Medizinische Universität Graz

Gastric Cancer

Minisympózium: Patológia Gastrointestinálneho Traktu

SD-IAP, Košice, Slovakia, 6-7 June 2019

European Network of Gastrointestinal Pathology
ENGIP

European Society of Pathology

Cord Langner MD
Diagnostic & Research Centre for Molecular BioMedicine
Institute of Pathology
Medical University of Graz, Austria

Agenda

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- **Classification of gastric cancer**
  - Histology
  - Molecular pathology
- **Predictive pathology**
  - HER2
  - PD-L1
  - Microsatellite instability
- **Sporadic *vs* hereditary gastric cancer**
- **Take home messages**

Agenda

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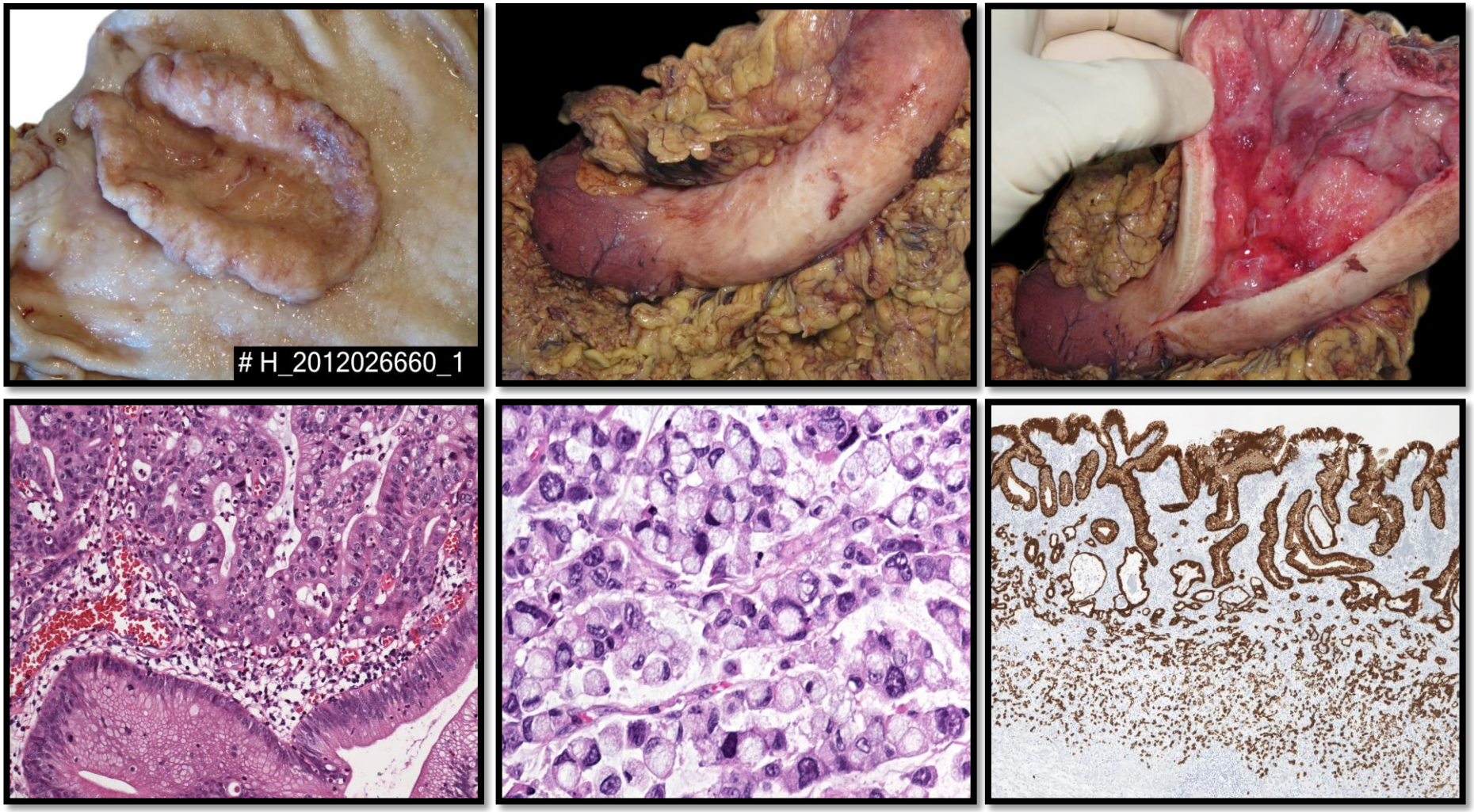
  - Microsatellite instability
- **Sporadic *vs* hereditary gastric cancer**
- **Take home messages**

Lauren Classification



Lauren. Acta Pathol Microbiol Scand 1965

Lauren classification and individualized chemotherapy in gastric cancer (Review)

JUNLI MA[1,2], HONG SHEN[2,3], LINDA KAPESA[1] and SHAN ZENG[1,2]

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	Intestinal Type GC	Diffuse Type GC
Macroscopy	Well circumscribed, antrum > corpus	Poorly circumscribed, corpus > antrum
Histology	Glandular (or solid), cell adhesion, often in association with intestinal metaplasia	Non-cohesive tumour cell growth, lack of cell adhesion, signet-ring cell carcinomas
Clinical characteristics	Elderly patients, male > female, more prevalent in high-risk areas	Younger patients, female > male, more prevalent in low-risk areas
Aetiology	Helicobacter pylori (Correa's cascade)	Chronic inflammation, HDGC
Metastasis	Liver > peritoneum	Peritoneum > liver
Biomarkers	CDX-2 (MUC2), MSI, HER2	CDH1 (loss of E-cadherin), MSS

Ma et al. Oncol Lett 2016

WHO Classification

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Epithelial tumours	
Intraepithelial neoplasia – Adenoma	8140/0[2]
Carcinoma	
Adenocarcinoma	8140/3
intestinal type	8144/3
diffuse type	8145/3
Papillary adenocarcinoma	8260/3
Tubular adenocarcinoma	8211/3
Mucinous adenocarcinoma	8480/3
Signet-ring cell carcinoma	8490/3
Adenosquamous carcinoma	8560/3
Squamous cell carcinoma	8070/3
Small cell carcinoma	8041/3
Undifferentiated carcinoma	8020/3
Others	
Carcinoid (well differentiated endocrine neoplasm)	8240/3

Non-epithelial tumours	
Leiomyoma	8890/0
Schwannoma	9560/0
Granular cell tumour	9580/0
Glomus tumour	8711/0
Leiomyosarcoma	8890/3
GI stromal tumour	8936/1
benign	8936/0
uncertain malignant potential	8936/1
malignant	8936/3
Kaposi sarcoma	9140/3
Others	
Malignant lymphomas	
Marginal zone B-cell lymphoma of MALT-type	9699/3
Mantle cell lymphoma	9673/3
Diffuse large B-cell lymphoma	9680/3
Others	
Secondary tumours	

[1] The classification is modified from the previous WHO histological classification of tumours {2066} taking into account changes in our understanding of these lesions. In the case of endocrine neoplasms, the classification is based on the recent WHO clinicopathological classification {1784}, but has been simplified to be of more practical utility in morphological classification.

[2] Morphology code of the International Classification of Diseases for Oncology (ICD-O) {542} and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /3 for malignant tumours, and /1 for unspecified, borderline or uncertain behaviour. Intraepithelial neoplasia does not have a generic code in ICD-O. ICD-O codes are available only for lesions categorized as glandular intraepithelial neoplaia grade III (8148/2), and adenocarcinoma in situ (8140/2).

Pathology and Genetics of Tumours of the Digestive System, 3rd edition (Hamilton SR & Aaltonen LA) IARC Press 2000

WHO Classification

Epithelial tumours	
Premalignant lesions	
Adenoma	8140/0
Intraepithelial neoplasia (dysplasia), low grade	8148/0*
Intraepithelial neoplasia (dysplasia), high grade	8148/2*
Carcinoma	
Adenocarcinoma	8140/3
Papillary adenocarcinoma	8260/3
Tubular adenocarcinoma	8211/3
Mucinous adenocarcinoma	8480/3
Poorly cohesive carcinoma (including signet ring cell carcinoma and other variants)	8490/3*
Mixed adenocarcinoma	8255/3
Adenosquamous carcinoma	8560/3
Carcinoma with lymphoid stroma (medullary carcinoma)	8512/3
Hepatoid adenocarcinoma	8576/3
Squamous cell carcinoma	8070/3
Undifferentiated carcinoma	8020/3
Neuroendocrine neoplasms[b]	
Neuroendocrine tumour (NET)	
NET G1 (carcinoid)	8240/3
NET G2	8249/3
Neuroendocrine carcinoma (NEC)	8246/3
Large cell NEC	8013/3
Small cell NEC	8041/3
Mixed adenoneuroendocrine carcinoma	8244/3
EC cell, serotonin-producing NET	8241/3
Gastrin-producing NET (gastrinoma)	8153/3
Mesenchymal tumours	
Glomus tumour	8711/0
Granular cell tumour	9580/0
Leiomyoma	8890/0
Plexiform fibromyxoma	8811/0*
Schwannoma	9560/0
Inflammatory myofibroblastic tumour	8825/1
Gastrointestinal stromal tumour	8936/3
Kaposi sarcoma	9140/3
Leiomyosarcoma	8890/3
Synovial sarcoma	9040/3
Lymphomas	
Secondary tumours	

WHO Classification of Tumours of the Digestive System, 4th edition (Bosmann FT, Carneiro F, Hruban RH, Theise ND) IARC Press 2010

WHO Classification

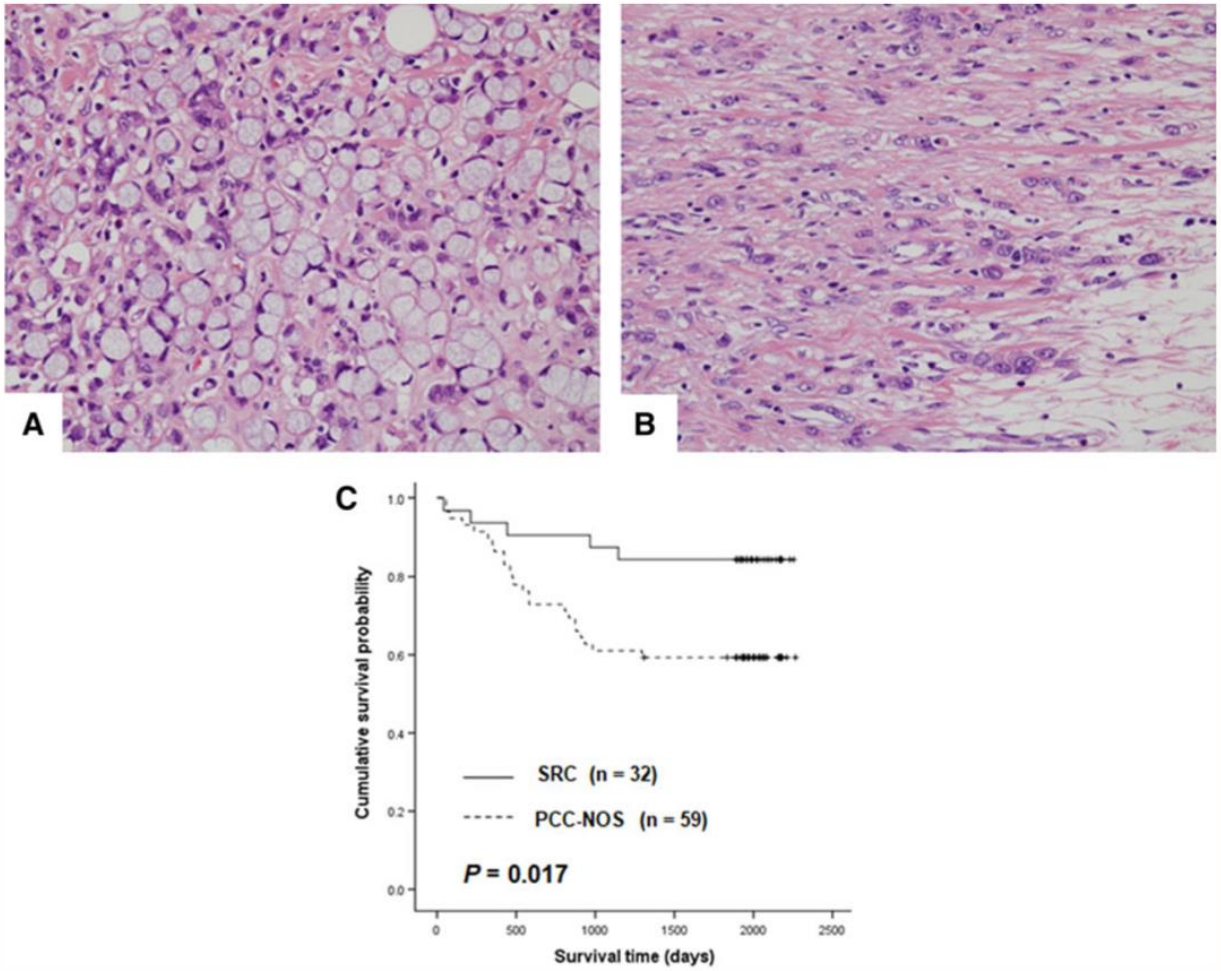
- **Poorly cohesive carcinomas** are composed of neoplastic cells that are isolated or arranged in small aggregates. These encompass:
  - **Signet-ring cell type** is defined as a tumour composed predominantly or exclusively of signet-ring cells, characterized by a central optically clear, globoid droplet of cytoplasmic mucin with an eccentrically placed nucleus
  - **Other cellular variants** of poorly cohesive carcinoma include tumours composed of neoplastic cells resembling histiocytes or lymphocytes; others have deeply eosinophilic cytoplasm; some poorly cohesive cells may show irregular bizarre nuclei
- **Mixed carcinomas** display a mixture of discrete morphologically identifiable glandular /tubular/papillary) and signet-ring/poorly cohesive cellular histological components (any component should be reported)

WHO Classification of Tumours of the Digestive System, 4th edition (Bosmann FT, Carneiro F, Hruban RH, Theise ND) IARC Press 2010

Gastric poorly cohesive carcinoma: a correlative study of mutational signatures and prognostic significance based on histopathological subtypes

Chae H Kwon,[1,2] Young K Kim,[1,2] Sojeong Lee,[1,2] Ahrong Kim,[1,2] Hye J Park,[1,2] Yuri Choi,[1,2] Yeo J Won,[1,2] Do Y Park[1,2] & Gregory Y Lauwers[3]

[1]*Department of Pathology, Pusan National University Hospital and Pusan National University School of Medicine,* [2]*BioMedical Research Institute, Pusan National University Hospital, Busan, Korea, and* [3]*Department of Pathology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33162, USA*



Kwon et al. Histopathology 2018

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Table 3. Relationship of five gene mutations and morphological features of 91 gastric poorly cohesive carcinomas

		Gastric poorly cohesive carcinoma, *n* (%)				
		SRC-predominant		PCC-NOS-predominant		
Genes	*n*	Pure SRC	Mixed SRC	Pure PCC-NOS	Mixed PCC-NOS	*P*-value*
RHOA						
Wild type	76	29 (38.2)	3 (3.9)	23 (30.3)	21 (27.6)	0.002
Mutant	15	0 (0.0)	0 (0.0)	1 (6.7)	14 (93.3)	
BRAF						
Wild type	75	27 (36.0)	3 (4.0)	21 (28.0)	24 (32.0)	0.036
Mutant	16	2 (12.5)	0 (0.0)	3 (18.3)	11 (68.8)	
PI3CA						
Wild type	71	27 (38.0)	2 (2.8)	19 (26.8)	23 (32.4)	0.033
Mutant	20	2 (10.0)	1 (5.0)	5 (25.0)	12 (60.0)	
SMAD4						
Wild type	79	28 (35.4)	3 (3.8)	21 (26.6)	27 (34.2)	0.050
Mutant	12	1 (8.3)	0 (0.0)	3 (25.0)	8 (66.7)	
TP53						
Wild type	54	21 (38.9)	3 (5.6)	15 (27.8)	15 (27.8)	0.025
Mutant	37	8 (21.6)	0 (0.0)	9 (24.3)	20 (54.1)	

PCC-NOS, Poorly cohesive carcinoma (not otherwise specified); SRC, Signet ring cell.

Kwon et al. Histopathology 2018

Gastric Cancer
https://doi.org/10.1007/s10120-018-0868-0

SPECIAL ARTICLE

Medizinische Universität Graz

Consensus on the pathological definition and classification of poorly cohesive gastric carcinoma

C. Mariette[1] · F. Carneiro[2] · H. I. Grabsch[3,4] · R. S. van der Post[5] · W. Allum[6] · Giovanni de Manzoni[7] on behalf of European Chapter of International Gastric Cancer Association

Table 2 Comparison of different classifications of gastric cancer (Laurén, Nakamura, WHO and Japanese classifications)

Laurén (1965)	Nakamura (1968)	WHO (2010)		Japanese classification (2017)	
Intestinal	Differentiated	Common type:	Papillary Tubular	Common type	Papillary: pap Tubular 1 (well-differentiated): tub1 Tubular 2 (moderately differentiated):
Intestinal/diffuse	Differentiate undifferent				ous
Diffuse	Undifferentia				arcinoma: sig 2 (non-solid type): por2
Mixed	Undifferentia				ption according to the propor-
Indeterminate	Undifferentia				or2 > sig > tub2) 1 (solid type): por1
					ierentiated carcinoma

To standardize the definition of SRC cancers, we propose that only WHO PC carcinomas with more than 90% poorly cohesive cells having classical signet ring cell morphology should be classified as SRC carcinomas.

