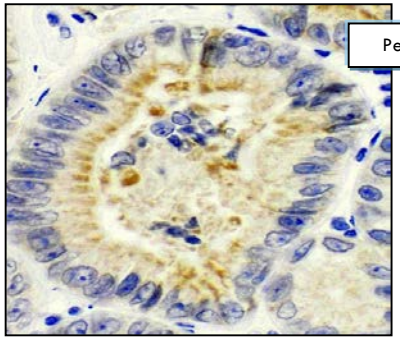
MOFFITT CANCER CENTER

Cell Type-Specific Expression of Muc-5AC & Muc-6



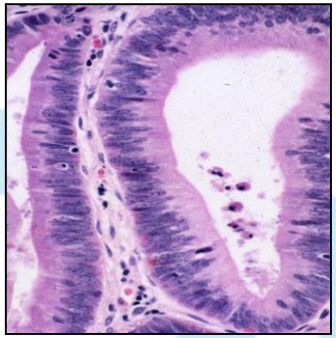




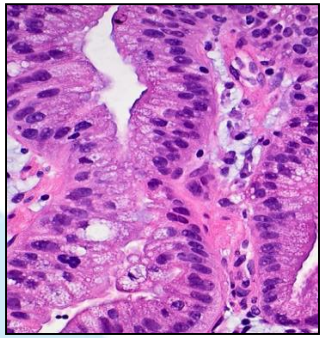


Phenotypic Diversity of Gastric Dysplasia

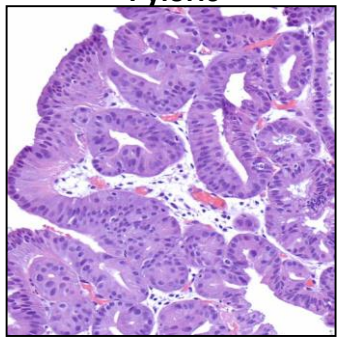
Adenomatous

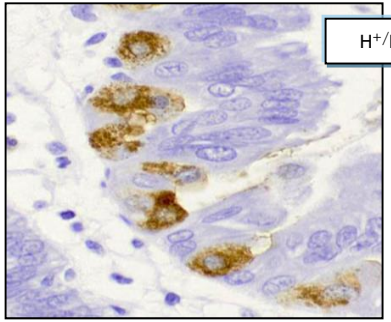


Foveolar



Pyloric





	CD10	MUC2	MUC5AC	MUC6
Intestinal	(+) (Apical membrane)	(+) (Goblet cells)	(-)	(-)
Foveolar	(-)	(-)	(+)	(+/-) (glands)
Pyloric	(-)	(-)	(+) (surface)	(+) (glands)

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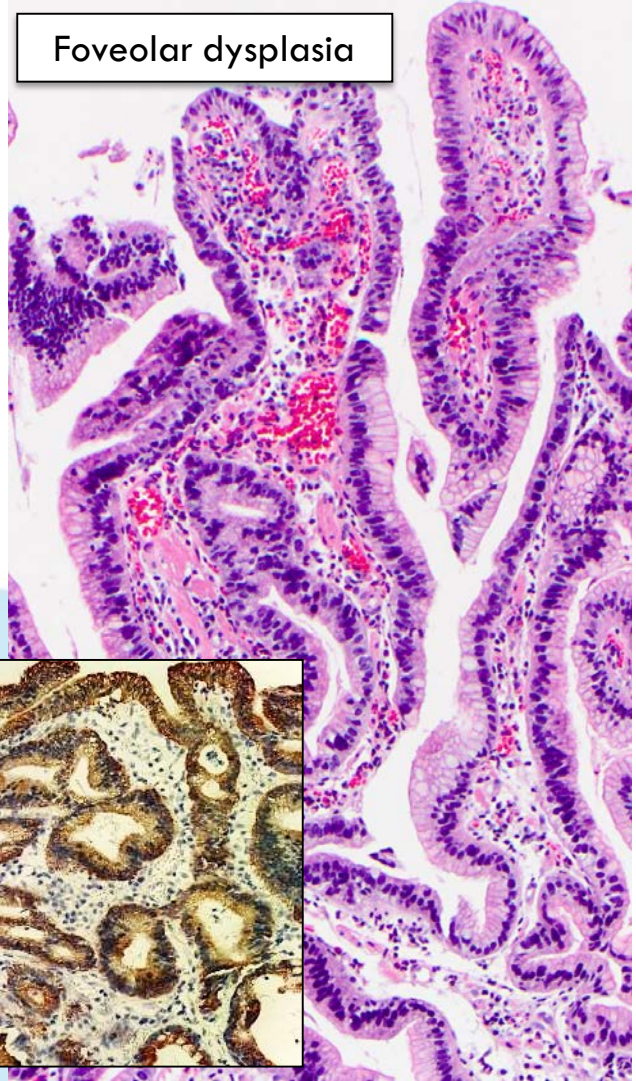
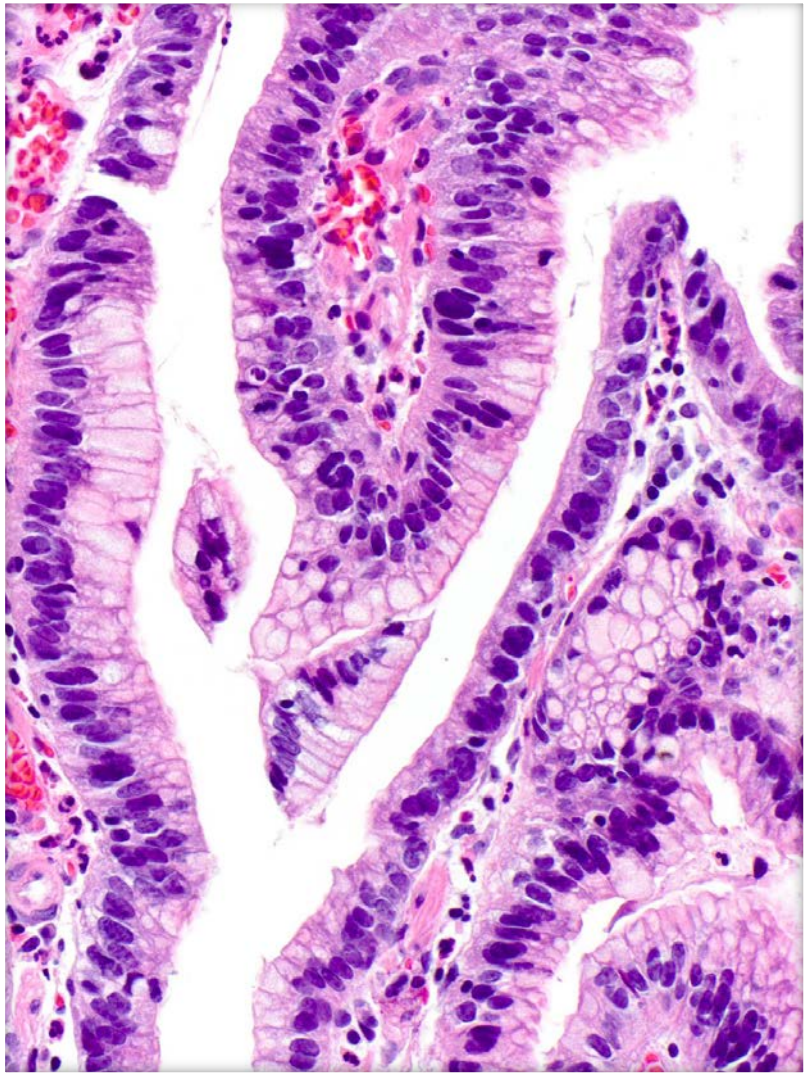
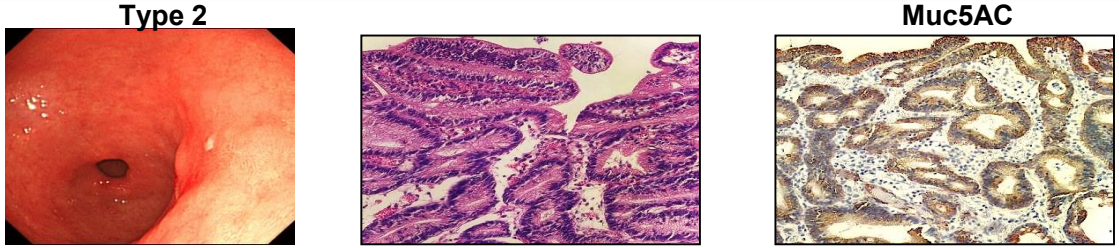
Foveolar dysplasia





MUC5AC

Prevalence of foveolar GED: 22% (Adenomatous: 45%, hybrid 33%) (n=69)



- Foveolar GED is often depressed/flat and associated w/ HGD (p= 0.046).
- HGD associated w/ MUC5AC expression regardless of the type (p=0.026).