We propose to use the following subclassification of PC and SRC carcinomas (Fig. 3):

- Signet ring cell (SRC) type (> 90% of signet ring cells)
- Combined poorly cohesive NOS and SRC carcinoma (PC-NOS/SRC; <90% but > 10% of signet ring cells)
- Poorly cohesive NOS (PC-NOS; <10% of signet ring cells)

Mariette et al. Gastric Cancer 2019

Gastric Cancer
https://doi.org/10.1007/s10120-018-0868-0

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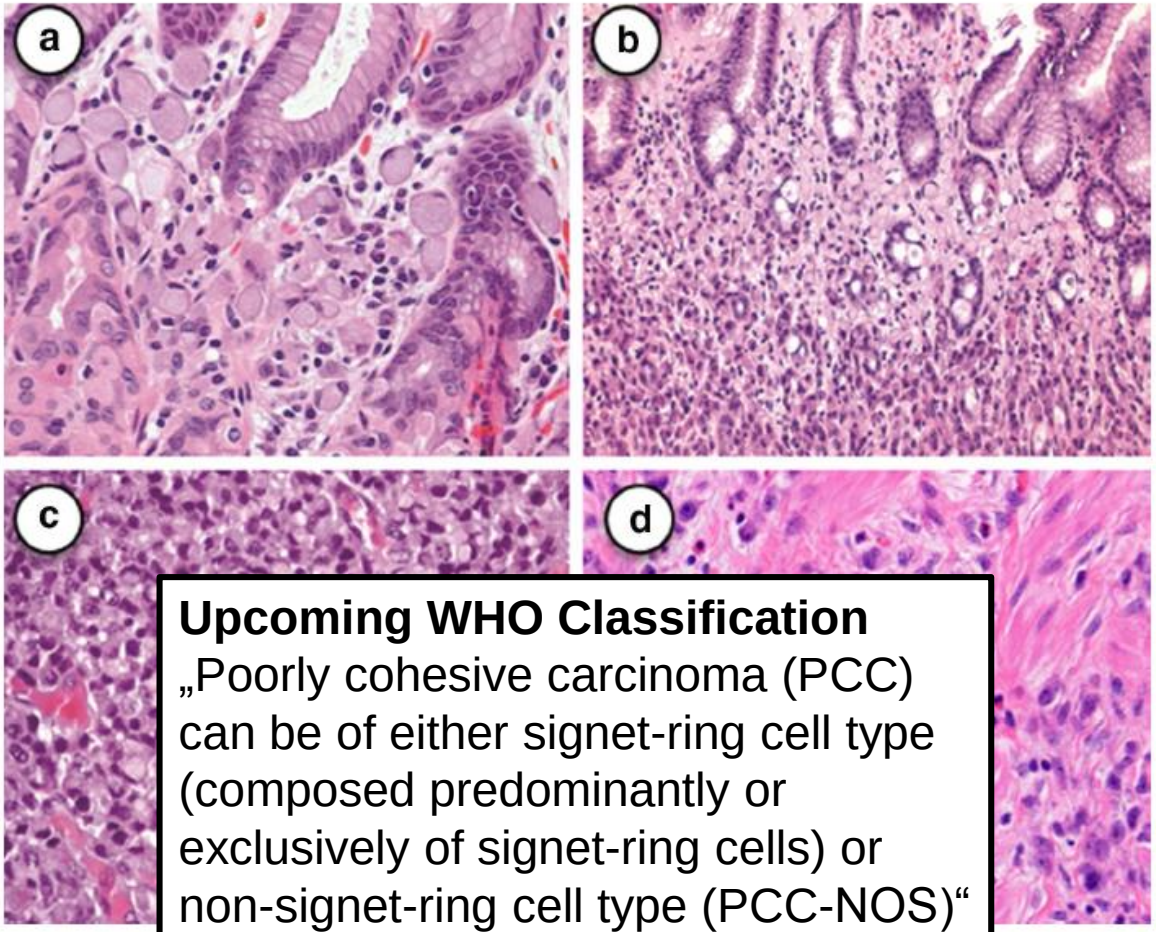


Fig. 3 Poorly cohesive gastric carcinoma, examples of morphology. **a** Signet ring cell carcinoma (SRCC) (> 90% of signet ring cells): classical signet ring cells are seen at the superficial layer of gastric mucosa; **b** combined PCC-NOS and SRCC (PCC-NOS/ SRC) (< 90% but > 10% of signet ring cells): this case has two components, the superficial part is composed of classical signet ring cells and the deeper part is composed by poorly cohesive, non-signet ring cells; **c** combined PCC-NOS and SRCC (PCC-NOS/ SRC) (< 90% but > 10% of signet ring cells): in this case, the two cell types (signet ring and poorly cohesive cells) are intermingled; **d** poorly cohesive carcinoma NOS (PCC-NOS) (< 10% of signet ring cells): the poorly cohesive, non-signet ring cells, are invading the muscle layer (H&E, original magnifications ×200–400)

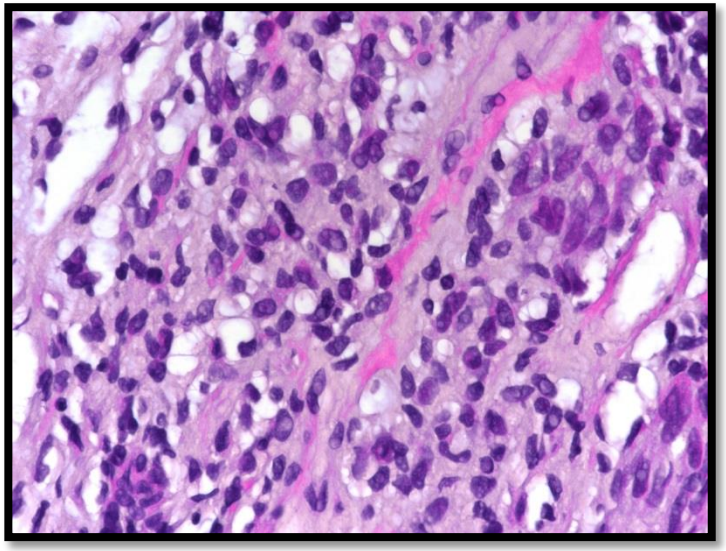
Upcoming WHO Classification

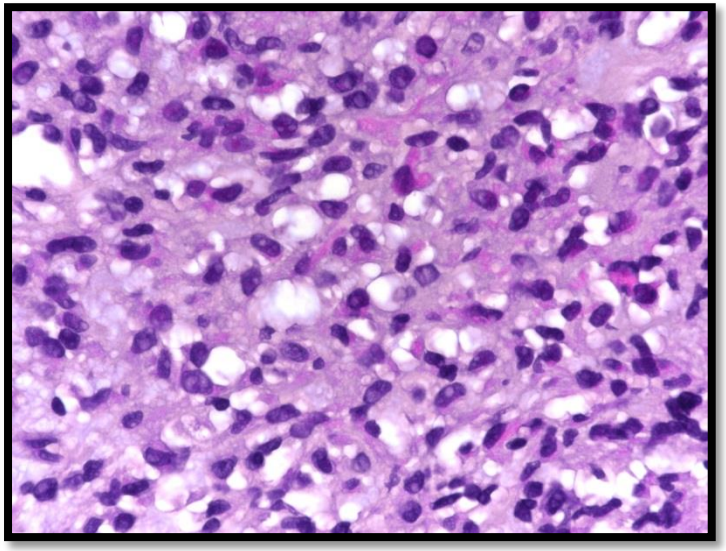
„Poorly cohesive carcinoma (PCC) can be of either signet-ring cell type (composed predominantly or exclusively of signet-ring cells) or non-signet-ring cell type (PCC-NOS)“

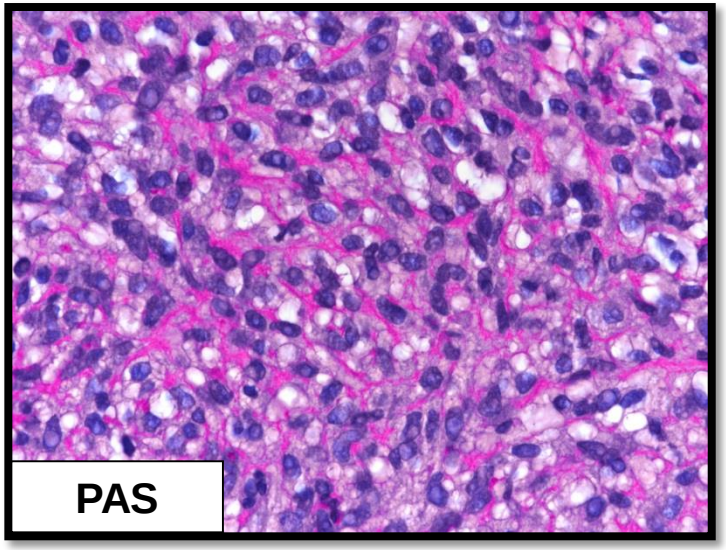
Mariette et al. Gastric Cancer 2019

A potential pitfall…

Gastric ulceration suspicious for malignancy



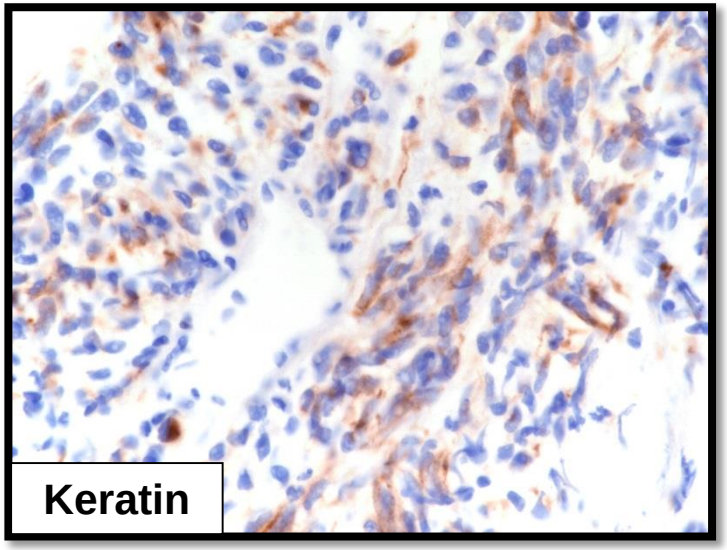


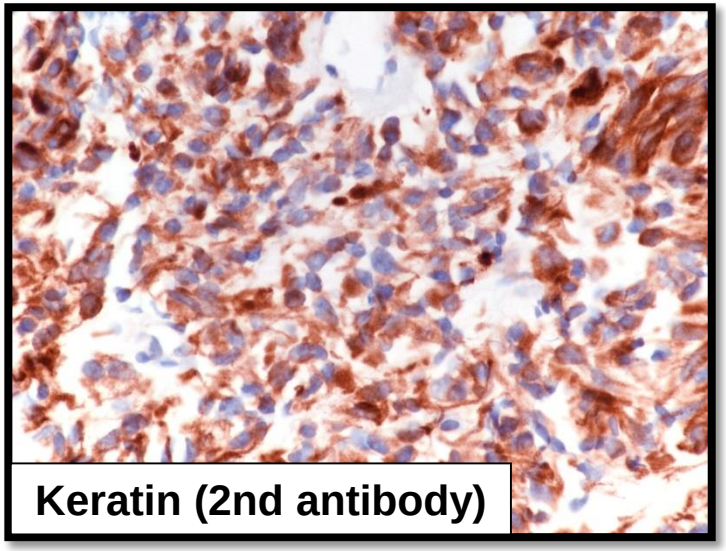


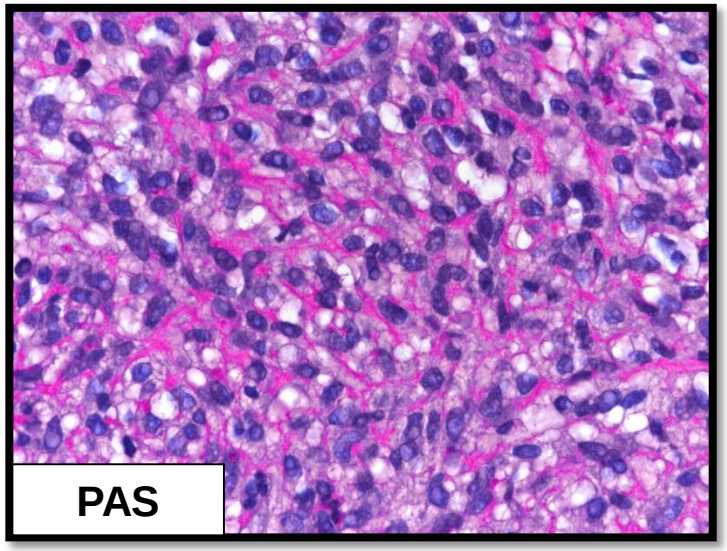
Poorly cohesive adenocarcinoma?

Better do keratin staining!

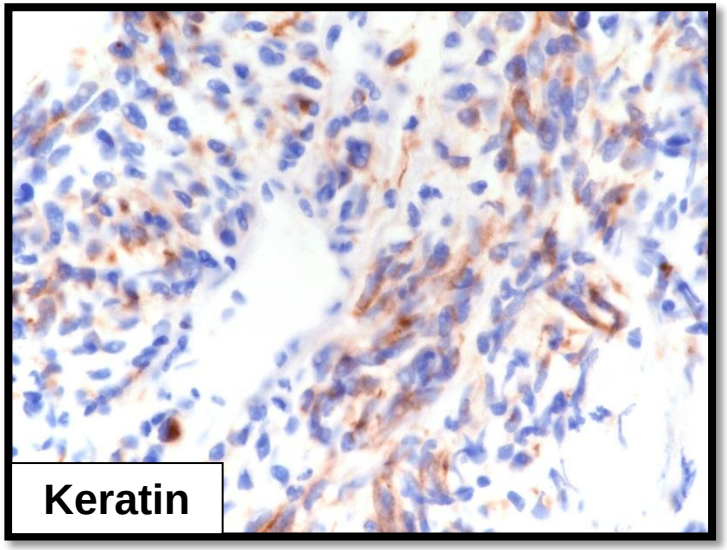
Gastric ulceration suspicious for malignancy

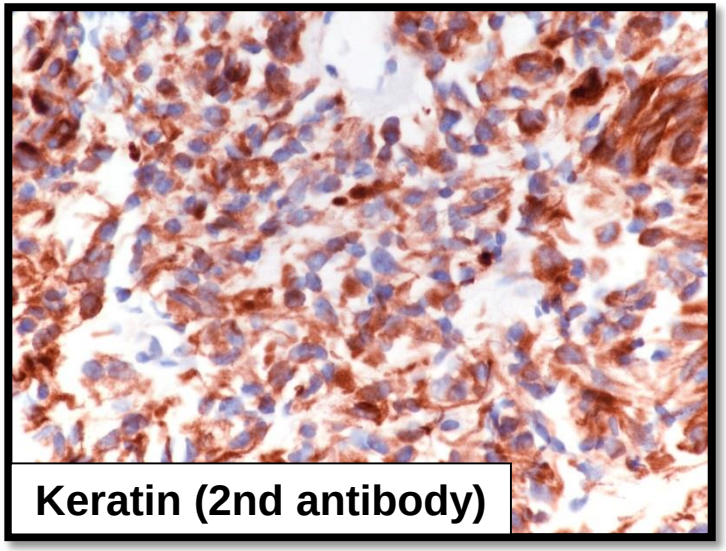


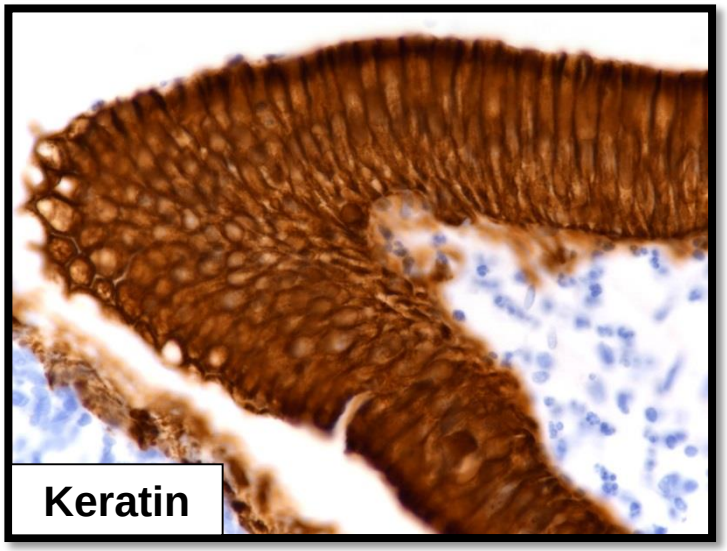




Gastric ulceration suspicious for malignancy



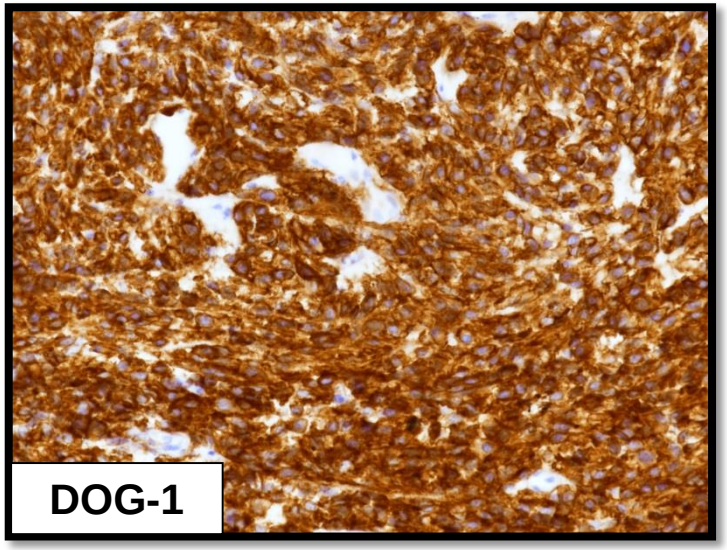


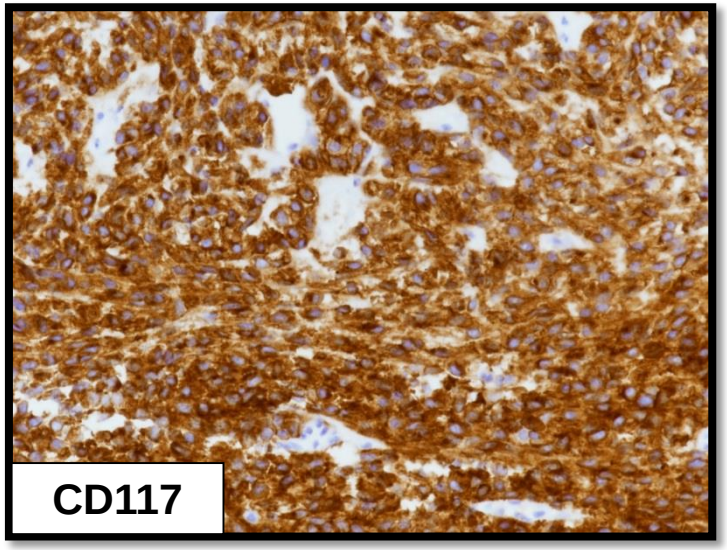


Poorly cohesive adenocarcinoma?

Better do additional staining!

Gastric ulceration suspicious for malignancy





Final diagnosis:

epitheloid (keratin positive) GIST

PDGFRalpha mutation positive

Towards a molecular classification of gastric cancer…

Gastroenterology 2015;149:1153–1162

Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma

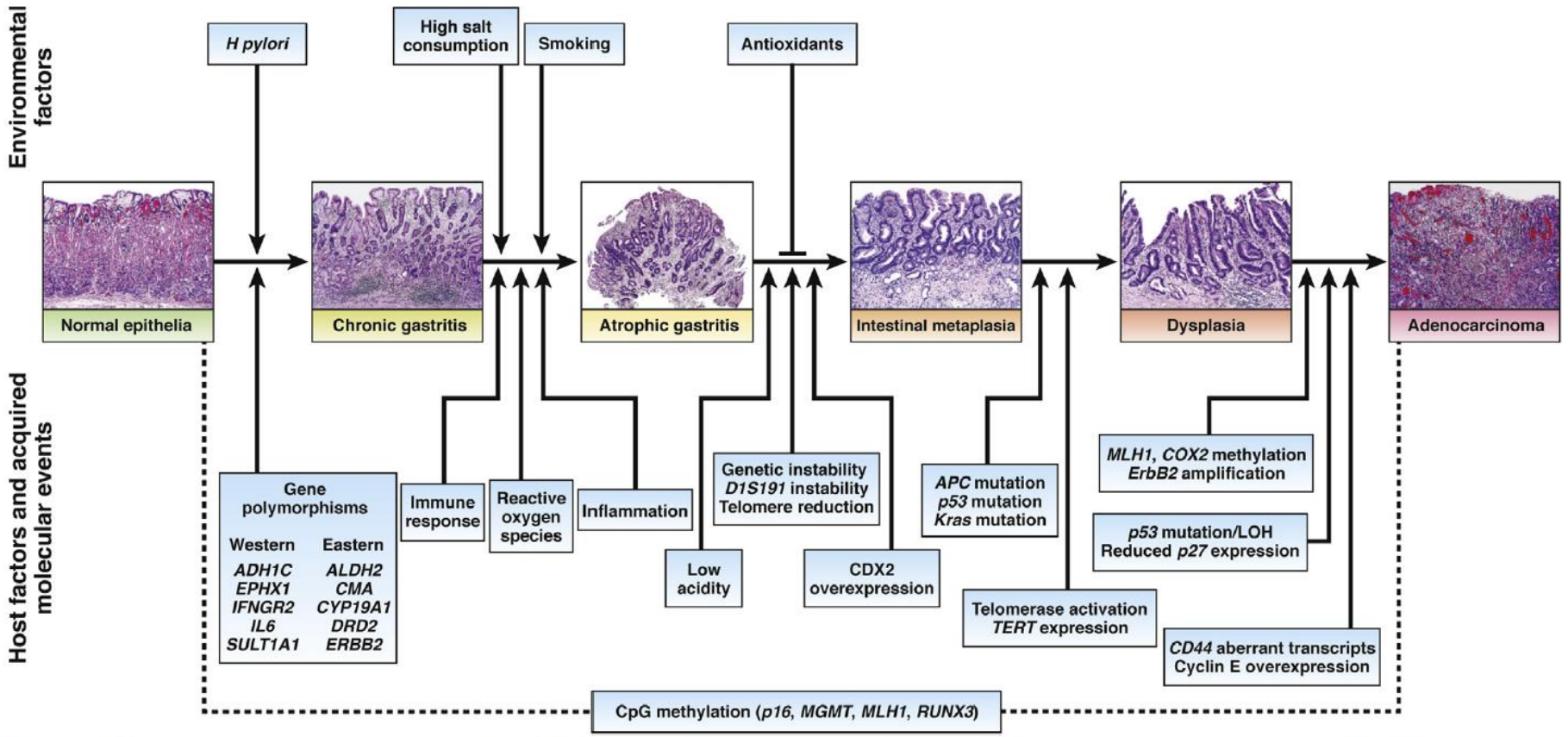


Figure 1. Cause and pathogenesis of intestinal-type GC. A summary of current knowledge of the cause and pathogenesis of intestinal type GC is shown, including host and environmental factors as well as acquired molecular events.[52,143–145] GC, gastric cancer.

Tan & Yeoh. Gastroenterology 2015

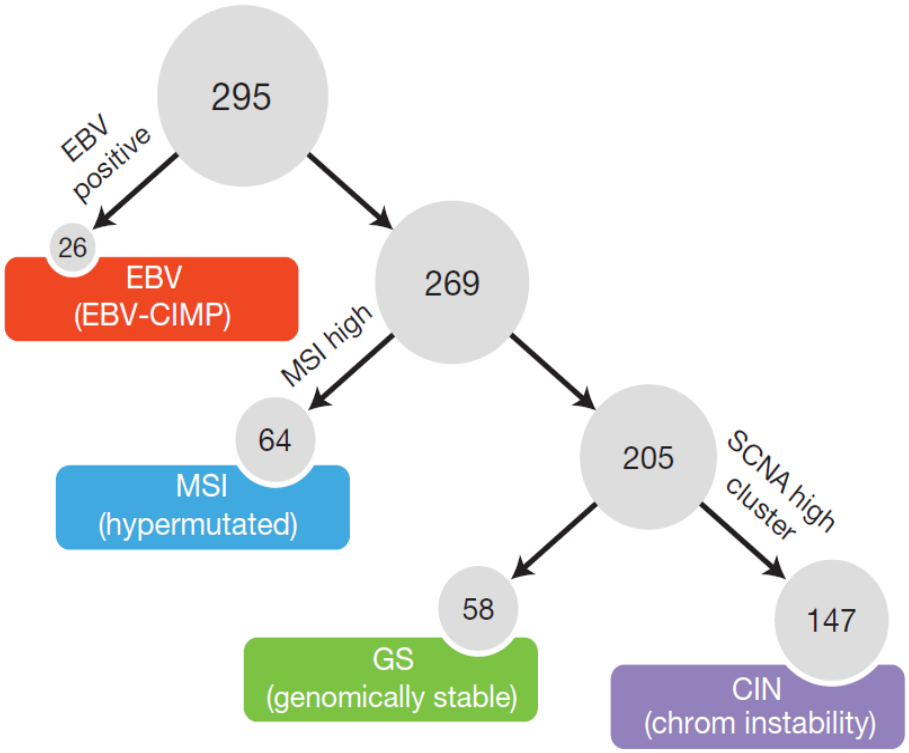
ARTICLE

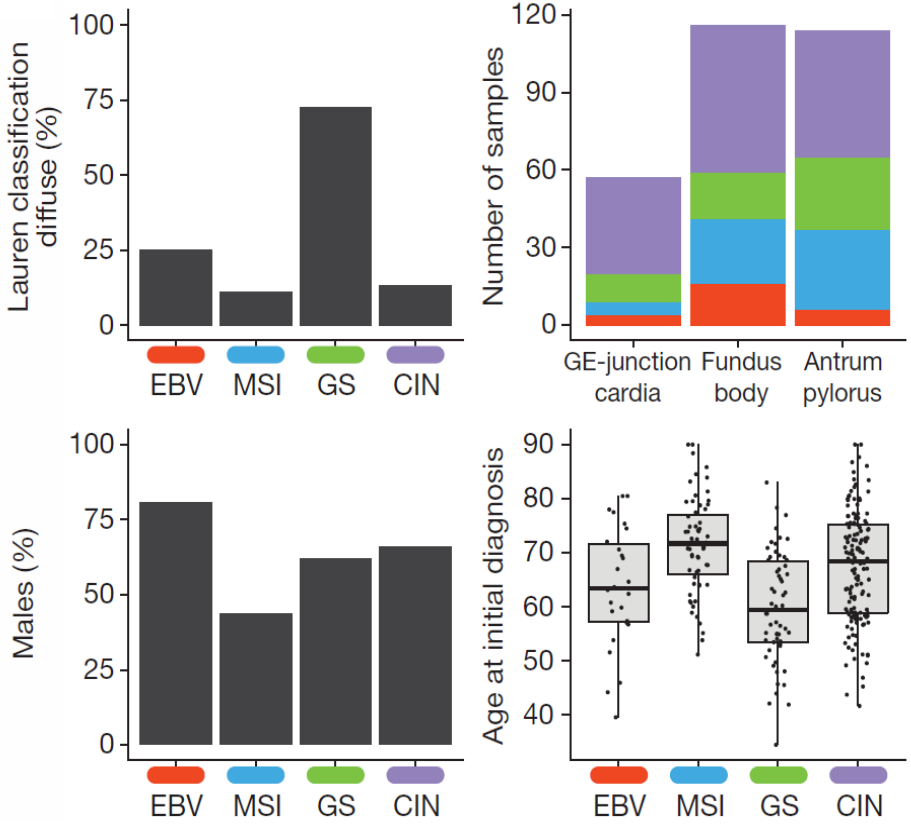
OPEN

doi:10.1038/nature13480

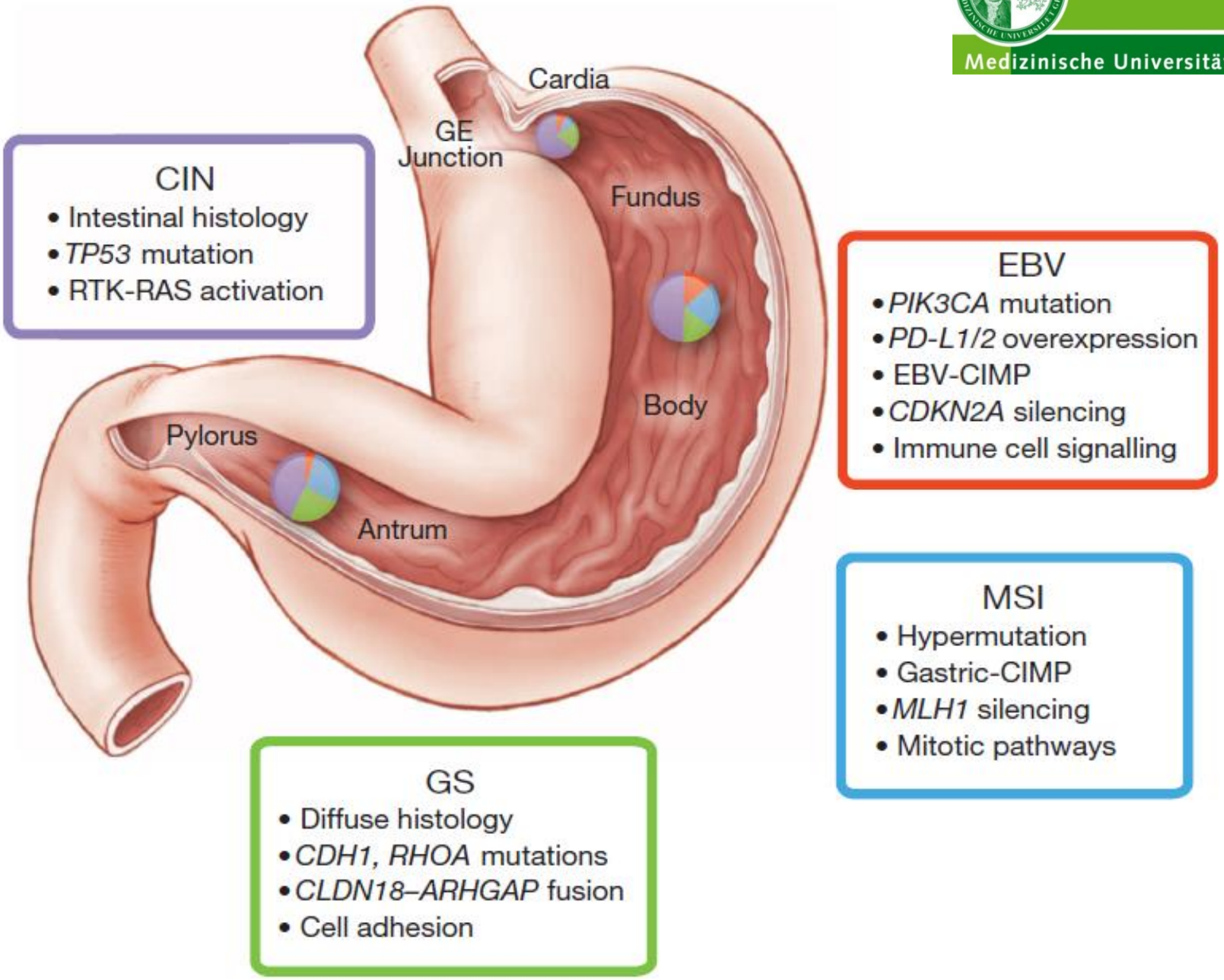
Comprehensive molecular characterization of gastric adenocarcinoma

The Cancer Genome Atlas Research Network*





Cancer Genome Atlas Research Network. Nature 2014



Cancer Genome Atlas Research Network. Nature 2014

Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes

Razvan Cristescu[1,12], Jeeyun Lee[2,12], Michael Nebozhyn[1,12], Kyoung-Mee Kim[3,12], Jason C Ting[4], Swee Seong Wong[4], Jiangang Liu[4], Yong Gang Yue[4], Jian Wang[4], Kun Yu[4,11], Xiang S Ye[4], In-Gu Do[3], Shawn Liu[5], Lara Gong[5], Jake Fu[6], Jason Gang Jin[6], Min Gew Choi[7], Tae Sung Sohn[7], Joon Ho Lee[7], Jae Moon Bae[7], Seung Tae Kim[2], Se Hoon Park[2], Insuk Sohn[8], Sin-Ho Jung[8], Patrick Tan[9,10], Ronghua Chen[1], James Hardwick[1,11], Won Ki Kang[2], Mark Ayers[1], Dai Hongyue[1,11], Christoph Reinhard[4], Andrey Loboda[1], Sung Kim[7] & Amit Aggarwal[4]

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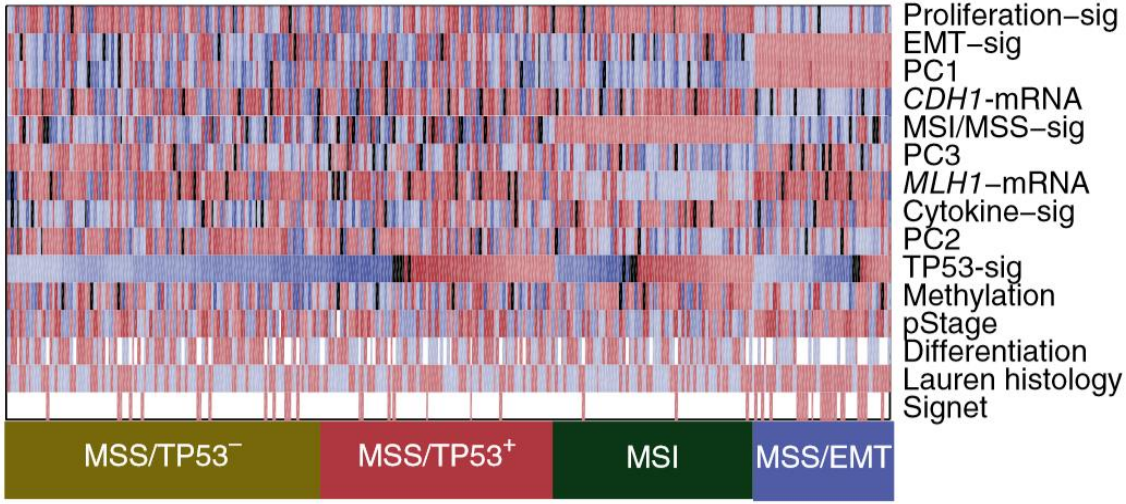
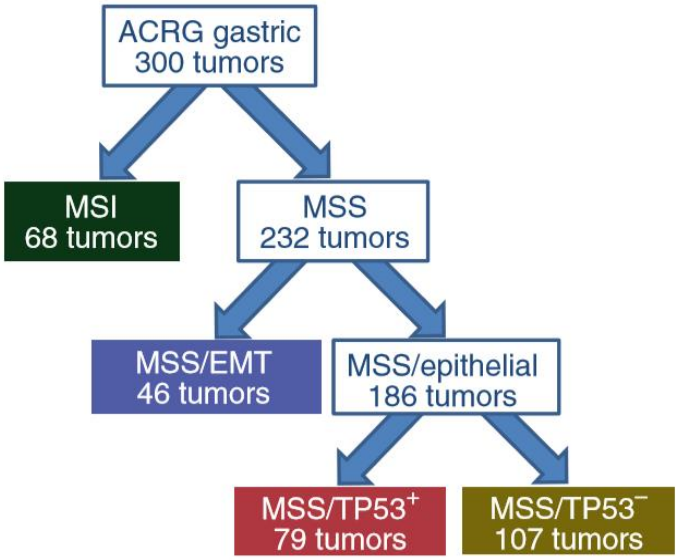
ACRG gastric tumors



ACRG gastric tumors



ACRG: Asian Cancer Research Group

Cristescu et al. Nat Med 2015

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Medizinische Universität Graz

Table 1 The four molecular subtypes and patient characteristics.