Grade	Immunophenotype			p value
	Foveolar (n=24)	Intestinal (n=22)	Hybrid (n=14)	
HGD (n=25)	15* (63%)	4 (18%)	6 (43%)	
Low grade (n=35)	9 (37%)	18 (82%)	8 (57%)	0.010

* coexistent intramucosal carcinoma in 8 cases

Foveolar differentiation is associated w/ HGD & coexistence of IMC

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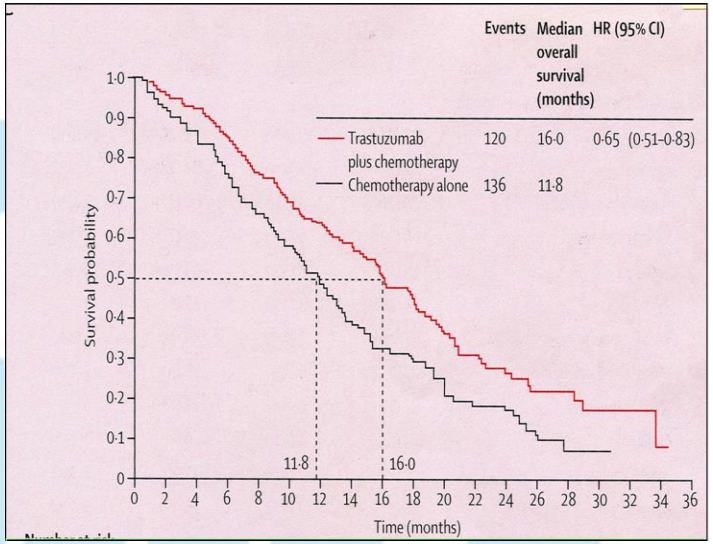
Valente P; Gastric Cancer 2014

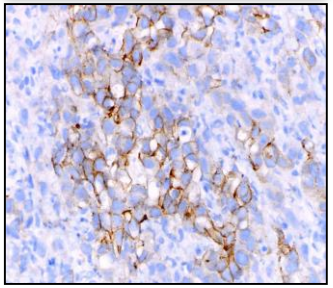
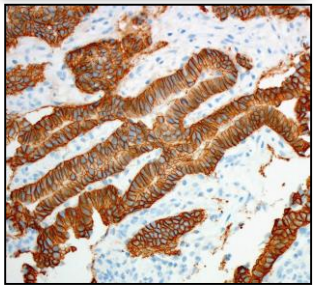
HER 2

- Receptor tyrosine kinase & member of EGFR family.
- 10-20% of CAs show ERBB2 amplifications resulting in protein overexpression.

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

The Lancet, 2010;376:687-697



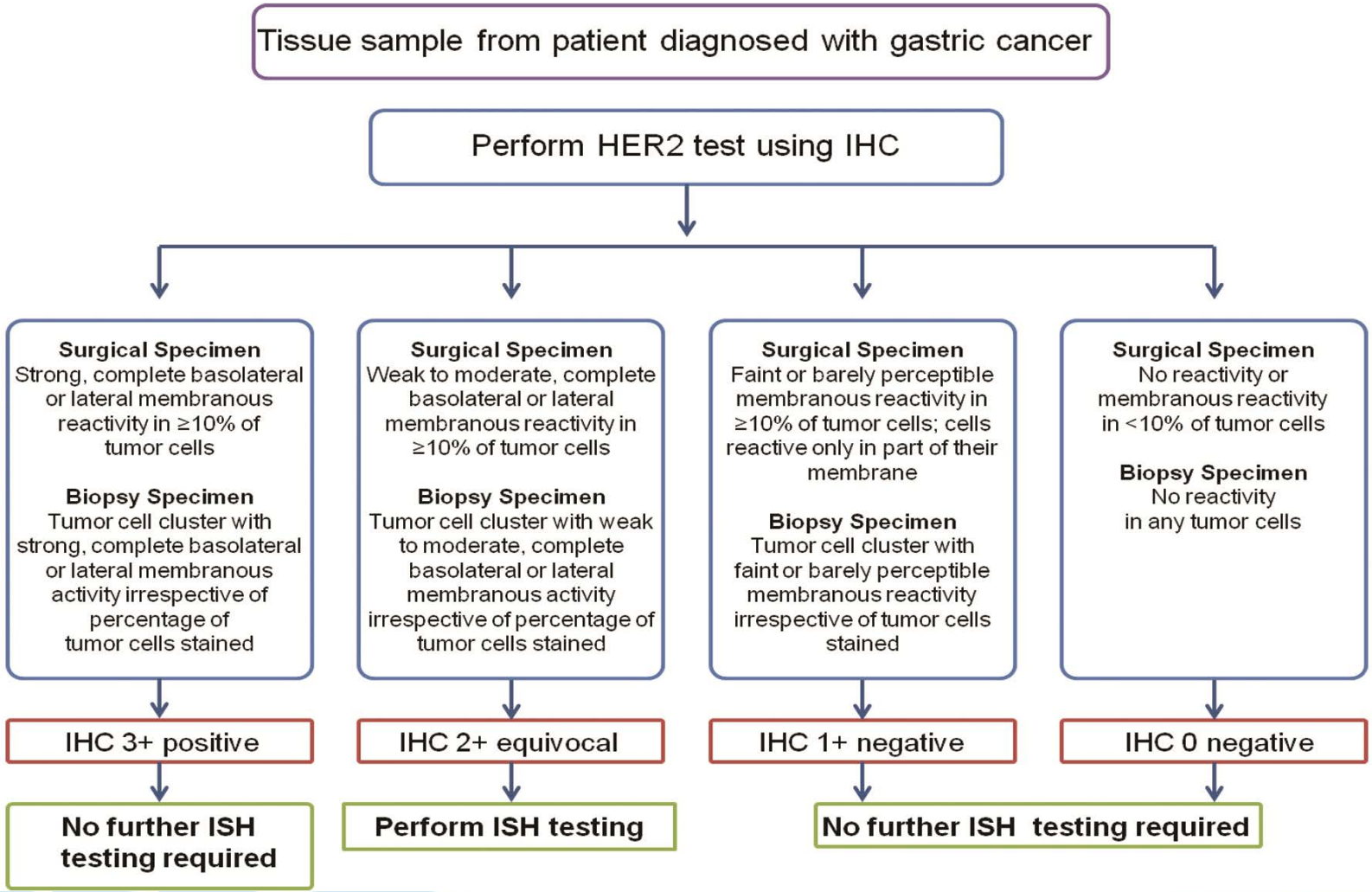
Weak to mod. complete, basolateral or lateral membranous reactivity	Strong complete or basolateral or lateral membranous reactivity
	

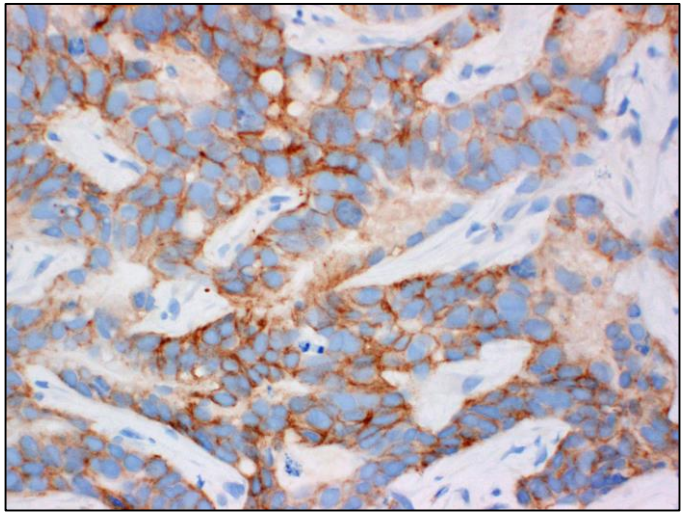
More common in CAs of GEJs than stomach

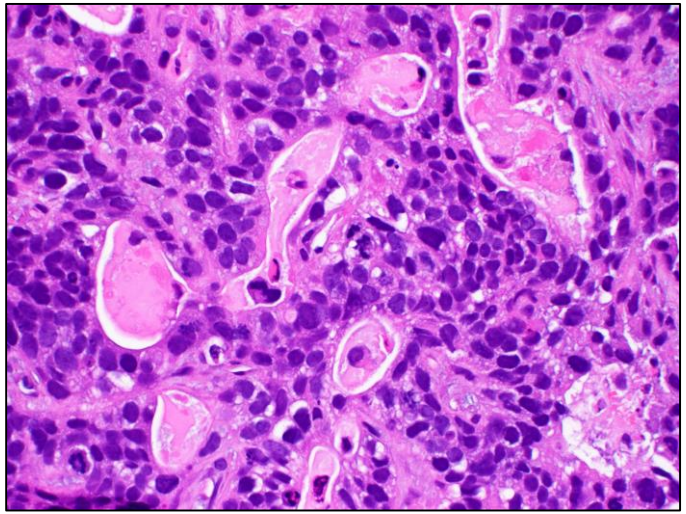
	IHC0	IHC1+	IHC2+	IHC3+
Diffuse (n=52)	73%	17%	6%	4%
Tubular (n=100)	61%	5%	7%	27%
Mixed (n=22)	73%	4%	14%	9%

Lee S. *Histopathology* 2011;59:832-840

MOFFITT CANCER CENTER


Tissue sample from patient diagnosed with gastric cancer
Perform HER2 test using IHC
Surgical Specimen
Strong, complete basolateral or lateral membranous reactivity in ≥10% of tumor cells
Biopsy Specimen
Tumor cell cluster with strong, complete basolateral or lateral membranous activity irrespective of percentage of tumor cells stained
IHC 3+ positive
No further ISH testing required
Surgical Specimen
Weak to moderate, complete basolateral or lateral membranous reactivity in ≥10% of tumor cells
Biopsy Specimen
Tumor cell cluster with weak to moderate, complete basolateral or lateral membranous activity irrespective of percentage of tumor cells stained
IHC 2+ equivocal
Perform ISH testing
Surgical Specimen
Faint or barely perceptible membranous reactivity in ≥10% of tumor cells; cells reactive only in part of their membrane
Biopsy Specimen
Tumor cell cluster with faint or barely perceptible membranous reactivity irrespective of tumor cells stained
IHC 1+ negative
Surgical Specimen
No reactivity or membranous reactivity in <10% of tumor cells
Biopsy Specimen
No reactivity in any tumor cells
IHC 0 negative
No further ISH testing required





- BX, resection specimens, metastatic bxs and cytology samples can be used.
- Heterogeneity in gastric & GE CAs: 5 to 40%.
- Negative result in fewer than 5 tumor fragments may not be accurate and warrants re-biopsy.