Characteristics	MSS/TP53⁻	MSS/TP53⁺	MSI	MSS/EMT	*P* value
N	107 (35.7%)	79 (26.3%)	68 (22.7%)	46 (15.3%)	
Median age	65 (30–82)	64 (24–81)	66 (31–84)	53 (28–86)	0.0324[a]
Sex					
Male	70 (65.4%)	57 (72.2%)	45 (66.2%)	27 (58.7%)	0.4863
Female	37 (34.6%)	22 (27.8%)	23 (33.8%)	19 (41.3%)	
Location of tumor					
Antrum	61 (57.0%)	26 (32.9%)	51 (75.0%)	17 (37.0%)	
Body	37 (34.6%)	36 (45.6%)	13 (19.1%)	21 (45.6%)	<0.0001
Cardia, GE junction	9 (8.4%)	14 (17.7%)	4 (5.9%)	5 (10.9%)	
Whole, multicentric	0 (0.0%)	3 (3.8%)	0 (0.0%)	3 (6.5%)	
Grade and WHO classification					
W/D and M/D tubular	54 (50.5%)	32 (40.5%)	33 (48.5%)	4 (8.7%)	
P/D tubular	37 (34.6%)	31 (39.2%)	29 (42.7%)	19 (41.3%)	< 0.0001
Signet ring cell	9 (8.4%)	5 (6.3%)	3 (4.4%)	20 (43.5%)	
Mucinous	4 (3.7%)	3 (3.8%)	0 (0.0%)	1 (2.2%)	
Others	3 (2.8%)	8 (10.1%)	3 (4.4%)	2 (4.3%)	

Cristescu et al. Nat Med 2015

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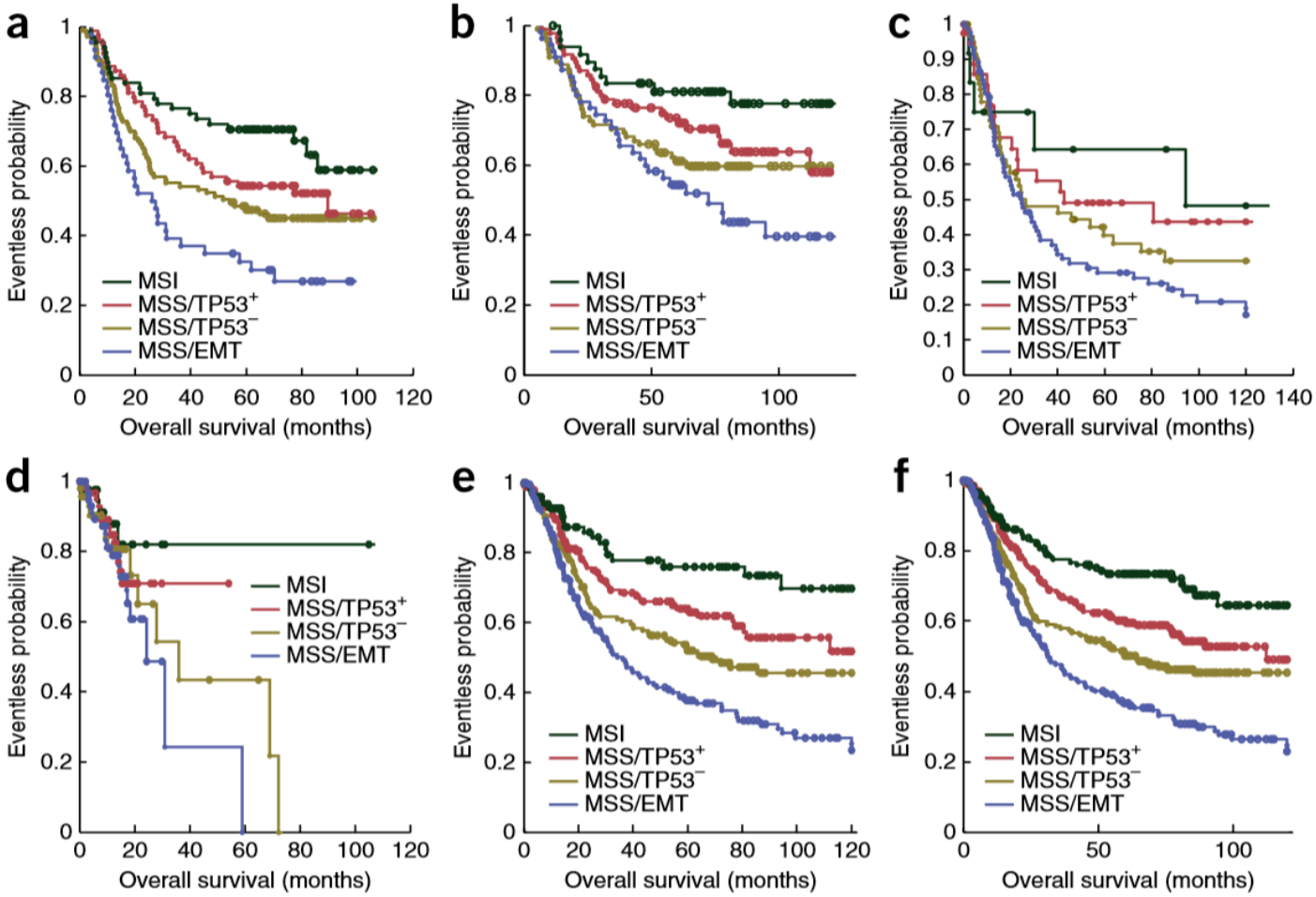
Characteristics	MSS/TP53⁻	MSS/TP53⁺	MSI	MSS/EMT	*P* value
Lauren type					
Intestinal	58 (54.2%)	38 (48.1%)	42 (61.8%)	8 (17.4%)	
Diffuse	42 (39.3%)	36 (45.6%)	20 (29.4%)	37 (80.4%)	<0.0001
Mixed	7 (6.5%)	4 (5.1%)	5 (7.4%)	1 (2.2%)	
Missing	0 (0.0%)	1 (1.2%)	1 (1.0%)	0 (0.0%)	
pT stage					
T2	70 (65.4%)	57 (72.1%)	47 (69.1%)	14 (30.4%)	
T3	29 (27.1%)	18 (22.8%)	17 (25.0%)	27 (58.7%)	0.0003
T4	8 (7.5%)	4 (5.1%)	4 (5.9%)	5 (10.9%)	
pN Stage					
N0	12 (11.2%)	4 (5.1%)	16 (23.5%)	6 (13.0%)	
N1	41 (38.3%)	45 (57.0%)	31 (45.6%)	14 (30.4%)	0.0058
N2	31 (29.0%)	19 (24.0%)	15 (22.1%)	15 (32.6%)	
N3	23 (21.5%)	11 (13.9%)	6 (8.8%)	11 (23.9%)	
EBV					
Positive	2 (1.9%)	12 (15.2%)	0 (0.0%)	4 (8.7%)	0.0002
Negative	94 (87.9%)	60 (75.9%)	63 (92.6%)	40 (87.0%)	
Missing	11 (10.2%)	7 (8.9%)	5 (7.4%)	2(4.3%)	

Cristescu et al. Nat Med 2015

Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes

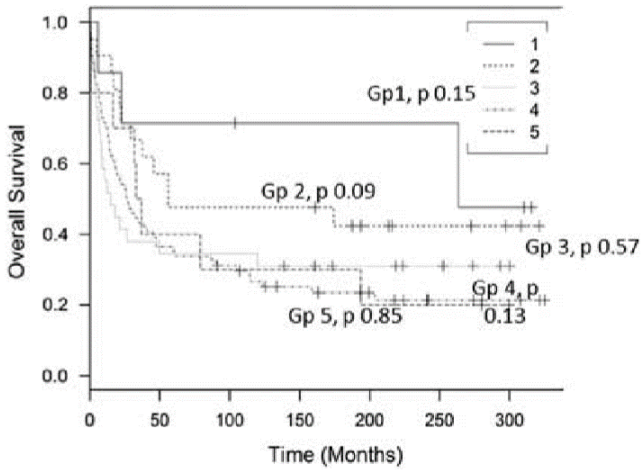
Razvan Cristescu[1,12], Jeeyun Lee[2,12], Michael Nebozhyn[1,12], Kyoung-Mee Kim[3,12], Jason C Ting[4], Swee Seong Wong[4], Jiangang Liu[4], Yong Gang Yue[4], Jian Wang[4], Kun Yu[4,11], Xiang S Ye[4], In-Gu Do[3], Shawn Liu[5], Lara Gong[5], Jake Fu[6], Jason Gang Jin[6], Min Gew Choi[7], Tae Sung Sohn[7], Joon Ho Lee[7], Jae Moon Bae[7], Seung Tae Kim[2], Se Hoon Park[2], Insuk Sohn[8], Sin-Ho Jung[8], Patrick Tan[9,10], Ronghua Chen[1], James Hardwick[1,11], Won Ki Kang[2], Mark Ayers[1], Dai Hongyue[1,11], Christoph Reinhard[4], Andrey Loboda[1], Sung Kim[7] & Amit Aggarwal[4]



Cristescu et al. Nat Med 2015

A protein and mRNA expression-based classification of gastric cancer

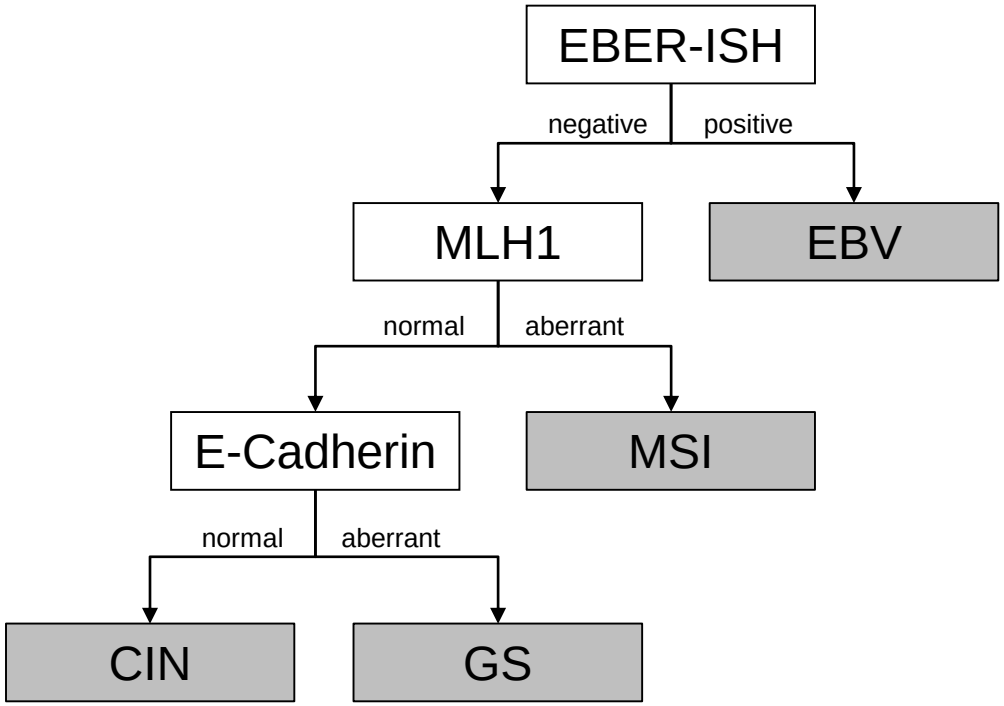
Namrata Setia[1], Agoston T Agoston[2], Hye S Han[1,3], John T Mullen[4], Dan G Duda[5], Jeffrey W Clark[6], Vikram Deshpande[1], Mari Mino-Kenudson[1], Amitabh Srivastava[2], Jochen K Lennerz[1], Theodore S Hong[5], Eunice L Kwak[6,*] and Gregory Y Lauwers[1,*]



Setia et al. Mod Pathol 2016

High-throughput Protein and mRNA Expression–based Classification of Gastric Cancers Can Identify Clinically Distinct Subtypes, Concordant With Recent Molecular Classifications

Sangjeong Ahn, MD, PhD,† So-Jeong Lee, MD,* Yonugkeum Kim, MD,* Ahrong Kim, MD,* Nari Shin, MD,‡ Kyung Un Choi, MD, PhD,* Chang-Hun Lee, MD, PhD,* Gi Yeong Huh, MD, PhD,§ Kyong-Mee Kim, MD, PhD,‖ Namrata Setia, MD,¶ Gregory Y. Lauwers, MD,# and Do Youn Park, MD, PhD**



Ahn et al. Am J Surg Pathol 2017

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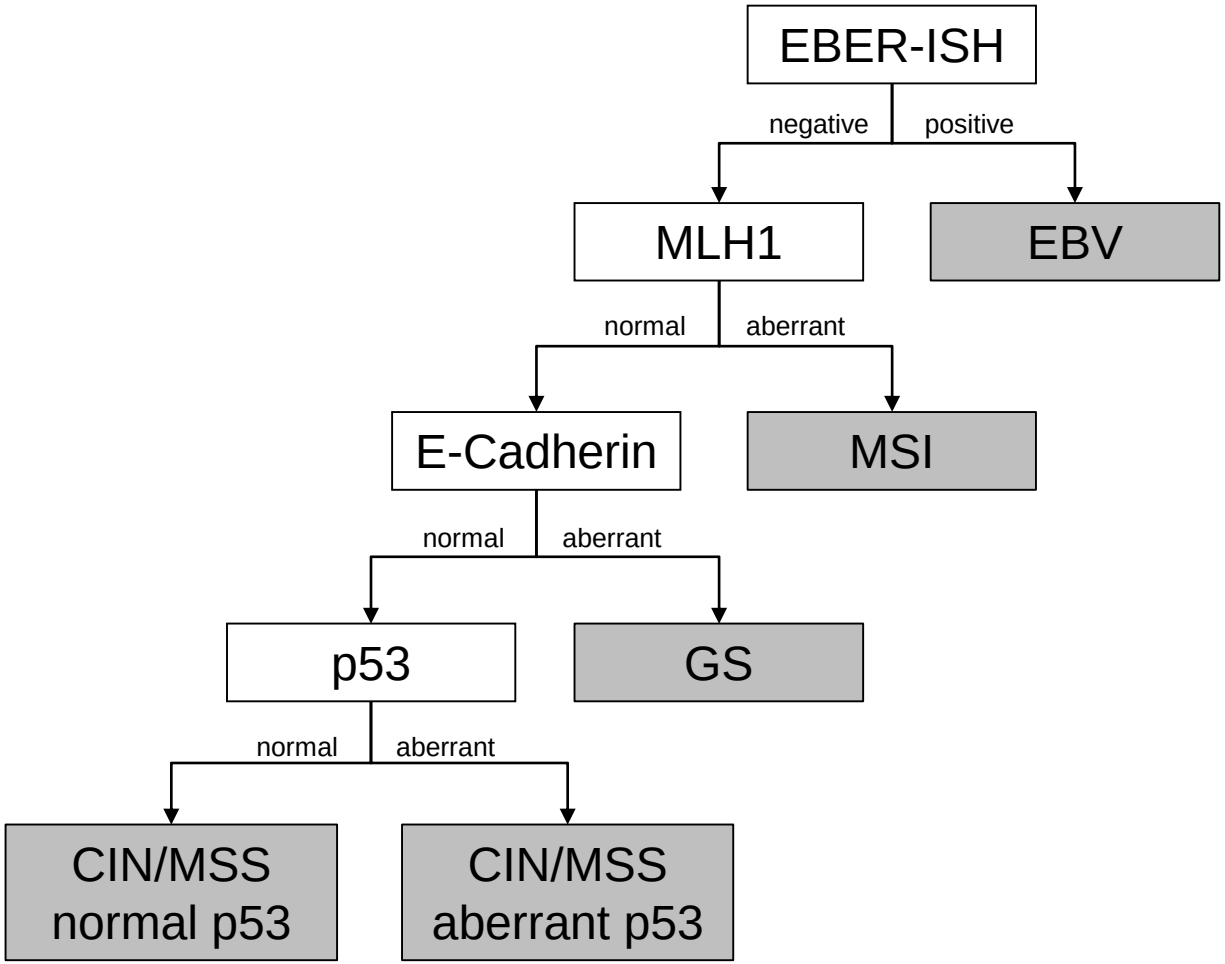

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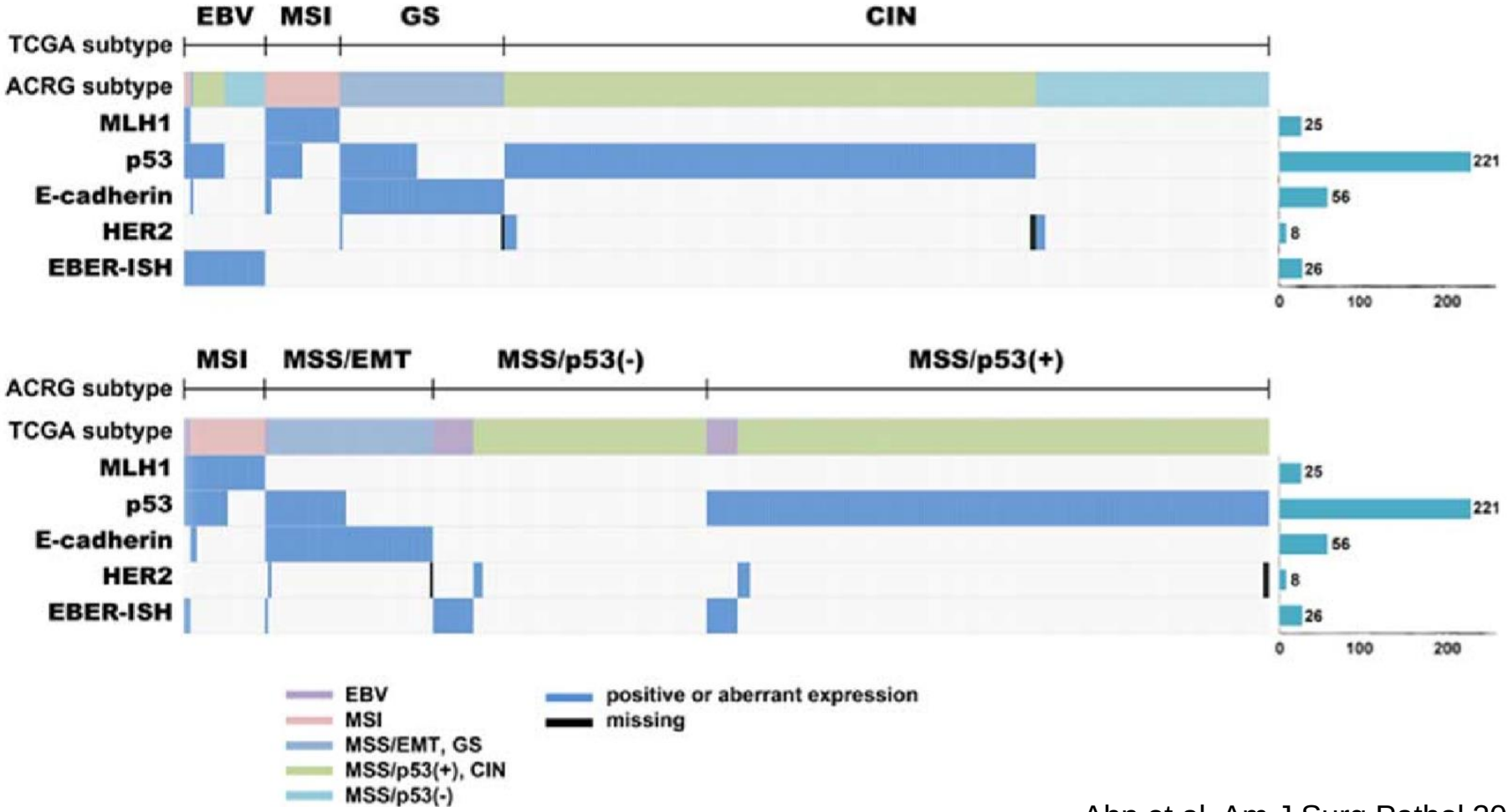