Wong DD,. Diagn Cytopathol. 2015 Jan;43(1):80-5),

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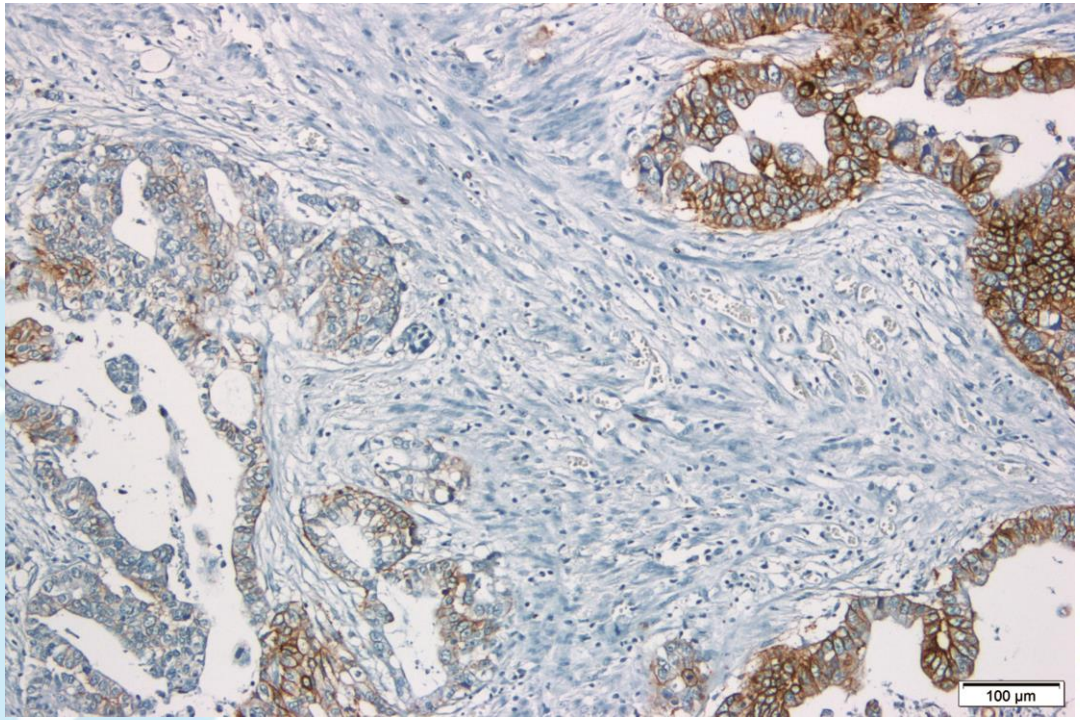
- 95% - 98% concordance rate between metastases and primaries
- Thus, heterogeneous amplification in primary lesions is responsible for discordant ERBB2 status of primary & metastases in gastric CAs

(*Br J Cancer 2011;104;1372-1376. -Pathology. 2015;7:641-6)*



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Genetic & Molecular Classifications of Gastric Cancer

THE CANCER GENOME ATLAS (TCGA)"
(Nature.2014)

EBV
- EBV-CIMP
- PIK3CA mut.
- PD-L1/2 expression

MSI
- Gastric-CIMP
- MLH1 silencing

GS
- Diffuse histology
- CDH, RHOA mutations

CIN
- Intestinal histology
- TP53 mutations

Drug Responsive Gastric Cancer Subtypes.
"Singapore-Duke" study
(Gastroenterology 2013)

MESENCHYMAL
- **Low TP53 mutations**
- **Low level of CDH1 (E-Cadherin)**

PROLIFERATIVE
- High number of TP53 mutations

METABOLIC
- Low TP53 mutations
- Expression of genes characteristic of normal gastric mucosa

LAUREN DIFFUSE

LAUREN INTESTINAL

No histological correlate

PIK3CA Mtor inhibitors

5-FU+Surgery

Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes.
(Nature Medicine.2015)

MSS/TP53+

MSI
Antrum
Intestinal type
Early stage Best Prognosis

Enriched in *ARID1A, KRAS, PIK3CA*

MSS/EMT
Loss of CDH1
Young age
Diffuse type Worst Prognosis

MSS/TP53-
Male Intestinal type
Adv. Stage Int. prognosis

Enriched in *APC, ARID1A, KRAS, PIK3CA, SMAD4*

MSS/TP53+
Male
Intestinal type
Int. prognosis

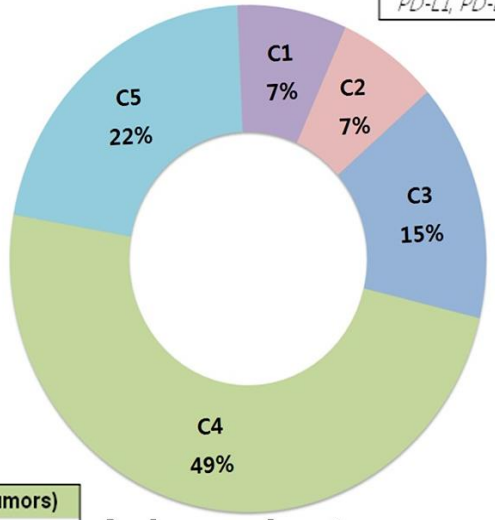
Enriched in *ERBB2, EGFR, CCND1,*

Ahn S. High-throughput protein and mRNA expression based classification of gastric cancers. Am J Surg Pathol . 2017;41;106-115

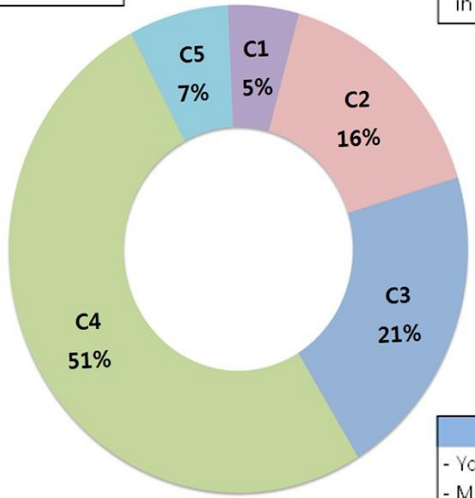
C5 (normal p53 expression tumors)
- Intermediate prognosis
- Moelcular features:
  intermediate level of mutations
  (*APC/KRAS/ARID1/PI3K/SMAD4*)

C1 (EBV tumors)
- Male predominance
- Body location
- Mostly poorly differentiated type by WHO classification
- Best prognosis
- Molecular features:
  PIK3CA, ARID1A, BCOR mutation
  CDKN2A (p16) promoter hypermethylation
  PD-L1, PD-L2, JAK2 amplification

C2 (MSI tumors)
- Old age
- Distal location
- Mostly Well- and moderaltely differentiated type by WHO classification
- Gastric type by mucin phenotype
- Better prognosis
- Molecular features:
  MLH1 promoter hypermethylation
  Hypermutation (occasional mutations in *PIK3CA, ERBB2, ERBB3, EGFR, ARID1*)



Asian cohort



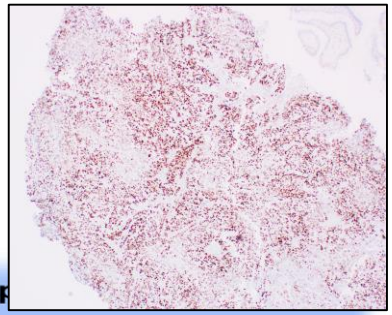
Western cohort

C4 (aberrant p53 expression tumors)
- Intermediate prognosis
- Moelcular features:
  high *TP53* mutation
  HER2, EGFR, CCNE1, CCND1, MDM2, ROBO2, GATA6, MYC amplifiction

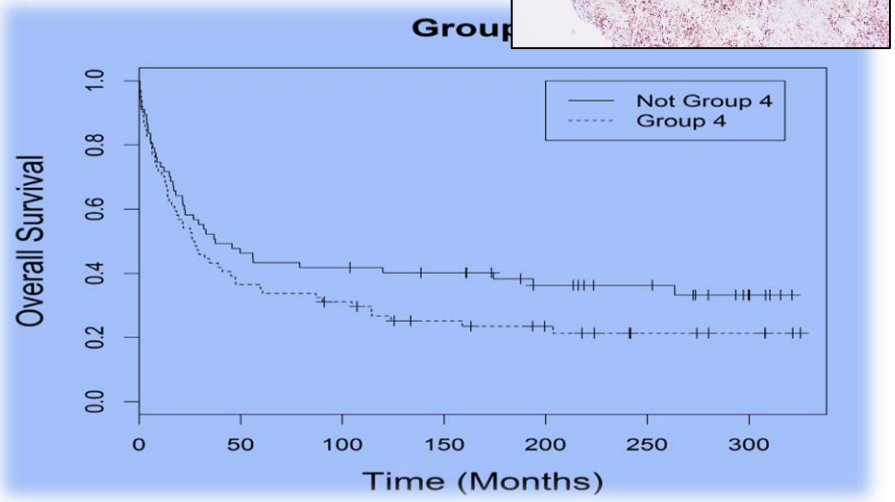
C3 (EMT tumors)
- Younger age
- Mostly diffuse type by Lauren classification
- Advanced T and N stage
- Worst prognosis
- Molecular features:
  CDH1, RHOA mutations
  lower number of mutations

Setia N. A protein and mRNA expression-based classification of gastric cancer. Mod Pathol. 2016;29:772-84.

Gastric CA w/ *Aberrant p53 Expression*



Frequency	45-51%
Age	Mean:68 years
Sex	M:F=2.5:1 to 1.5:1
Localization	Variable
Histology	Intestinal (~80%)



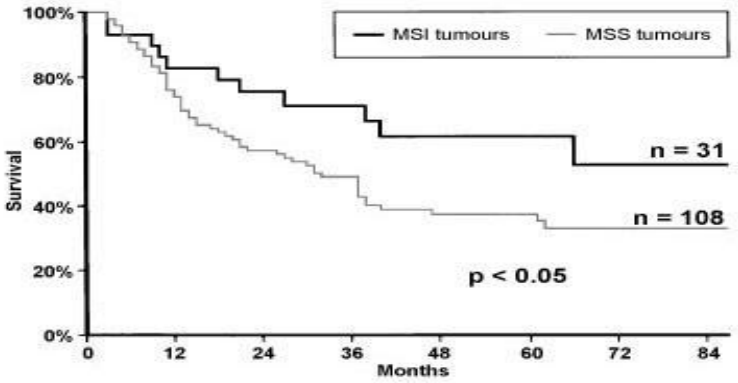
[1]Bass et al. Nature.2014. [2]Lei et al. Gastroenterology.2013.

- Associated with higher nodal status >N0
- A trend towards increased Her2 staining
- No significant survival difference (p = 0.13, median survival: 26.8 months) (N.Setia 2016)

Microsatellite Instability: a marker of good prognosis

15-38% of gastric cancers

Univariate analysis

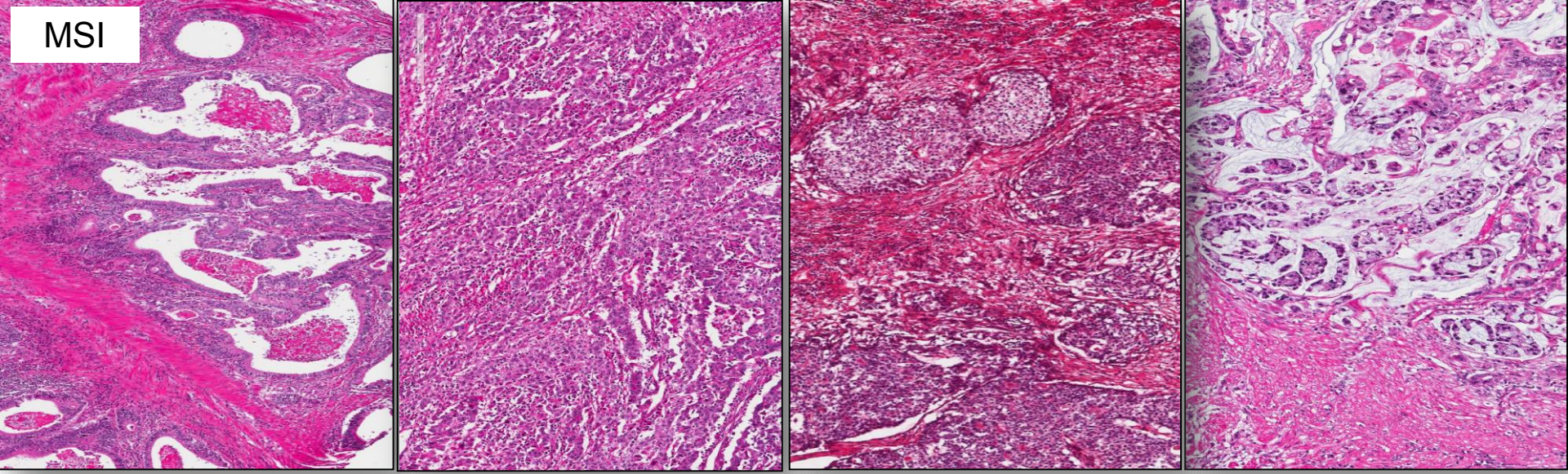


Multivariate analysis

- Staging (pTNM) (p< 0.0008)
- Venous invasion (p= 0.004)
- Histological classification (p=0.08)
- Microsatellite instability (p=0.04)

Location	Antrum+
***H. Pylori* infection**	Common
Patient age	Older patients (>65)
Histology	Tubular , **Papillary, solid, mucinous, poorly cohesive**
Node metastasis	Infrequent
Genetic changes	Associated w/ TGFpR11, BAX, hMSH3 gene mutation.
Epigenetic changes	Associated w/ CpG island hypermethylation (CIMP) of hMLH1

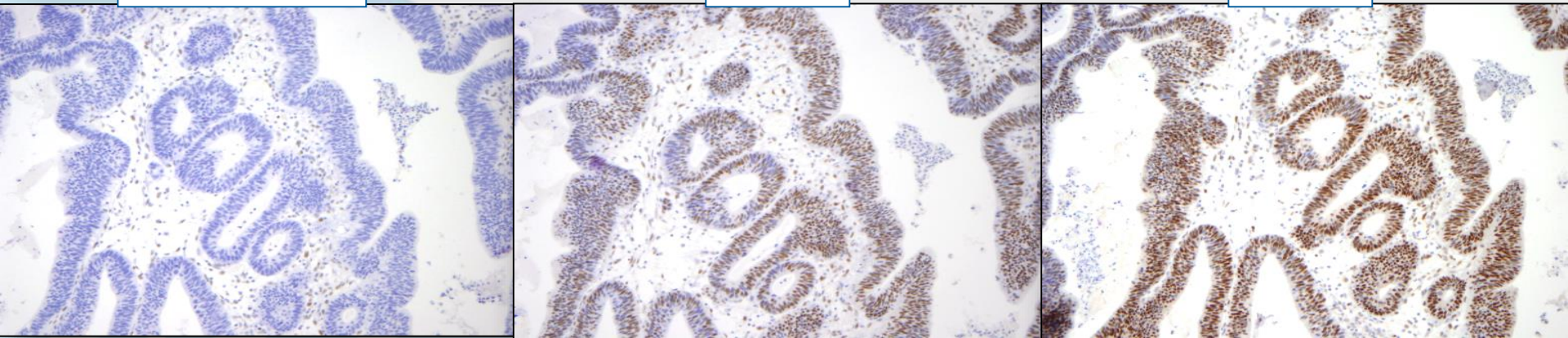
MSI



MLH1/PMS2

MSH2

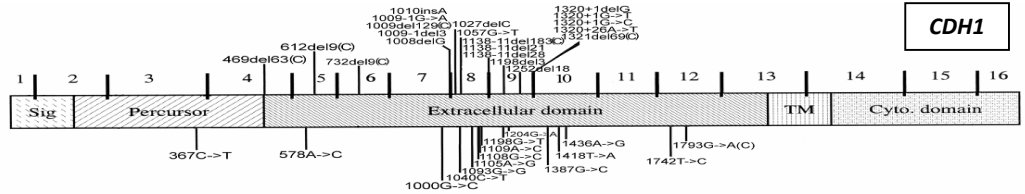
MSH6



Gastric CA w/ *Aberrant E-cadherin Expression*

Frequency	15%-21%
Age	Mean:67.23 years (22-83 years)
Sex	Male-predominant (M:F=1.6:1)
Localization	Less commonly involving cardia (cardia-23%)
Histology	Diffuse/poorly cohesive (60-90%)

[1]Bass et al. Nature.2014. [2]Lei et al. Gastroenterology.2013.