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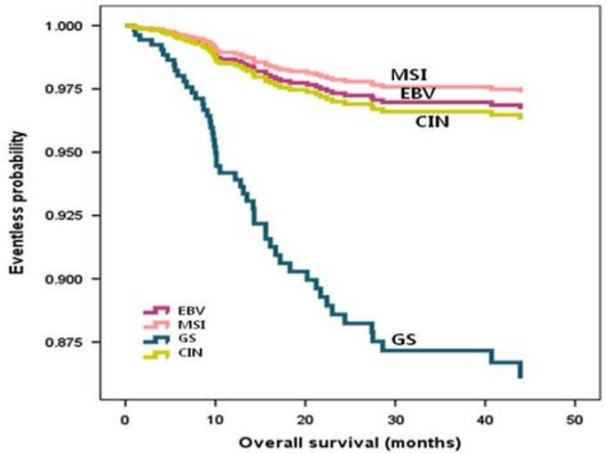
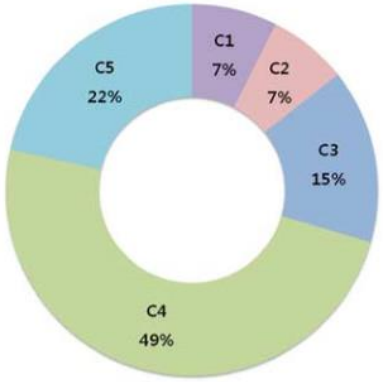
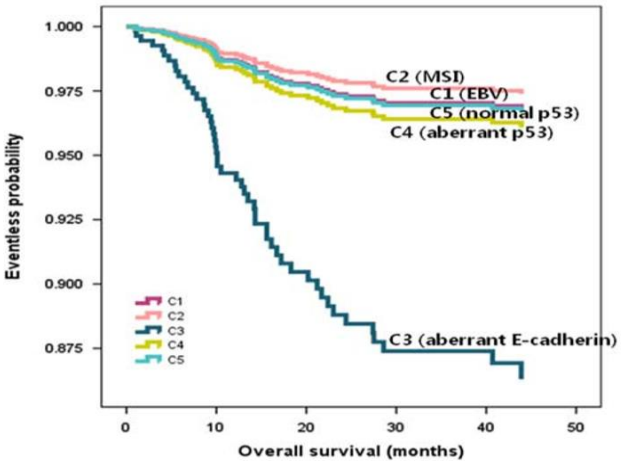
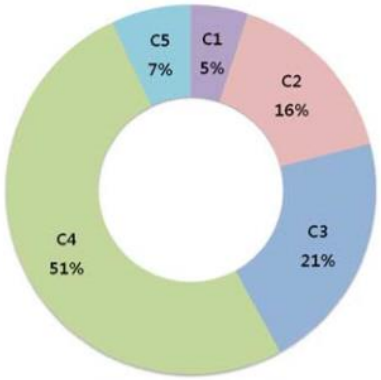




Asian cohort (current study)





Western cohort [16]

C1 (EBV tumors)
- Male predominance
- Body location
- Mostly poorly differentiated type
- 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
- Gastric type by mucin phenotype
- Better prognosis
- Molecular features:
  MLH1 promoter hypermethylation
  Hypermutation (occasional mutations in *PIK3CA, ERBB2, ERBB3, EGFR, ARID1*)

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

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

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

Ahn et al. Am J Surg Pathol 2017

Agenda

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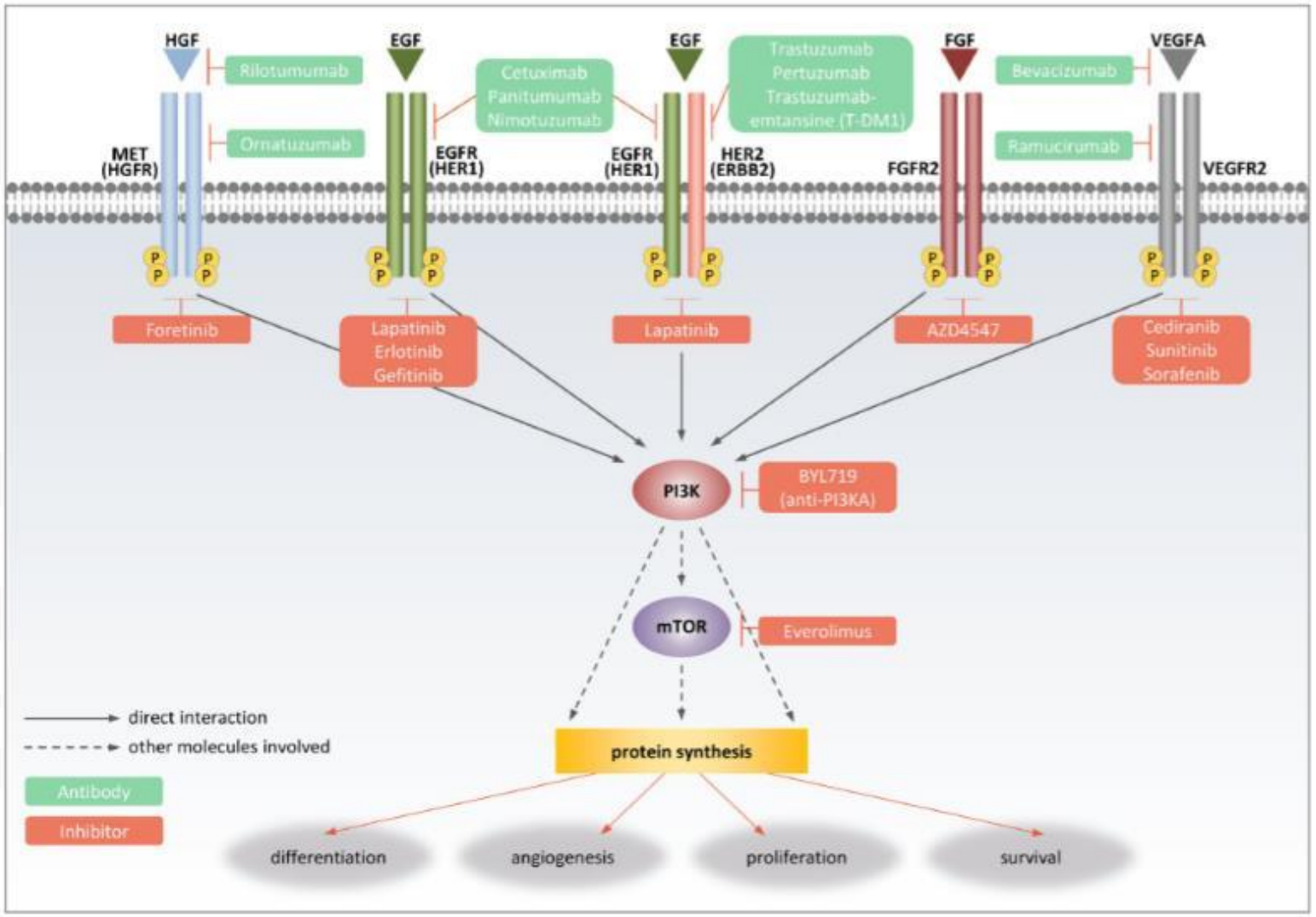
- Classification of gastric cancer
  - Histology
  - Molecular pathology
- **Predictive pathology**
  - HER2
  - PD-L1
  - Microsatellite instability
- Sporadic *vs* hereditary gastric cancer
- Take home messages

Biomarkers for gastric cancer: prognostic, predictive or targets of therapy?

Cecília Durães · Gabriela M. Almeida · Raquel Seruca · Carla Oliveira · Fátima Carneiro



Duraes et al. Virchows Arch 2014

Modern Pathology (2012) 25, 637–650

Medizinische Universität Graz

HER2 testing in gastric cancer: a practical approach

Josef Rüschoff[1,2], Wedad Hanna[3], Michael Bilous[4], Manfred Hofmann[2], Robert Y Osamura[5], Frédérique Penault-Llorca[6], Marc van de Vijver[7] and Giuseppe Viale[8]

Table 2 Comparison of differences between human epidermal growth factor receptor 2 (HER2) scoring in gastric and breast cancer[29]

		Gastric cancer	*Breast cancer*
Immunohistochemical scoring	Extent	Biopsy specimens ≥5 cells	≥10% (≥30%)[a]
	(Area cutoff)	Resection specimens: ≥10%	
	Circularity	Mostly missing (often only lateral in immunohistochemistry 2+/3+)	A must in immunohistochemistry 2+/3+
(Fluorescence) *in situ* hybridization analysis	Cell number	20 cohesive tumor cells showing highest gene count (add 20 new if ratio 1.8–2.2)	20 cohesive tumor cells showing highest gene count (add 20 new if ratio 1.8–2.2)
	Amplification	Ratio ≥2.0	Ratio ≥2.0 (≥2.2)[a]
HER2 positivity	Tumor type	About 30% of intestinal type gastric cancer about 15% of mixed type about 5% of diffuse type (signet ring type typically negative)	15–25% of ductal type (grade 2/3); almost never in subtypes such as lobular, medullary, and ductal grade 1
	Tumor location	About 30% at cardiac/gastro–esophageal junction About 15% of gastric cancer	No correlation
Patient selection	Fluorescence *in situ* hybridization *vs* immunohistochemistry	Immunohistochemistry more predictive than fluorescence *in situ* hybridization: immunohistochemistry primary test[b] fluorescence *in situ* hybridization only if immunohistochemistry 2+	Fluorescence *in situ* hybridization/immunohistochemistry equally predictive

[a]According to the American Society of Clinical Oncology/College of American Pathologists.[30]
[b]According to approval by the European Medicines Agency.[27]

Rüschoff et al. Mod Pathol 2012

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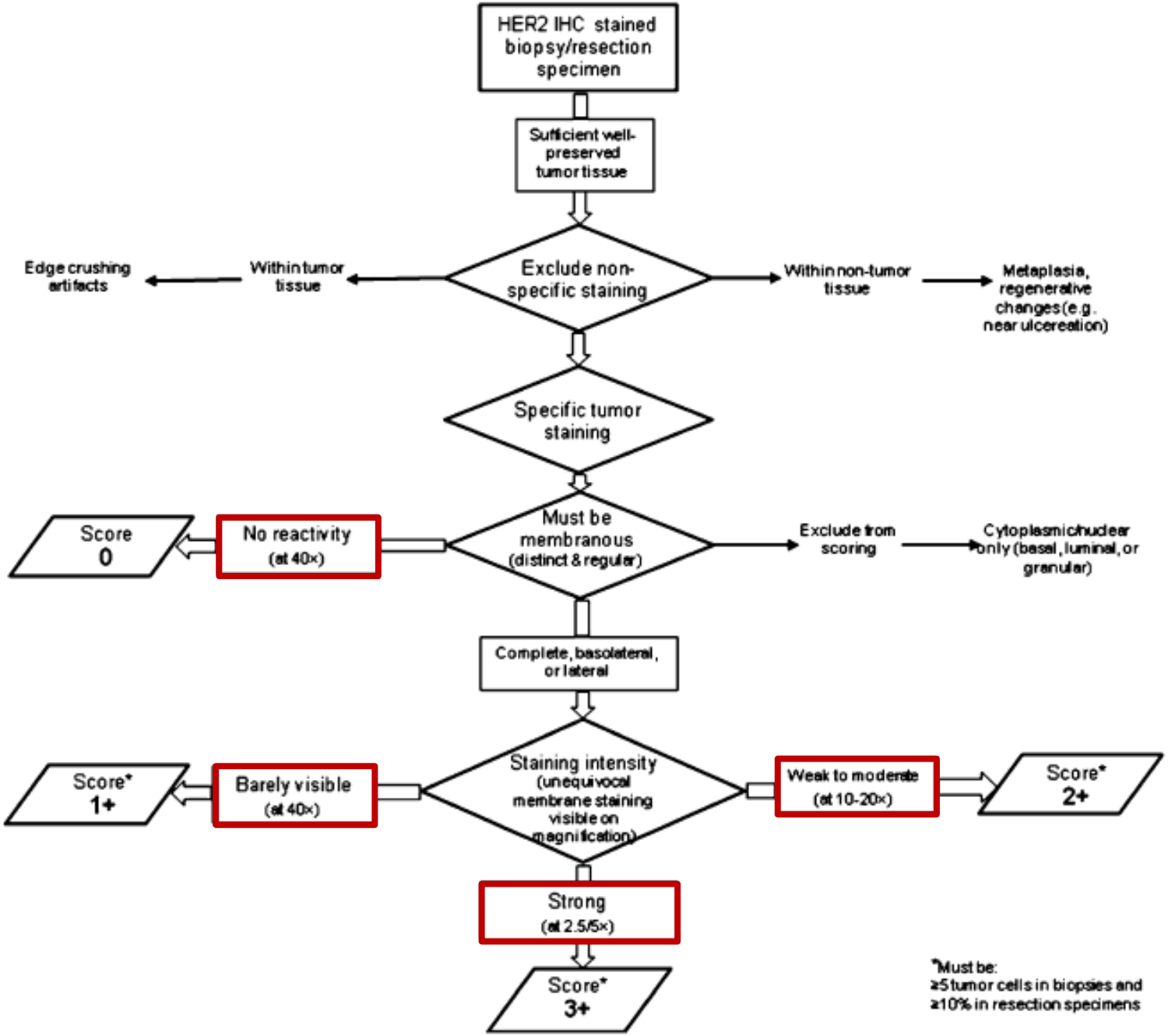
Table 1 Human epidermal growth factor receptor 2 (HER2) scoring criteria for gastric cancer

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

The „magnification rule“ should be used to assess HER2 positivity

Rüschoff et al. Mod Pathol 2012



Rüschoff et al. Virchows Arch 2010

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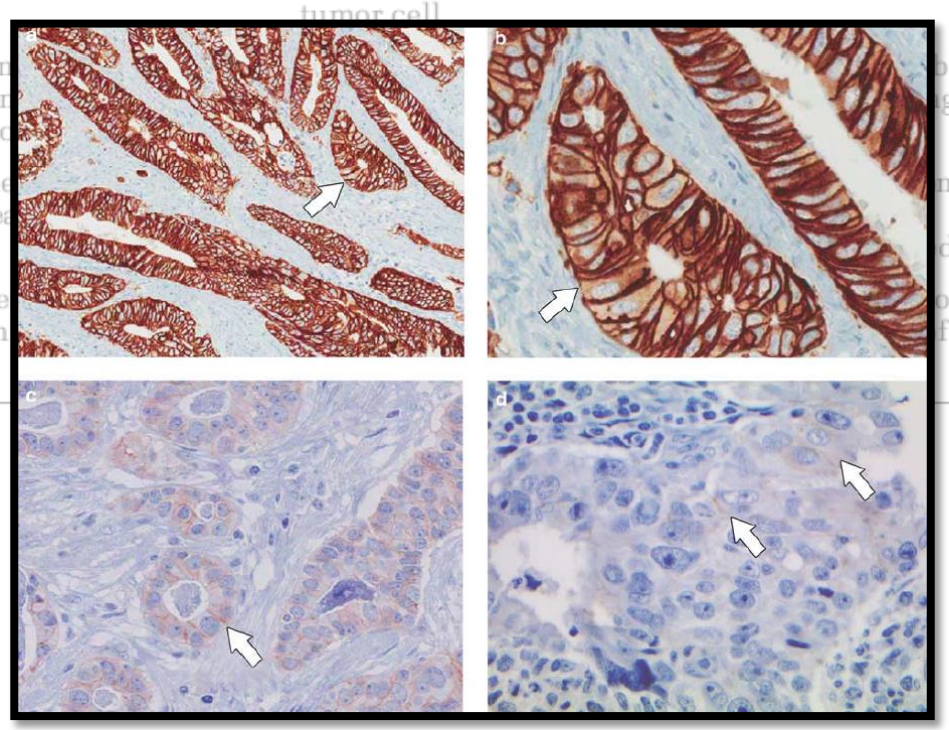
2+	Weak to moderate compl or lateral membranous re of tumor cells	mplete, d	Equivocal
3+	Strong complete, basolate membranous reactivity in tumor cells	olateral, f	Positive