- Low frequency of aberrant p53 expression

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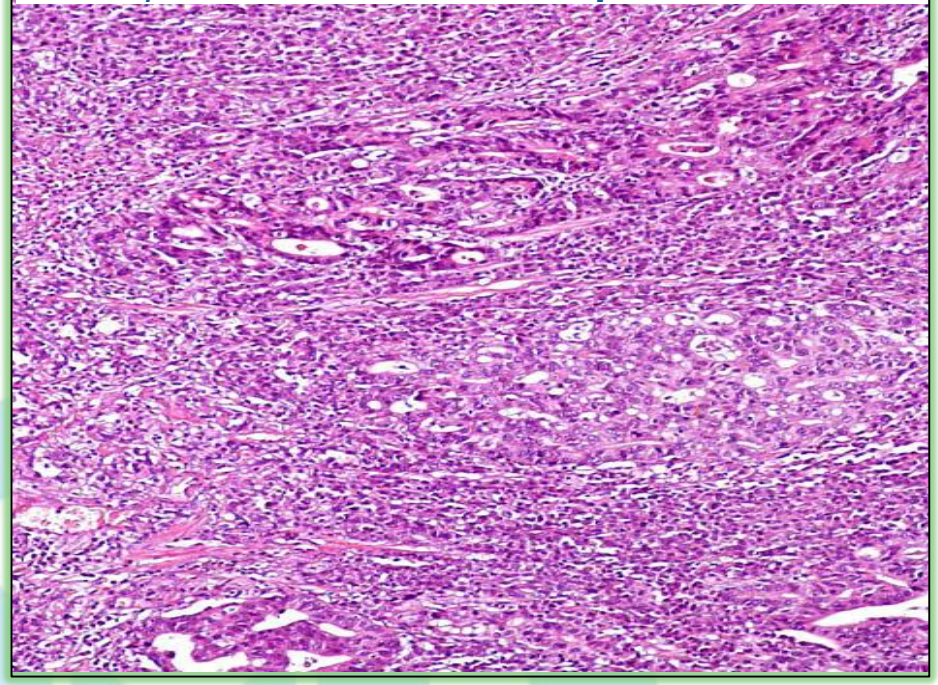
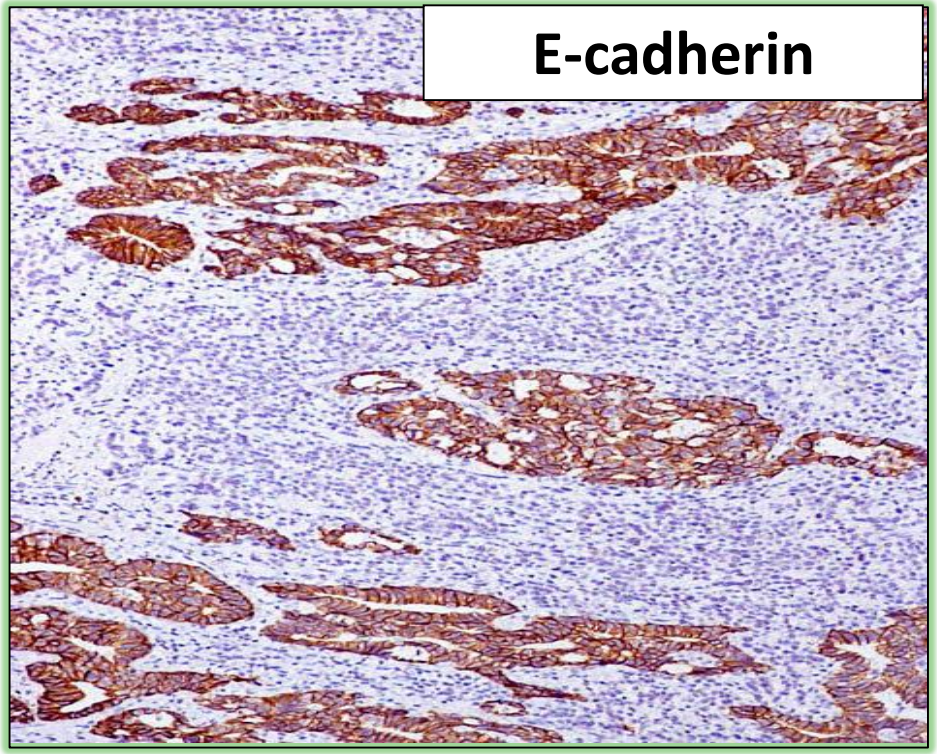
CA w/ *Aberrant E-cadherin Expression*





- No survival difference VS worse prognosis (?)

Machado JC *et al: Lab Invest* 79: 459, 1999

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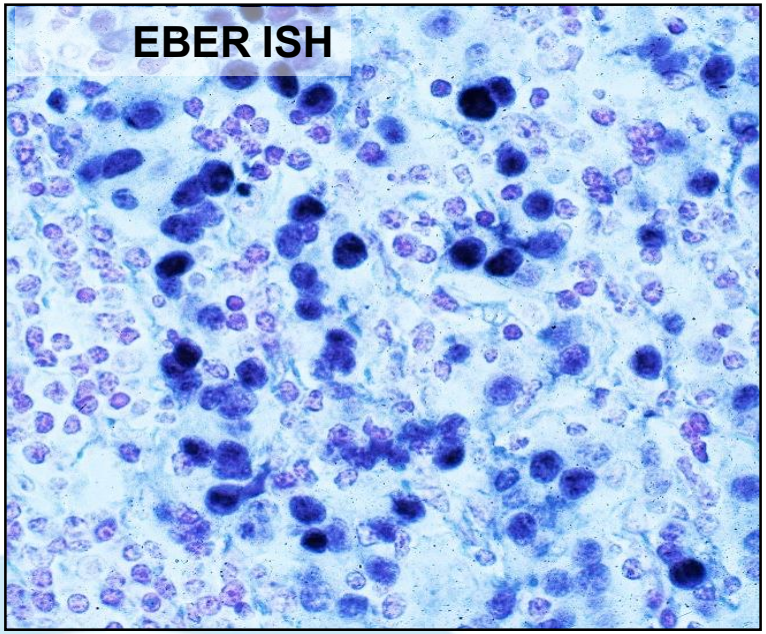
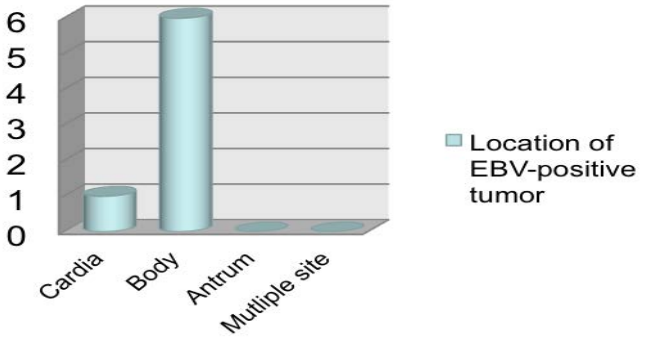
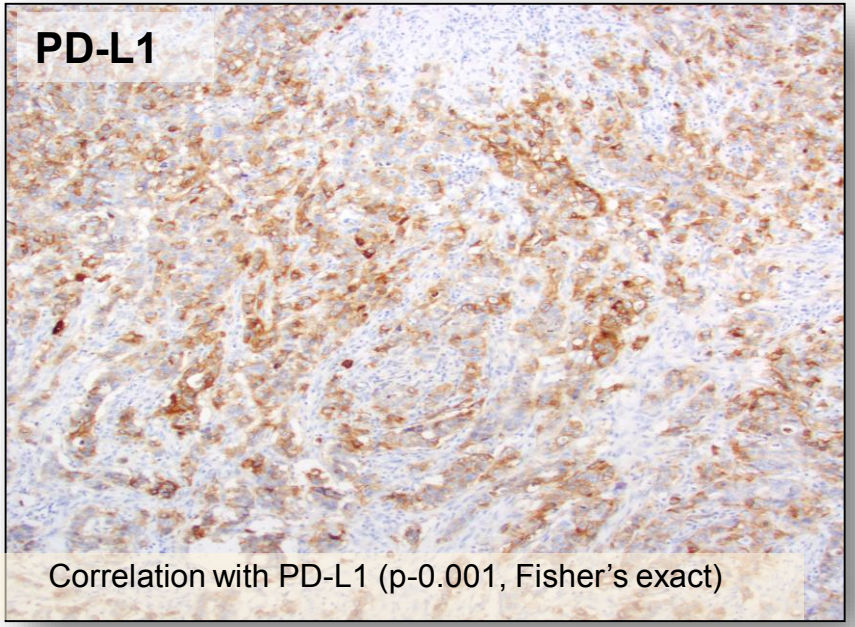


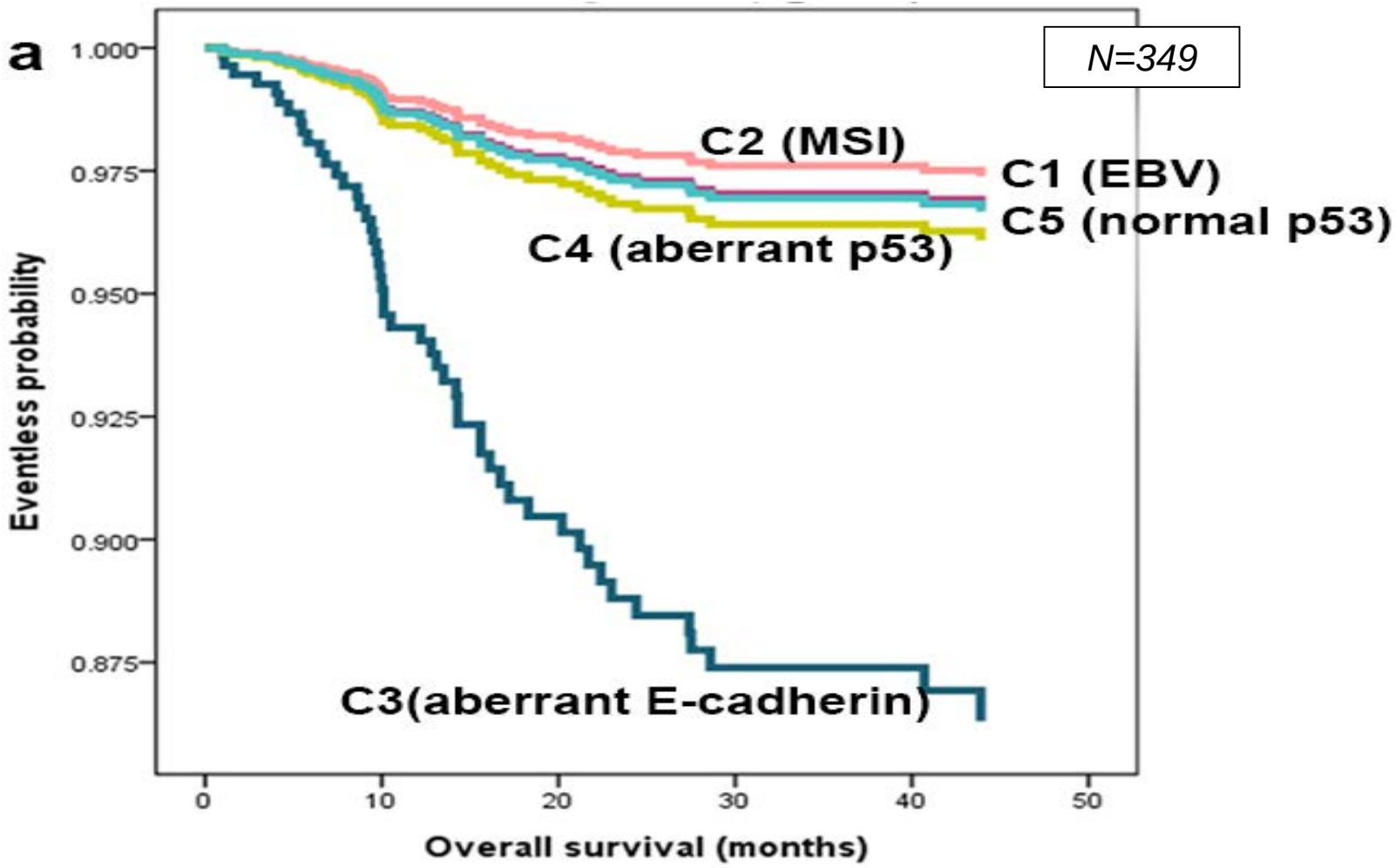


- Trend towards better survival

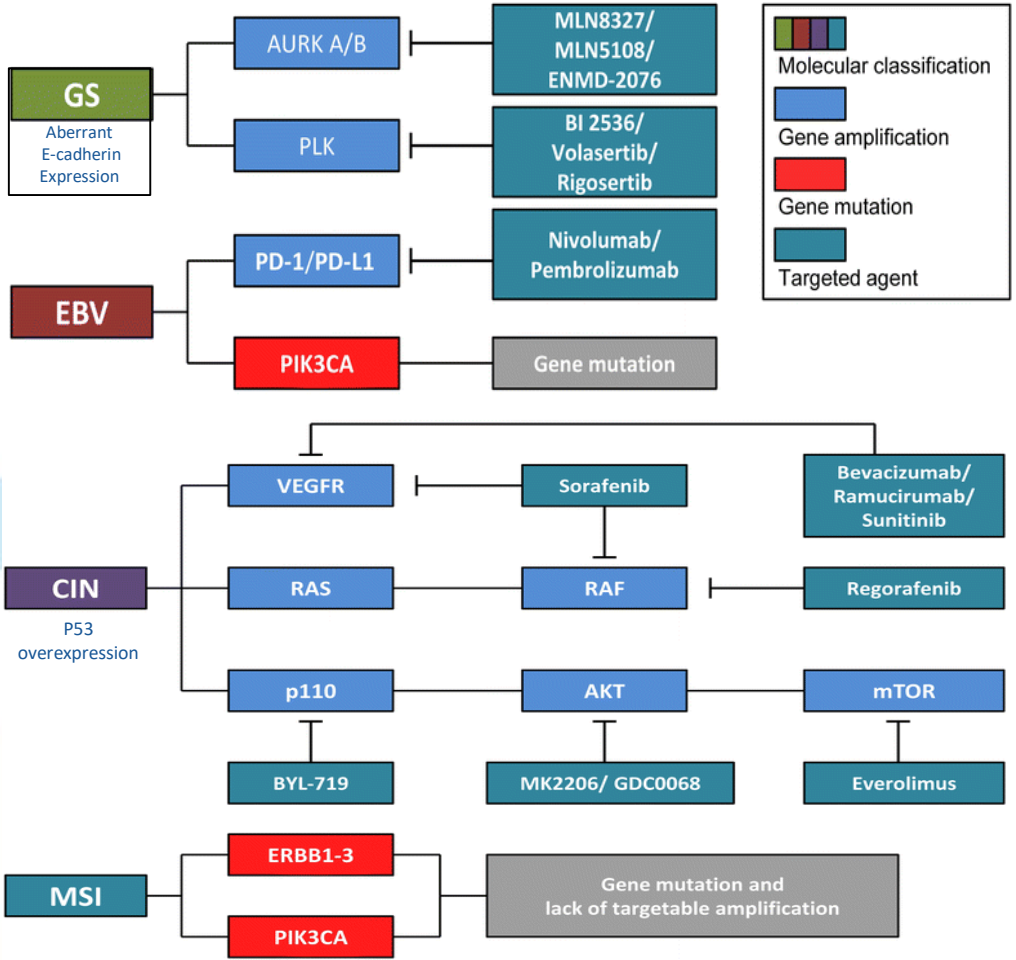
Frequency	**5-10%**
Age	**Mean:64.85 years**
Sex	**Male-predominant (M:F=1.3:1)**



Correlation with PD-L1 (p-0.001, Fisher's exact)


a
N=349
C2 (MSI)
C1 (EBV)
C5 (normal p53)
C4 (aberrant p53)
C3(aberrant E-cadherin)
Eventless probability
1.000
0.975
0.950
0.925
0.900
0.875
0
10
20
30
40
50
Overall survival (months)

Specific adjuvant therapy for each type of gastric cancer



PD-L1 positive or MSI high or Mismatch repair deficient advanced cancers that have previously been treated with at least 2 prior systemic therapies

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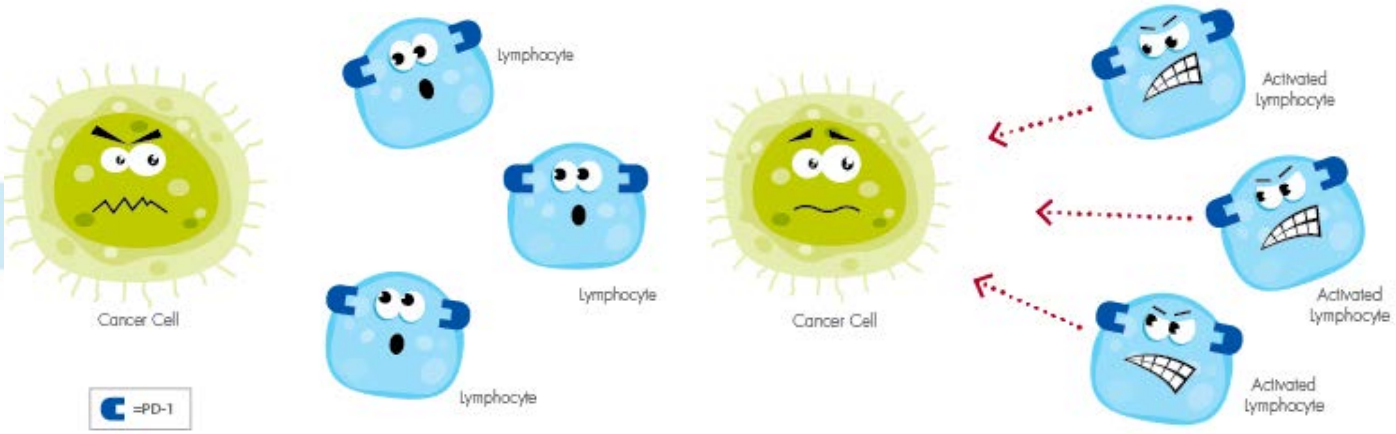
Immunosurveillance

What is supposed to happen…..