Rüschoff et al. Mod Pathol 2012

HER2 Testing Algorithm

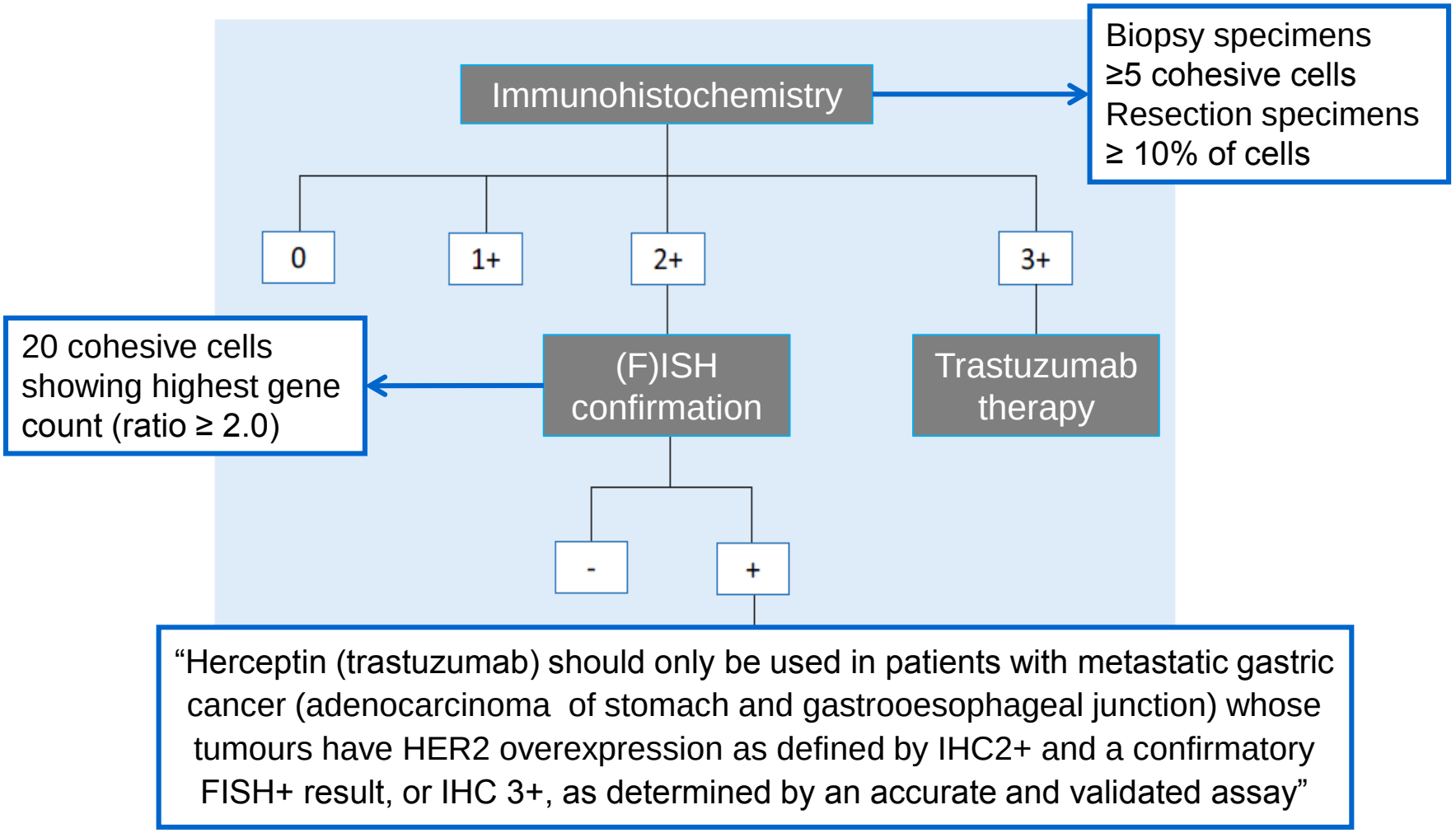


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Table 3 Human epidermal growth factor receptor 2 (HER2) testing recommendations in gastric cancer, (a) immunohistochemistry and (b) *in situ* hybridization

(a) *Immunohistochemistry*

Testing recommendations

- Representative surgical samples or an adequate number of viable biopsy specimens (ideally six to eight) are required
  - If few biopsies are available, all viable specimens should be tested
- Immunohistochemistry should be the initial HER2 testing methodology for gastric cancer and bright-field methodologies are preferred wherever possible
  - HER2-positive per European Medicines Agency license: immunohistochemistry 3+ or immunohistochemistry 2+/fluorescence *in situ* hybridization-positive or immunohistochemistry 2+/silver *in situ* hybridization-positive
  - Borderline immunohistochemistry 1+/immunohistochemistry 2+ cases and samples with focal and intense membranous reactivity in $<10\%$ cells may also be retested with fluorescence *in situ* hybridization or silver *in situ* hybridization (scores for both assays should be indicated separately on the report)
- Validated immunohistochemistry HER2 assays should be used

Scoring recommendations

- Due to the tumor heterogeneity (focal areas of positivity) and incomplete membrane staining commonly seen in gastric cancer, the gastric cancer-specific scoring criteria should be adhered to:
  - Surgical specimen cutoff: complete, basolateral, or lateral membranous reactivity in $\geq 10\%$ of cells
  - Biopsy specimen cutoff: complete, basolateral, or lateral membranous reactivity in ≥ 5 clustered cells
- The 'magnification rule' should be used in conjunction with the scoring criteria
- Borderline cases (immunohistochemistry 1+/immunohistochemistry 2+ or focal staining in $<10\%$ cells) that score fluorescence *in situ* hybridization-positive or silver *in situ* hybridization-positive may be considered HER2-positive (scores for both assays should be indicated separately on the report)

Rüschoff et al. Mod Pathol 2012

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(b) In situ hybridization

Testing recommendations

- Tumor samples classified as immunohistochemistry 2+ should be retested by fluorescence *in situ* hybridization or silver *in situ* hybridization to assess HER2 status
- Silver *in situ* hybridization is a more suitable methodology than fluorescence *in situ* hybridization for assessing HER2 status in gastric tumor samples as it is a bright-field methodology and thus allows for rapid identification of HER2-positive tumor foci within a heterogeneous sample
- Validated *in situ* hybridization HER2 assays should be used

Scoring recommendations

- The definition of fluorescence *in situ* hybridization or silver *in situ* hybridization positivity in gastric or gastro–esophageal junction cancer is a HER2:chromosome 17 ratio of ≥ 2.0
- The entire case should be screened for amplified regions (particularly important for fluorescence *in situ* hybridization samples where a bright-field image is not available)
- At least 20 evaluable, non-overlapping cells in the invasive component should be counted initially
- In borderline amplification cases, ~20 additional cells should be recounted or scoring should be performed in an alternative area of tissue
- The overall *HER2* gene count is important:
  - >6 *HER2* gene copies using single probe: considered positive
  - Four to six *HER2* gene copies: dual probe test advised and the ratio should be recalculated by counting an additional 20 cells

Ensuring quality and timely HER2 testing results

- The use of validated immunohistochemistry and *in situ* hybridization tests is strongly recommended and appropriate controls should be included in each run
- Turnaround time from initial diagnosis to reporting of results should ideally not exceed 5 working days and a multidisciplinary approach is required
- Centralized testing is recommended wherever possible and all laboratories should participate in validated quality assurance programs

Rüschoff et al. Mod Pathol 2012

Cancer Science
Japanese Cancer Association JCA
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Significant intratumoral heterogeneity of human epidermal growth factor receptor 2 status in gastric cancer: A comparative study of immunohistochemistry, FISH, and dual-color *in situ* hybridization

Kazuki Kanayama,[1] Hiroshi Imai,[1] Misao Yoneda,[2] Yoshifumi S. Hirokawa[1] and Taizo Shiraishi[1]

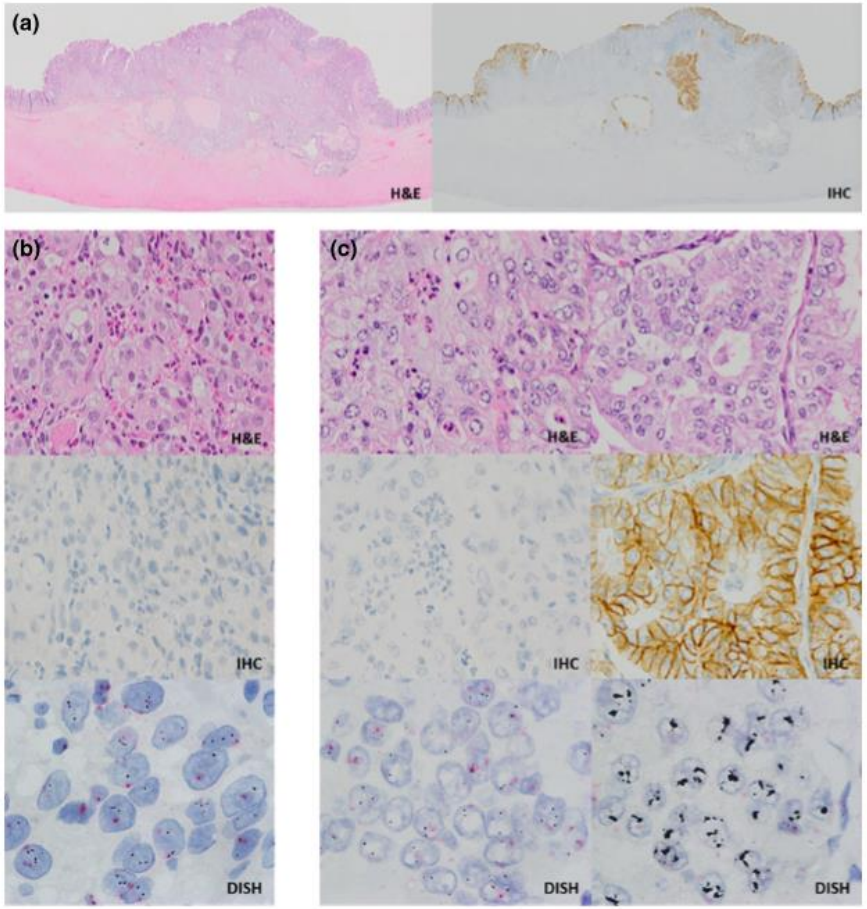
Table 2. Comparison of FISH and dual-color *in situ* hybridization (DISH) results for assessment of human epidermal growth factor receptor 2 status in biopsy specimens

IHC score	FISH		DISH		Total
	Unamplified	Amplified	Unamplified	Amplified	
0	74	0	74	0	74
1+	18	0	18	0	18
2+	5	4	5	4	9
3+	0	7	0	7	7
Total	97	11	97	11	108

IHC, immunohistochemistry.

Table 3. Concordance of human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) between biopsy and surgical resection specimens

	Score	HER2 IHC in surgical resection			Total
		0 or 1+	2+	3+	
HER2 IHC in biopsy	0 or 1+	50	9	1	60
	2+	2	4	1	7
	3+	0	1	2	3
Total		52	14	4	70



Kanayama et al. Cancer Sci 2016

Cancer Science
Japanese Cancer Association JCA
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Significant intratumoral heterogeneity of human epidermal growth factor receptor 2 status in gastric cancer: A comparative study of immunohistochemistry, FISH, and dual-color *in situ* hybridization

Kazuki Kanayama,[1] Hiroshi Imai,[1] Misao Yoneda,[2] Yoshifumi S. Hirokawa[1] and Taizo Shiraishi[1]

Table 5. Discordant and concordant cases of human epidermal growth factor receptor 2 (HER2) status between biopsy and surgical resection specimens

Case no.	Biopsy			Surgical resection			Amplified area, %
	HER2 IHC	HER2 status (HER2/CEP17 ratio)	WHO classification	HER2 IHC	HER2 status (HER2/CEP17 ratio)	WHO classification	
Discordant cases							
10	0	Unamplified (1.13)	Tubular	2+ Heterogeneous	Heterogeneous (2.96)	Tubular	17.4
15	0	Unamplified (1.17)	Tubular	2+ Heterogeneous	Heterogeneous (3.36)	Tubular	5.4
38	0	Unamplified (1.13)	Poorly cohesive	2+ Heterogeneous	Heterogeneous (4.02)	Poorly cohesive (tubular component)	9.3
71	2+ Heterogeneous	Unamplified (1.11)	Tubular	2+	Homogeneous (3.61)	Tubular	89.9
79	1+	Unamplified (1.10)	Tubular	2+ Heterogeneous	Heterogeneous (2.68)	Papillary	12.2
87	0	Unamplified (1.36)	Tubular	3+ Heterogeneous	Heterogeneous (7.69)	Tubular	5.3
Concordant cases							
6	3+	Amplified (7.62)	Poorly cohesive	3+	Homogeneous (8.69)	Tubular (poorly component)	100.0
41	3+ Heterogeneous	Amplified heterogeneous (8.92)	Tubular	3+ Heterogeneous	Heterogeneous (8.86)	Tubular	12.4
58	2+ Heterogeneous	Amplified (3.25)	Tubular	2+ Heterogeneous	Homogeneous (3.42)	Tubular	100.0
64	2+ Heterogeneous	Amplified (2.60)	Tubular	2+ Heterogeneous	Homogeneous (2.20)	Tubular	100.0
72	2+	Amplified (4.29)	Tubular	3+	Homogeneous (4.60)	Tubular	100.0
85	3+ Heterogeneous	Amplified heterogeneous (4.40)	Tubular	2+ Heterogeneous	Heterogeneous (2.75)	Tubular	47.9

Kanayama et al. Cancer Sci 2016

HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology

Angela N. Bartley, Mary Kay Washington, Carol Colasacco, Christina B. Ventura, Nofisat Ismaila, Al B. Benson III, Alfredo Carrato, Margaret L. Gulley, Dhanpat Jain, Sanjay Kakar, Helen J. Mackay, Catherine Streutker, Laura Tang, Megan Troxell, and Jaffer A. Ajani

Medizinische Universität Graz

Key Points and Recommendations for Pathologists

Recommendation 2.1: Laboratories/pathologists must specify the antibodies and probes used for the test and ensure that assays are appropriately validated for HER2 IHC and ISH on GEA specimens (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).

Recommendation 2.2: When GEA HER2 status is being evaluated, laboratories/pathologists should perform/order IHC testing first, followed by ISH when IHC result is 2+ (equivocal). Positive (3+) or negative (0 or 1+) HER2 IHC results do not require further ISH testing (Type: evidence based; Quality of evidence: high; Strength of recommendation: strong).

Recommendation 2.3: Pathologists should use the Ruschoff/Hofmann method in scoring HER2 IHC and ISH results for GEA (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).

Recommendation 2.4: Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than one tissue block may be selected if different morphologic patterns are present (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: recommendation/moderate).

Recommendation 2.5: Laboratories should report HER2 test results in GEA specimens in accordance with the CAP "Template for Reporting Results of *HER2 (ERBB2)* Biomarker Testing of Specimens From Patients With Adenocarcinoma of the Stomach or Esophagogastric Junction" (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).

Recommendation 2.6: Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by IHC in GEA specimen for subsequent ISH scoring when required (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).

(continued on following page)

Bartley et al. J Clin Oncol 2017

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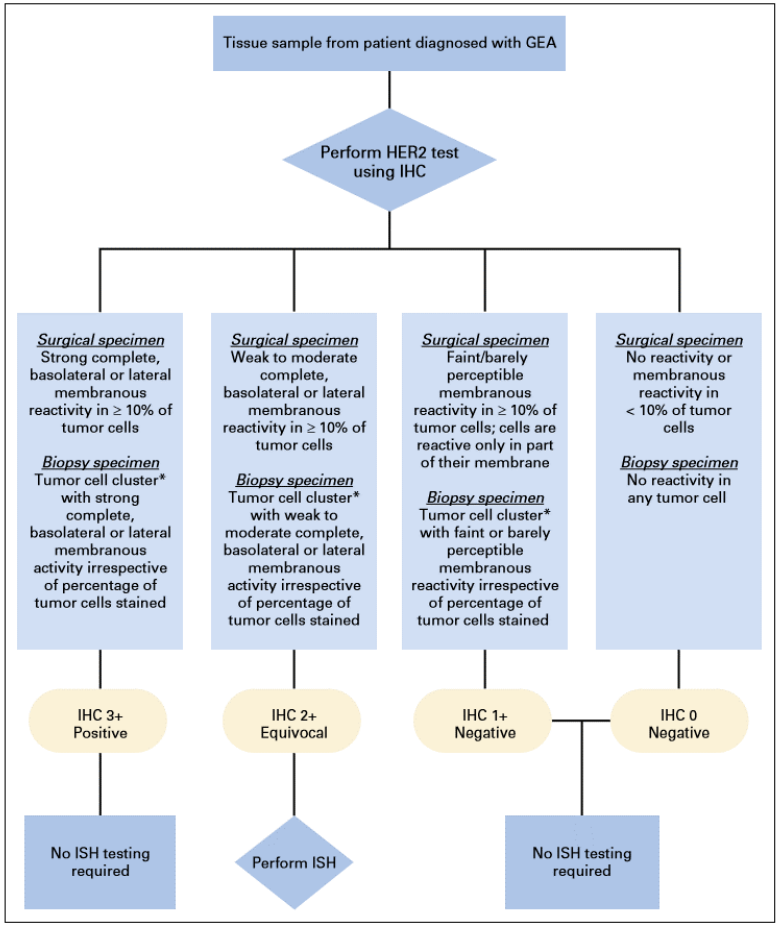
Recommendation 2.4

Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than one tissue block may be selected if different morphologic patterns are present (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: recommendation/moderate).

As mentioned previously, studies show that HER2 overexpression is strongly associated with the intestinal phenotype, and less frequently with the diffuse (signet ring cell) phenotype of GEA. The rates of HER2 positivity vary for intestinal (3% to 23.5%), diffuse (0% to 6%), and mixed histology (0% to 20%) cancers.[36,85-88]

HER2 positivity seems to be more strongly associated with low-grade than high-grade tumors and varies from 15% to 45% for low grade, and 6% to 28% for high grade, in different studies.[36,86,89-91] When choosing a tissue block, selecting one with the lower grade or intestinal morphology appears more likely to yield HER2-positive results and is thus recommended. If the cancer comprises substantially different grades or histologic patterns, it is reasonable to test different areas, which may require selection of more than one block.