Lymphocytes recognize the cancer cell as foreign…

….and become activated to help destroy the cancer cell

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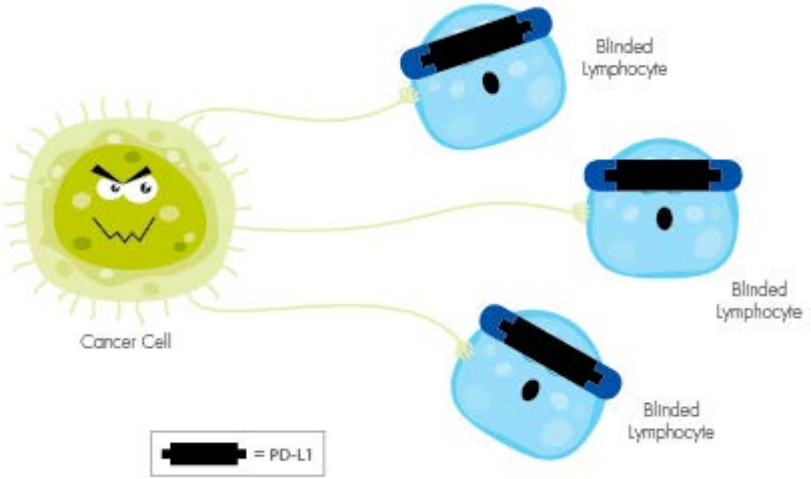
Immunotherapy Evasion

But what happens sometimes…..



Cancer cell secretes proteins that make lymphocytes and other immune cells unable to "see" the cancer cells.

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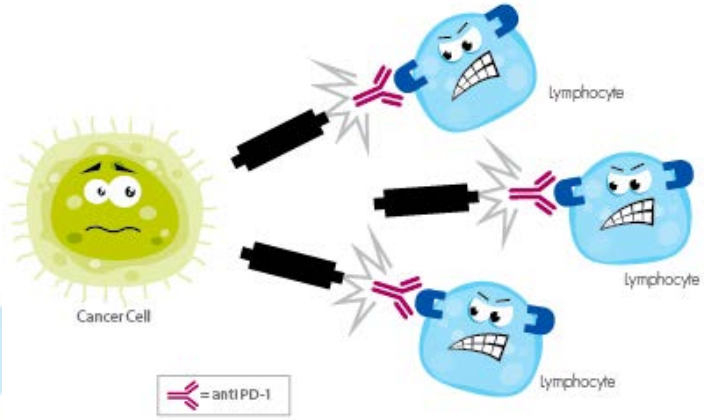
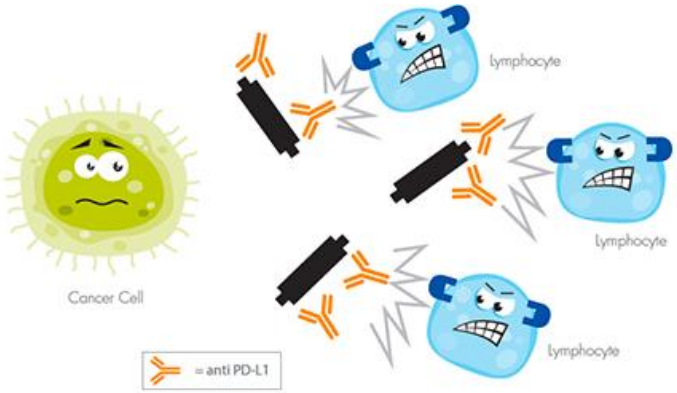
Immunotherapy





Immunotherapy agents such as Opdivo and Keytruda stick to PD-1 so it can't interact with PD-L1

Some other immunotherapies in development bind to PD-L1 and inhibit PD-1 from binding to PD-L1

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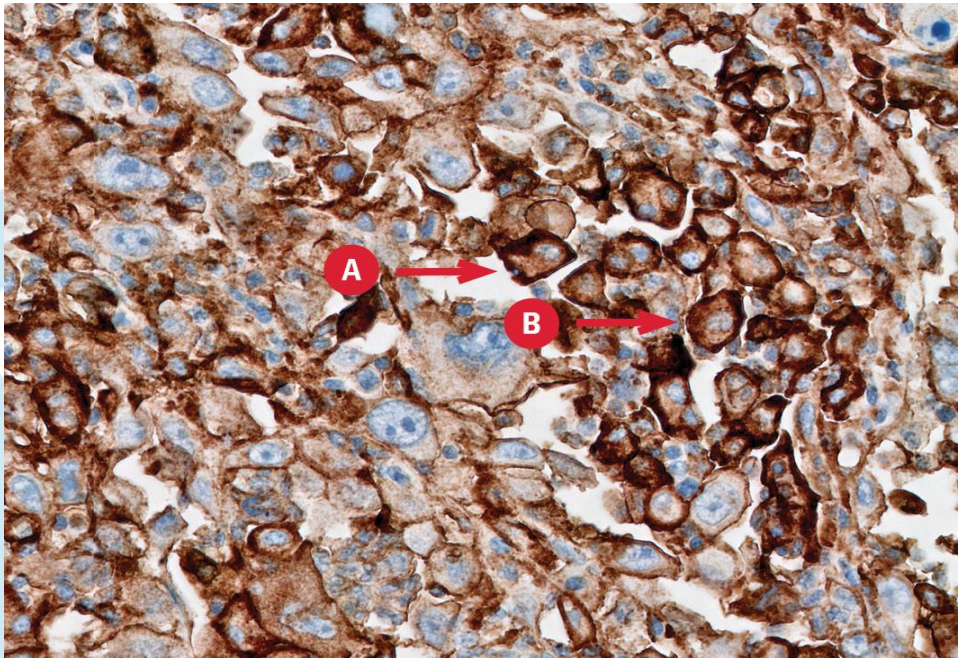
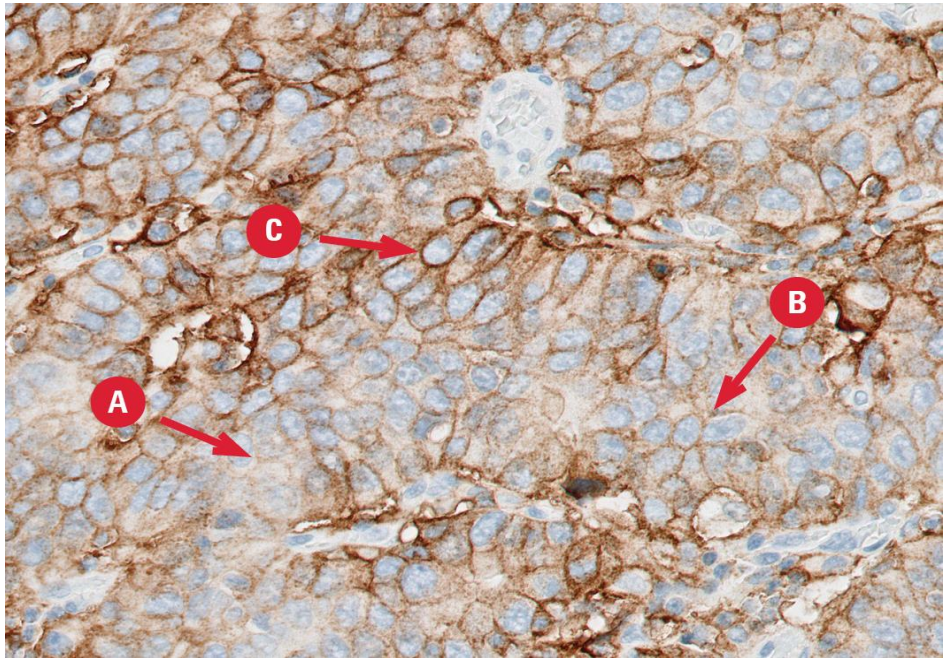
PD-L1 IHC 22C3 pharmDx

- Qualitative immunohistochemistry assay

PD-L1 antibody shows linear membrane staining distinct from cytoplasmic staining (20×).