Bartley et al. J Clin Oncol 2017

HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology

Angela N. Bartley, Mary Kay Washington, Carol Colasacco, Christina B. Ventura, Nofisat Ismaila, Al B. Benson III, Alfredo Carrato, Margaret L. Gulley, Dhanpat Jain, Sanjay Kakar, Helen J. Mackay, Catherine Streutker, Laura Tang, Megan Troxell, and Jaffer A. Ajani

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Table 3. Scoring Guidelines for Interpretation of HER2 Immunohistochemistry in Gastric Carcinoma

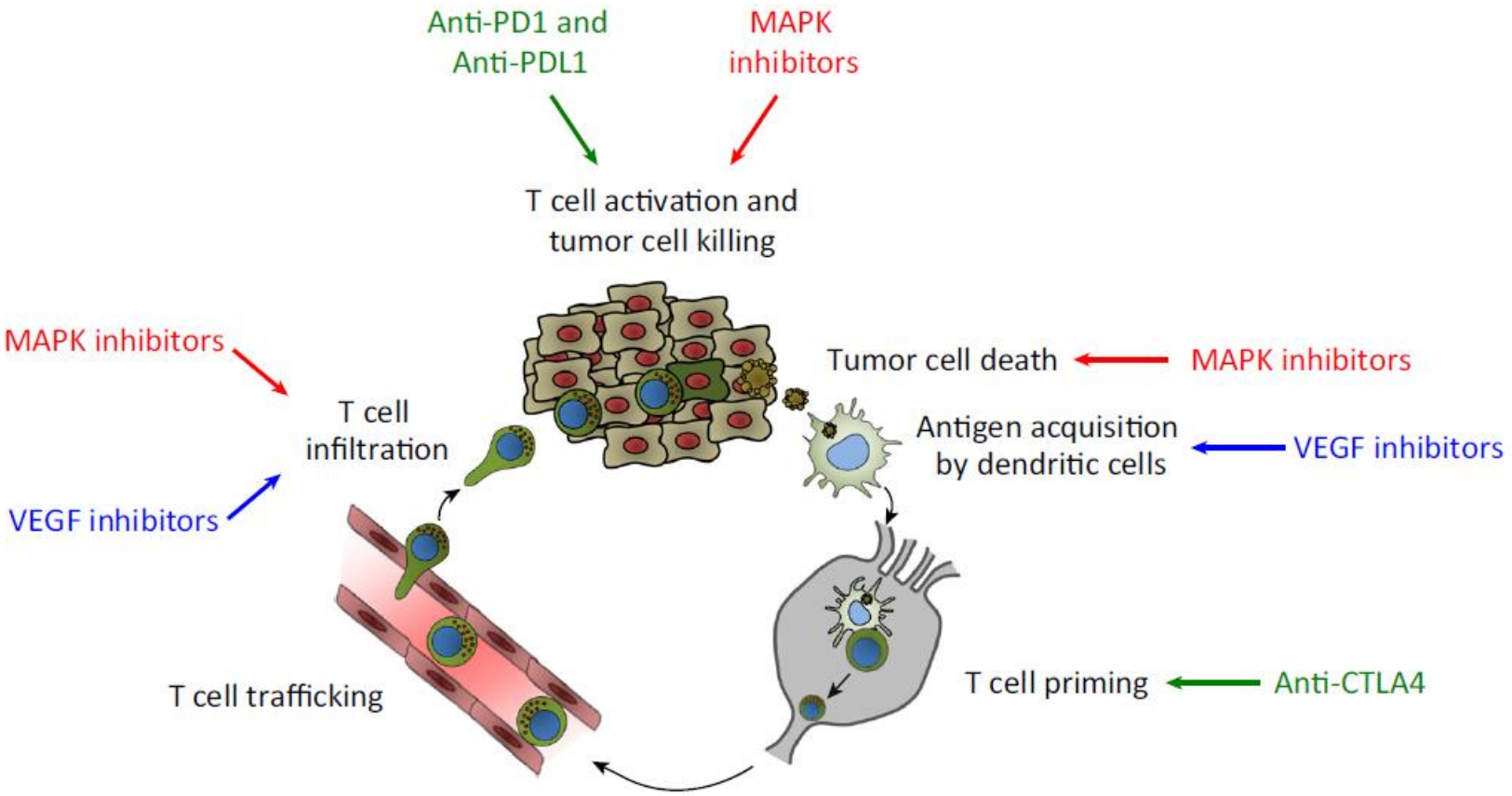
Surgical Specimen-Staining Pattern	Biopsy Specimen-Staining Pattern	Score	HER2 Expression Assessment
No reactivity or membranous reactivity in < 10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	0	Negative
Faint/barely perceptible membranous reactivity in ≥ 10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster* with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	1+	Negative
Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster* with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	2+	Equivocal
Strong complete, basolateral or lateral membranous reactivity in ≥ 10% of tumor cells	Tumor cell cluster* with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	3+	Positive

NOTE. Hofmann M, et al: Histopathology 52:797-805, 2008.[13]
*Tumor cell cluster (≥ 5 neoplastic cells).

Bartley et al. J Clin Oncol 2017

Targeted Therapy and Checkpoint Immunotherapy Combinations for the Treatment of Cancer

Paul E. Hughes,[1] Sean Caenepeel,[1] and Lawren C. Wu[2,*]



Hughes et al. Trends Immunol 2016

Gastric Cancer
DOI 10.1007/s10120-016-0631-3

ORIGINAL ARTICLE

Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein–Barr virus status in a large cohort of gastric cancer patients

Akihito Kawazoe[1,2,3] · Takeshi Kuwata[1,5] · Yasutoshi Kuboki[2] · Kohei Shitara[2] · Akiko Kawano Nagatsuma[1] · Masaaki Aizawa[4] · Takayuki Yoshino[2] · Toshihiko Doi[2] · Atsushi Ohtsu[2,3] · Atsushi Ochiai[1]

Table 1 Prevalence of programmed death ligand 1 (*PD-L1*) expression according to mismatch repair (*MMR*) status and Epstein–Barr virus (*EBV*) status

	All ($N = 487$)	P-MMR ($n = 462$)	D-MMR ($n = 25$)	*P*	EBV negative ($n = 462$)	EBV positive ($n = 25$)	*P*
TCs							
No PD-L1 expression in TCs	376 (77.2 %)	369 (79.9 %)	7 (28.0 %)	<0.001	364 (78.8 %)	12 (48.0 %)	0.001
PD-L1 expression in TCs	111 (22.8 %)	93 (20.1 %)	18 (72.0 %)		98 (21.2 %)	13 (52.0 %)	
IHC score 1+	67 (13.8 %)	58 (12.5 %)	9 (36.0 %)	<0.001	60 (13.0 %)	7 (28.0 %)	0.022
IHC score 2+	23 (4.7 %)	19 (4.1 %)	4 (16.0 %)		20 (4.3 %)	3 (12.0 %)	
IHC score 3+	21 (4.3 %)	16 (3.5 %)	5 (20.0 %)		18 (3.9 %)	3 (12.0 %)	
TIICs							
No PD-L1 expression in TIICs	188 (38.6 %)	187 (40.5 %)	1 (4.0 %)	<0.001	183 (39.6 %)	5 (20.0 %)	0.050
PD-L1 expression in TIICs	299 (61.4 %)	275 (59.5 %)	24 (96.0 %)		279 (60.4 %)	20 (80.0 %)	
IHC score 1+	278 (57.1 %)	260 (56.3 %)	18 (72.0 %)	<0.001	259 (56.1 %)	19 (76.0 %)	1.00
IHC score 2+	19 (3.9 %)	14 (3.0 %)	5 (20.0 %)		18 (3.9 %)	1 (4.0 %)	
IHC score 3+	2 (0.4 %)	1 (0.2 %)	1 (4.0 %)		2 (0.4 %)	0 (0 %)	

D-MMR MMR deficient, *IHC* immunohistochemistry, *P-MMR* MMR proficient, *TCs* tumor cells, *TIICs* tumor-infiltrating immune cells

Kawazoe et al. Gastric Cancer 2016

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MSI-H GC

EBV pos GC

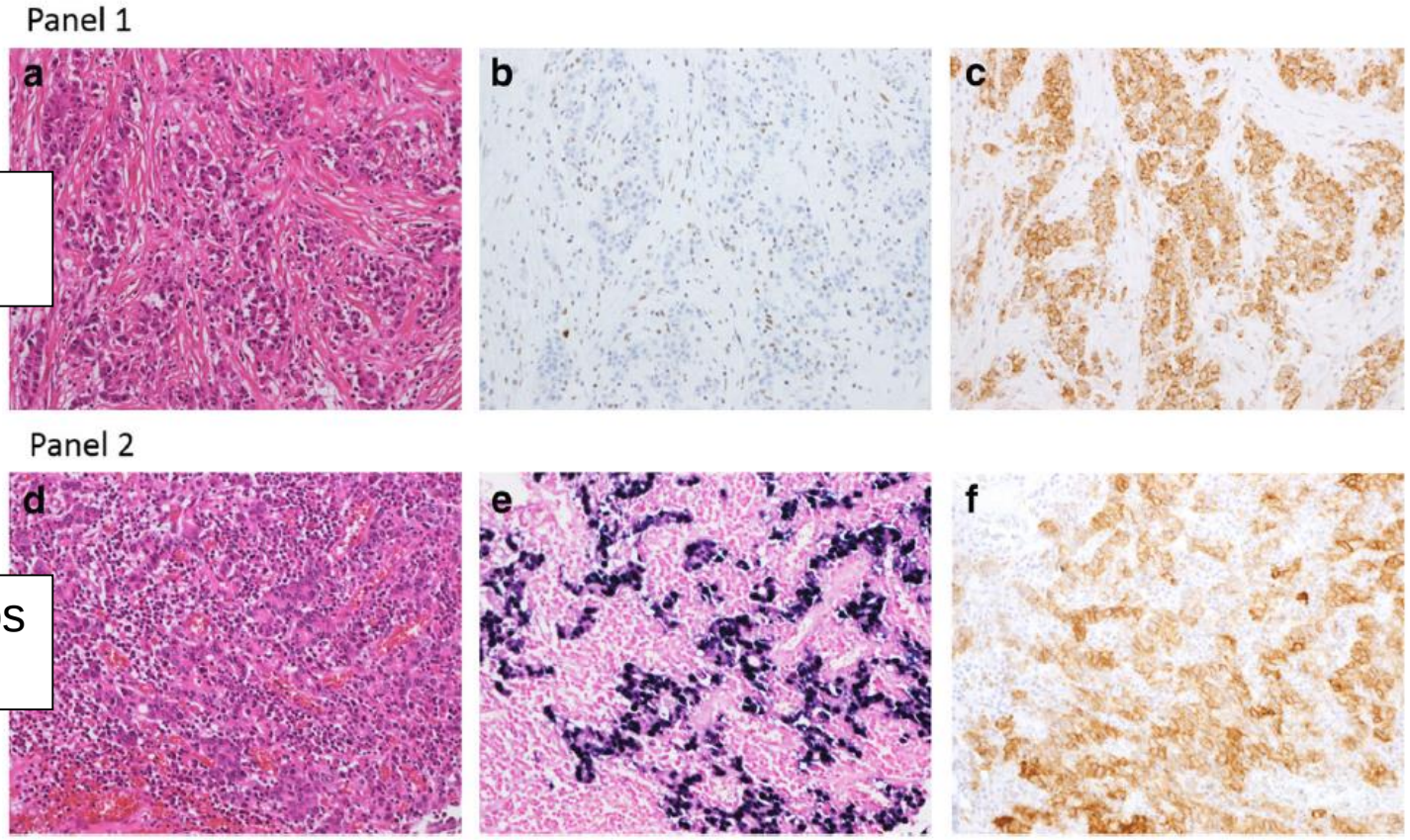

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Table 3 Association between programmed death ligand 1 (*PD-L1*) expression and tumor-infiltrating lymphocytes

	CD3		CD4		CD8		FOXP3	
	High	Low	High	Low	High	Low	High	Low
PD-L1 in TCs								
Positive	86 (77 %)	25 (23 %)	69 (62 %)	42 (38 %)	87 (78 %)	24 (22 %)	84 (76 %)	27 (24 %)
Negative	157 (42 %)	219 (58 %)	175 (47 %)	201 (53 %)	157 (42 %)	219 (58 %)	158 (42 %)	218 (58 %)
P	<0.001		0.004		<0.001		<0.001	
PD-L1 in TIICs								
Positive	188 (63 %)	111 (37 %)	164 (55 %)	135 (45 %)	193 (65 %)	106 (35 %)	179 (60 %)	120 (40 %)
Negative	55 (29 %)	133 (71 %)	80 (43 %)	108 (57 %)	51 (27 %)	137 (73 %)	63 (33 %)	125 (67 %)
P	<0.001		0.008		<0.001		<0.001	

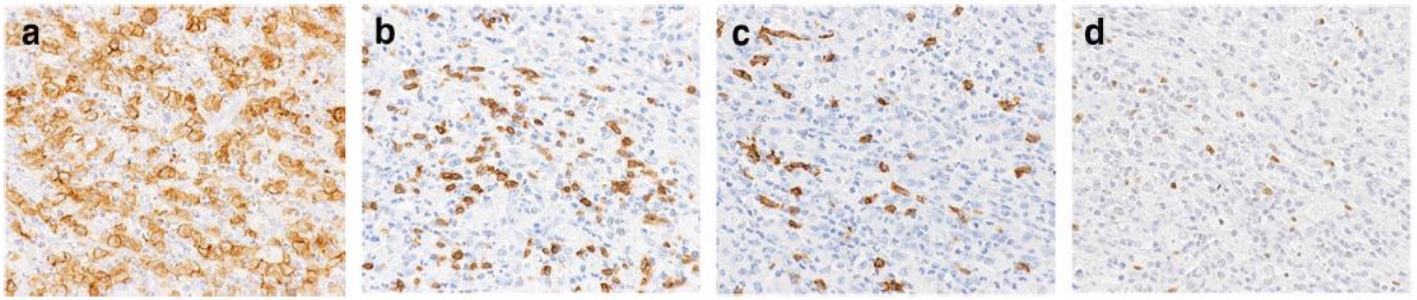

Fig. 3 A strong association was observed between programmed death ligand 1 (PD-L1) expression and high densities of $CD3^+$, $CD8^+$, and forkhead box P3 (FOXP3)-positive tumor-infiltrating lymphocytes (TILs). Representative images of a case with **a** PD-L1 immunohistochemistry score 3+ on tumor cells and high densities of **b** $CD3^+$, **c** $CD8^+$, and **d** $FOXP3^+$ TILs

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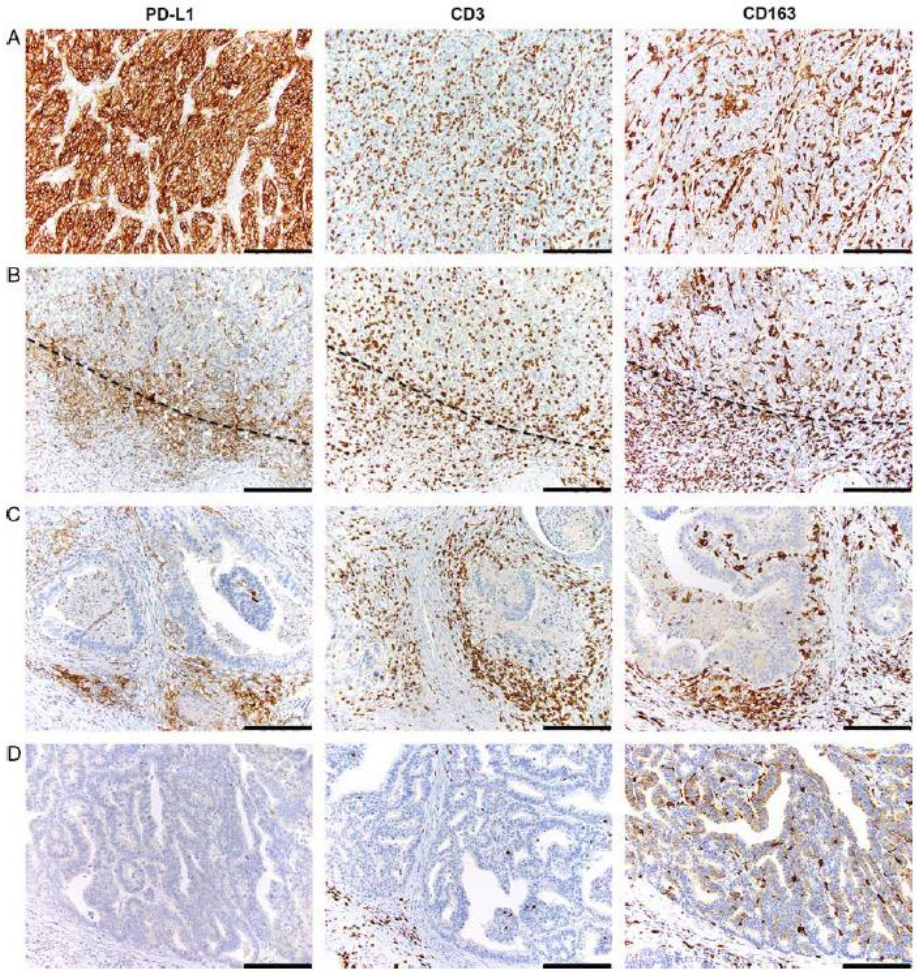
Programmed Death-Ligand 1 Expression Is Common in Gastric Cancer Associated With Epstein-Barr Virus or Microsatellite Instability

Changqing Ma, MD, PhD, Krishna Patel, MD,† Aatur D. Singhi, MD, PhD,* Bing Ren, MD, PhD,* Benjamin Zhu, BA,* Fyza Shaikh, MD,† and Weijing Sun, MD†*



A, Diffuse membranous PD-L1 staining in an MSI cancer.

B, Membranous PD-L1 staining at the invasive front in an MSI cancer. The dashed line in each photomicrograph indicates the invasive front of the tumor/tumor-stroma interface.

C, Membranous PD-L1 staining in immune infiltrates in an EBV/MSS cancer.

D, Negative/no PDL1 staining in tumor cells and immune infiltrates in an EBV/MSS cancer.