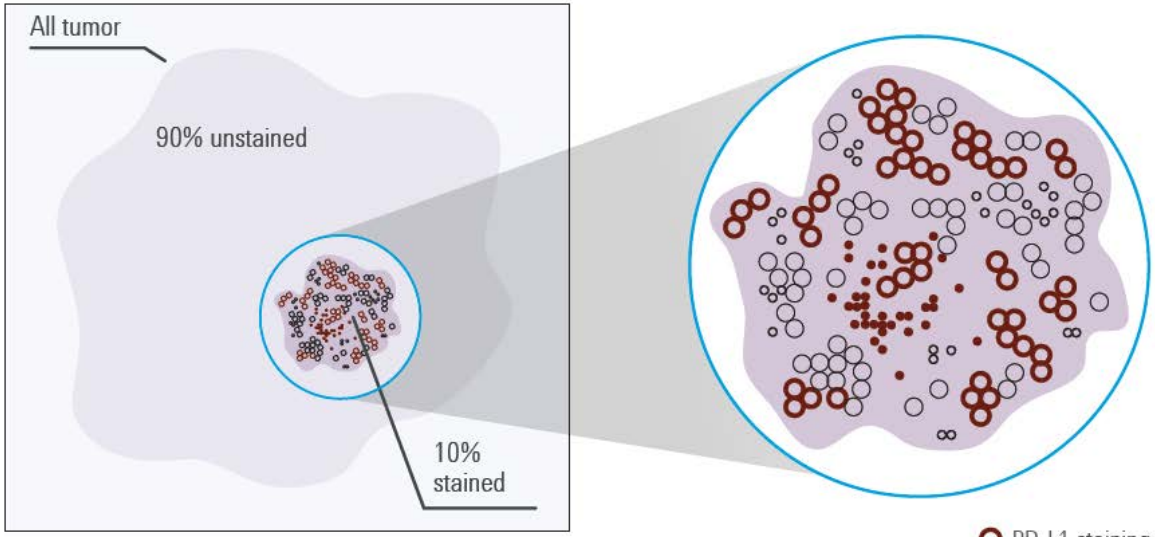


Heterogeneous staining intensities
A: 1+ intensity, **B**: 2+ intensity, **C**: 3+ intensity



A minimum of 100 viable tumor cells must be present for the specimen to be considered adequate for PD-L1 evaluation.

Assessment: *There are approximately 100 viable tumor cells and about 80 PD-L1 staining cells (per the CPS numerator)*



Any convincing partial or complete linear membrane staining (≥ 1+) of viable tumor cells that is perceived as distinct from cytoplasmic staining is considered PD-L1 staining and should be included in scoring.

Any convincing membrane and/or cytoplasmic staining (≥ 1+) of lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within tumor nests and/or adjacent supporting stroma is considered PD-L1 staining and should be included in scoring. Only MICs directly associated with the response against the tumor are scored.

- PD-L1 staining tumor cell
- PD-L1 non-staining tumor cell
- PD-L1 staining mononuclear inflammatory cell
- PD-L1 non-staining mononuclear inflammatory cell

Calculate the Combined Positive Score of the entire tumor area:

Assessment: CPS of area with staining:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells*}}{\text{Total \# viable tumor cells}} \times 100 = \frac{\sim 80 \text{ PD-L1 staining cells}}{100 \text{ tumor cells}} \times 100 = 80$$

CPS of entire tumor area: 10% x 80 = ~CPS 8

or

- CPS < 1: No PD-L1 expression
- CPS ≥ 1: PD-L1 expression

DUODENAL NEOPLASIA

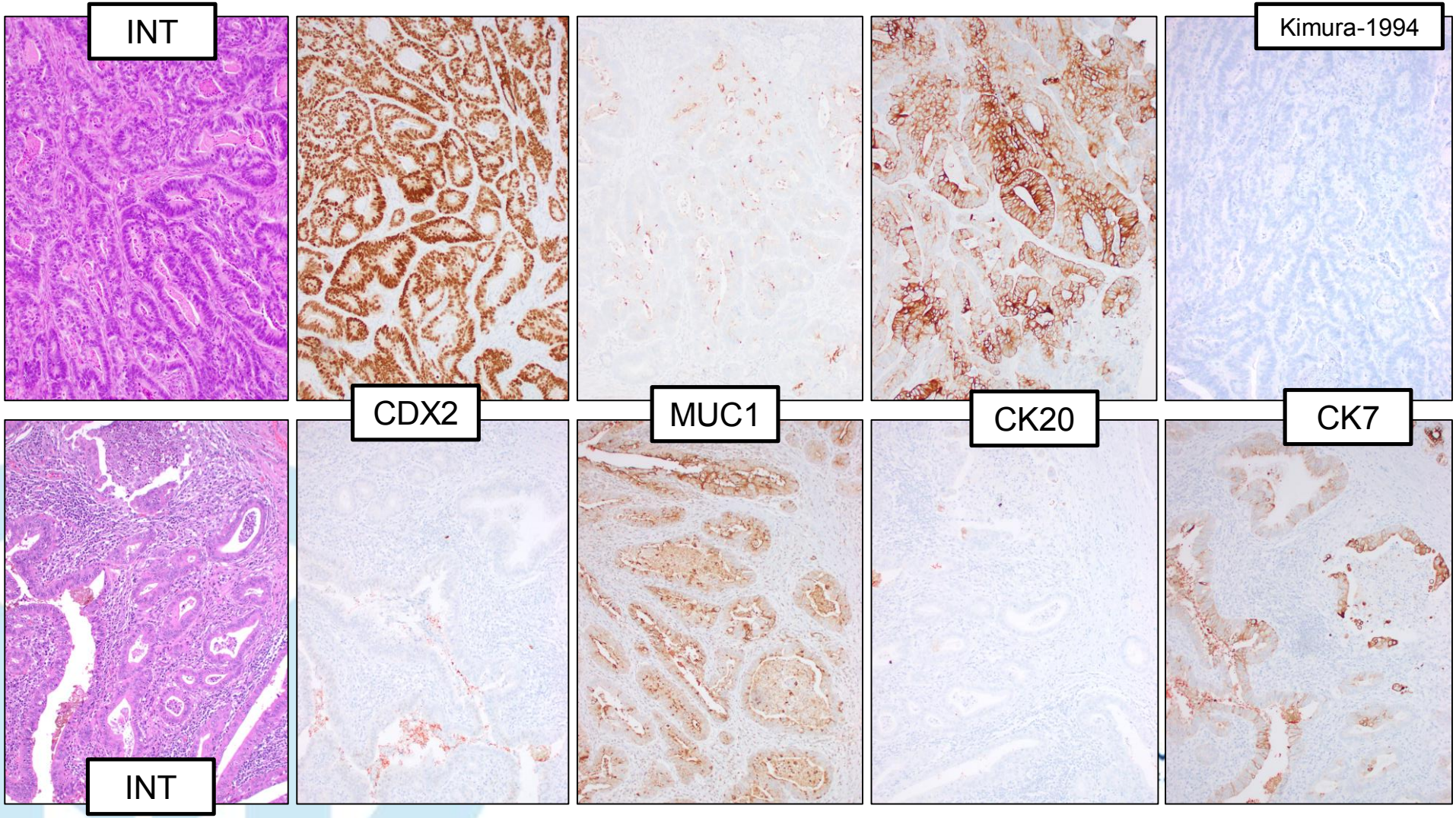
- CA Ampullary of Vater
  - Pancreatic, duodenal or biliary origin / type can be uncertain.
  - Heterogeneous of clinical behavior

- IMMUNOPHENOTYPE /PROGNOSIS
- GUIDE TO THERAPY

CA of Ampulla of Vater

- Histologic sub-classification [Kimura]
  - CAs w/ intestinal phenotype fare better than those w/ biliary or pancreatic phenotype.
  - MUC2 [+] and CDX2 [+] tend to correlate w/ better survival
  - MUC1 [+] correlate with worse survival.

Jpn J Cancer 1994;85:161-166


INT
CDX2
MUC1
CK20
Kimura-1994
CK7
INT

JOURNAL OF CLINICAL ONCOLOGY
2013;31:1348-1356

ORIGINAL REPORT

Histomolecular Phenotypes and Outcome in Adenocarcinoma of the Ampulla of Vater

David K. Chang, Nigel B. Jamieson, Amber L. Johns, Christopher J. Scarlett, Marina Pajic, Angela Chou, Anthony J. Gill, Aldo Scarpa, Colin J. McKay, and Andrew V. Biankin

72 pts / validated in 136 pts

Validation of histomolecular classification utilizing histological subtype, MUC1, and CDX2 for prognostication of resected ampullary adenocarcinoma

154 pts

British Journal of Cancer (2015) 113, 64–68 | doi: 10.1038/bjc.2015.172

- Cases classified using histology, CDX2 & MUC1*
  - Pancreaticobiliary histo-molecular phenotype : PB histology, MUC1 [+] and [-] CDX2 staining.
  - Others: intestinal histo-molecular phenotype (INT).
  - PB phenotype and LN positivity were indicators of poor OS [in multivariate analysis] & verified across all cohorts.

CDX2 score >35 & MUC1>10%

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Histomorphologic and molecular phenotypes predict gemcitabine response and overall survival in adenocarcinoma of the ampulla of Vater

Tobias S. Schiergens, MD,[a,b] Simone Reu, MD,[b,c] Jens Neumann, MD,[b,c] Bernhard W. Renz, MD,[b,d] Hanno Niess, MD,[a,b] Stefan Boeck, MD,[b,e] Volker Heinemann, MD,[b,e] Christiane J. Bruns, MD,[f] Karl-Walter Jauch, MD,[a,b] *and* Axel Kleespies, MD,[a,b] *Munich and Magdeburg, Germany, and New York, NY*

Surgery 2015;158:156-161

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Conclusion

- Re-enforce the central role of pathology beyond Dx and ‘static’ prognosis, by offering therapeutic guidance and predictability of response to Rx.
- This comes at a price: need to exercise strict ‘quality control’, not only of the ‘test’, but also of the *ordering*, of the *sample* being tested, of the *validity of results*/*scoring* and of the *cost* – to generate the best results with the most appropriate use of resources.

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