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TABLE 2. PD-L1 Expression and Tumor Immune Microenvironment Stratified by EBV and MSI Status

	All (N = 44)	EBV + (N = 7)	MSI (N = 16)	EBV −/MSS (N = 21)	*P*
Pattern of PD-L1 staining (n [%])					
Diffuse	10 (23)	4 (57)	5 (31)	1 (5)	< 0.001
Invasive front	14 (32)	2 (29)	9 (56)	3 (14)	
Immune infiltrates	8 (18)	1 (14)	0	7 (33)	
Negative	12 (27)	0	2 (13)	10 (48)	
Portion of cells in tumor with PD-L1 staining (n [%])					
0% (PD-L1 −)	12 (27)	0	2 (13)	10 (48)	0.013
> 0% (PD-L1 +)	32 (72)	7 (100)	14 (87)	11 (52)	
> 0, < 5%	16	2	5	9	
≥ 5%, < 10%	6	1	4	1	
≥ 10%, < 20%	3	1	2	0	
≥ 20%, < 30%	3	1	2	0	
≥ 30%, < 40%	1	1	0	0	
≥ 40%	3	1	1	1	
T cells at the invasive front (mean/high-power field [range])					
CD3	436 (112-865)	580 (265-865)	527 (149-780)	318 (112-654)	< 0.001
CD4	249 (51-516)	310 (164-516)	287 (51-489)	200 (52-514)	0.011
CD8	246 (35-640)	366 (129-512)	306 (63-640)	161 (35-338)	< 0.001
PD-1	97 (5-331)	137 (48-230)	127 (5-331)	60 (17-178)	0.002
CD4/CD8 ratio (n [%])					
CD4 ≥ CD8	29 (66)	3 (43)	8 (50)	18 (86)	0.028
CD4 < CD8	15 (34)	4 (57)	8 (50)	3 (14)	
Portion of immune cells expressing PD-1 (n [%])					
< 5%, > 0%	3 (7)	0	2 (13)	1 (5)	0.481
≥ 5%	41 (93)	7 (100)	14 (87)	20 (95)	

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Article

EBV$^+$ and MSI Gastric Cancers Harbor High PD-L1/PD-1 Expression and High CD8$^+$ Intratumoral Lymphocytes

Simona De Rosa [1], Nora Sahnane [2], Maria Grazia Tibiletti [1], Francesca Magnoli [1,2], Alessandro Vanoli [3], Fausto Sessa [1,2] and Anna Maria Chiaravalli [1,*]

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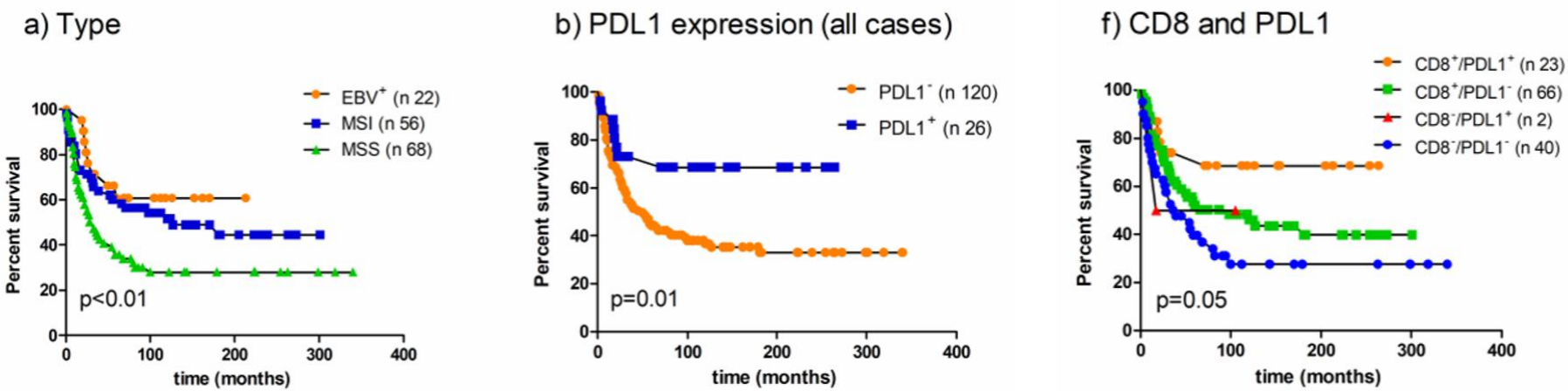
Table 2. Immunophenotype of TILs and PD-L1 expression on tumor and immune cells.

Feature	Total	EBV$^+$ Cases	MSI Cases	MSS/EBV$^-$ Cases	*p*
N. of cases	169	33	59	77	
PD-1$^+$ cases	137 (81%)	30 (91%)	51 (86%)	56 (73%)	*
PD-1$^+$ TILs Mean (range)	7 (0–74.6)	17.5 (0–74.6)	7 (0–35.3)	3.1 (0–26.7)	***
CD8$^+$ TILs Mean (range)	28.9 (0–167.1)	64.2 (14.4–167.1)	23.1 (0–69.2)	15.8 (0–94.4)	*
PDL1$^+$ TC cases	31 (18%)	15 (46%)	14 (24%)	2 (3%)	***
PDL1$^+$ TC Mean % ^ (range)	25 (1–90)	33 (1–90)	18 (1–40)	17.5 (15–20)	**
PDL1$^+$ IC cases	72 (42%)	31 (94%)	22 (37%)	19 (25%)	***
PDL1$^+$ TI	31/72 (43%)	18/31 (58%)	12/22 (54%)	1/19 (5%)	***
PDL1$^+$ IM	41/72 (57)	13/31 (42%)	10/22 (46%)	18/19 (95%)	

Legend. TC, tumor cells; IC, immune cells; TI, tumor infiltrating pattern; IM, invasive margin * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001; ^ in positive cases.



Intratumoral Immune Response to Gastric Cancer Varies by Molecular and Histologic Subtype

Teresa S. Kim, MD, Edaise da Silva, PhD,† Daniel G. Coit, MD,‡ and Laura H. Tang, MD, PhD†*

TABLE 1. Clinicopathologic Characteristics by Tumor Subtype

	N (%)					
Characteristic	**Total (43)**	**EBV (6)**	**MSI (11)**	**Intestinal (14)**	**Diffuse (12)**	***P***
Age (median [range]) (y)	68 (20-92)	72 (40-85)	74 (55-85)	78 (52-92)	48 (20-67)	<0.01
Sex						0.02
Male	24 (55.8)	5 (83.3)	5 (45.5)	11 (78.6)	3 (25.0)	
Female	19 (44.2)	1 (16.7)	6 (54.5)	3 (21.4)	9 (75.0)	
Tumor location						<0.01
Upper	2 (4.7)	1 (16.7)	0	0	1 (8.3)	
Middle	14 (32.6)	4 (66.7)	5 (45.5)	0	5 (41.7)	
Lower	25 (58.1)	1 (16.7)	6 (54.5)	14 (100)	4 (33.3)	
Entire	2 (4.7)	0	0	0	2 (16.7)	
Helicobacter pylori infection						0.03
Absent	32 (74.4)	2 (33.3)	7 (63.6)	12 (85.7)	11 (91.7)	
Present	11 (25.6)	4 (66.7)	4 (36.4)	2 (14.3)	1 (8.3)	
NLR (mean [range])	3.0 (1.1-6.8)	3.8 (2.0-5.6)	3.1 (1.2-6.8)	3.0 (1.3-4.8)	2.5 (1.1-4.1)	0.19
Tumor stage						0.39
T1	9 (20.9)	3 (50.0)	3 (27.3)	3 (21.4)	0	
T2	7 (16.3)	1 (16.7)	2 (18.2)	3 (21.4)	1 (8.3)	
T3	15 (34.9)	2 (33.3)	3 (27.3)	4 (28.6)	6 (50.0)	
T4	12 (27.9)	0	3 (27.3)	4 (28.6)	5 (41.7)	
Lymphovascular invasion						0.07
Absent	13 (30.2)	4 (66.7)	2 (18.2)	2 (14.3)	5 (41.7)	
Present	30 (69.8)	2 (33.3)	9 (81.8)	12 (85.7)	7 (58.3)	
Perineural invasion						0.01
Absent	17 (39.5)	3 (50.0)	7 (63.6)	7 (50.0)	0	
Present	26 (60.5)	3 (50.0)	4 (36.4)	7 (50.0)	12 (100.0)	
Nodal stage						0.45
N0	18 (41.9)	4 (66.7)	5 (45.5)	5 (35.7)	4 (33.3)	
N1	6 (14.0)	1 (16.7)	2 (18.2)	3 (21.4)	0	
N2	10 (23.3)	0	3 (27.3)	4 (28.6)	3 (25.0)	
N3	9 (20.9)	1 (16.7)	1 (9.1)	2 (14.3)	5 (41.7)	

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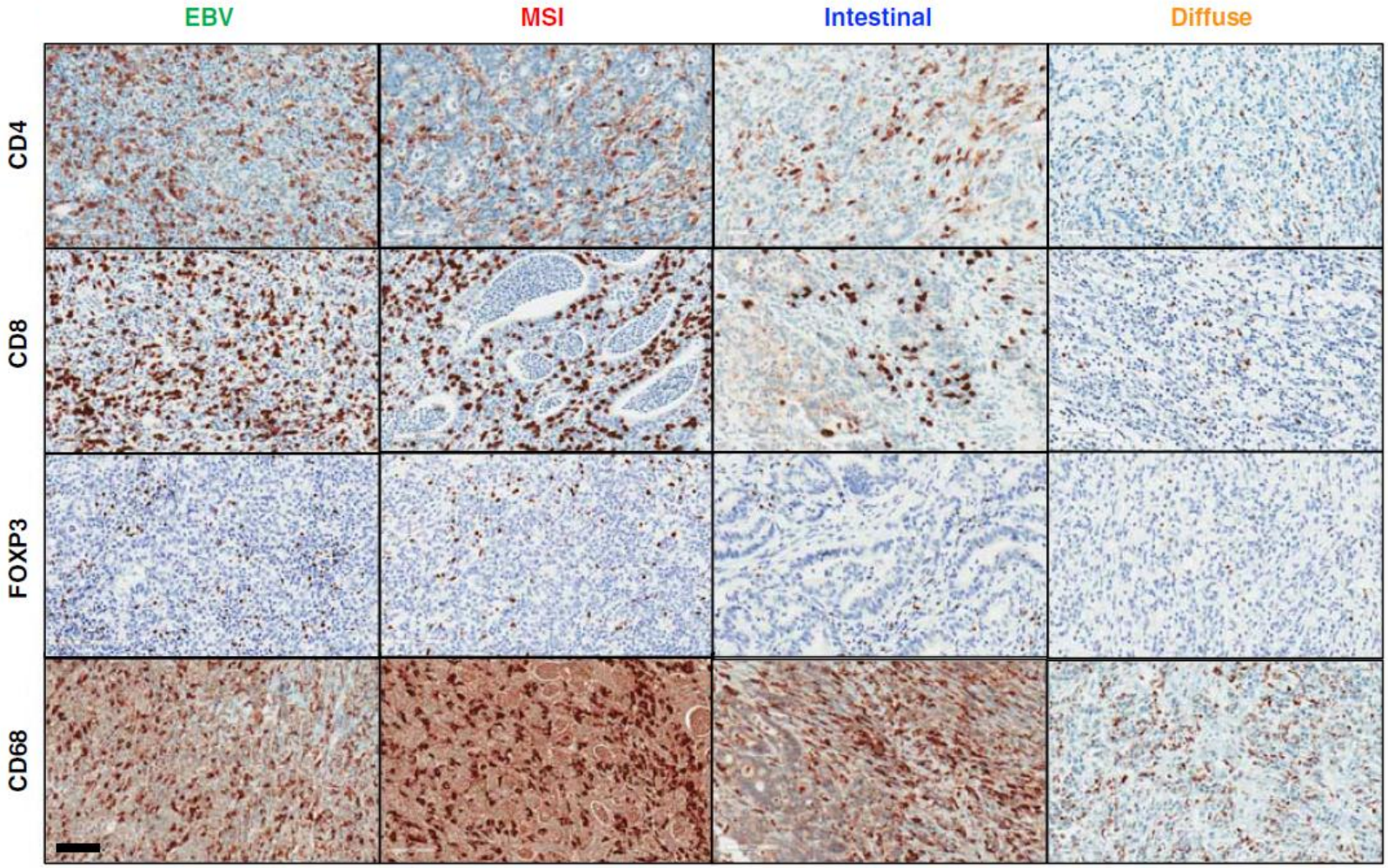

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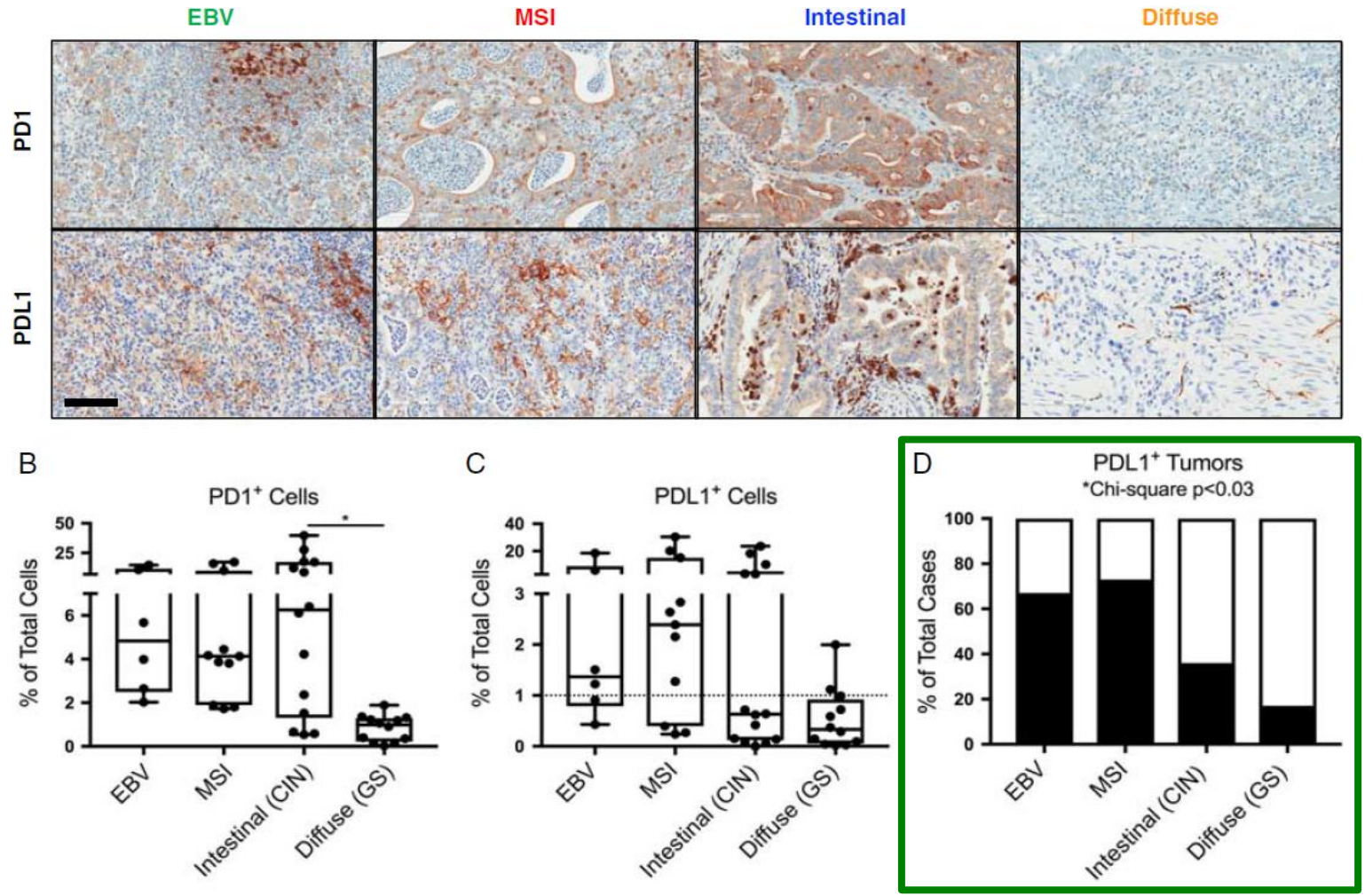

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Clinical Utility of the Combined Positive Score for Programmed Death Ligand-1 Expression and the Approval of Pembrolizumab for Treatment of Gastric Cancer

Karina Kulangara, PhD; Nancy Zhang, MD; Ellie Corigliano, PhD; Lindsay Guerrero, MS; Stephanie Waldroup, BSc; Dipeshkumar Jaiswal, MS; Malinka Jansson, MS; Supriya Shah, PhD; Debra Hanks, MD; Jiangdian Wang, PhD; Jared Lunceford, PhD; Mary J. Savage, PhD; Jonathan Juco, MD; Kenneth Emancipator, MD

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Scoring Methods

The CPS is given by summing the number of PD-L1–stained cells (tumor cells, lymphocytes, macrophages) and dividing the result by the total number of viable tumor cells, multiplied by 100, and is expressed by the following formula:

$$\text{CPS} = \frac{\text{No. PD-L1–stained cells (tumor cells, lymphocytes, macrophages)}}{\text{Total No. of viable tumor cells}} \times 100$$

Although theoretically that quantity can exceed 100, the maximum score is defined as 100. A minimum of 100 viable tumor cells must be present in the PD-L1–stained slide (sectioned tumor biopsy or resection tissue) for the specimen to be considered adequate for evaluation. Tumor cells must show partial or complete membrane staining ($\geq 1^+$) to be counted as "stained," whereas immune cells are counted if there is any staining.

Kulangara et al. Arch Pathol Lab Med 2019

Clinical Utility of the Combined Positive Score for Programmed Death Ligand-1 Expression and the Approval of Pembrolizumab for Treatment of Gastric Cancer

Karina Kulangara, PhD; Nancy Zhang, MD; Ellie Corigliano, PhD; Lindsay Guerrero, MS; Stephanie Waldroup, BSc; Dipeshkumar Jaiswal, MS; Malinka Jansson, MS; Supriya Shah, PhD; Debra Hanks, MD; Jiangdian Wang, PhD; Jared Lunceford, PhD; Mary J. Savage, PhD; Jonathan Juco, MD; Kenneth Emancipator, MD

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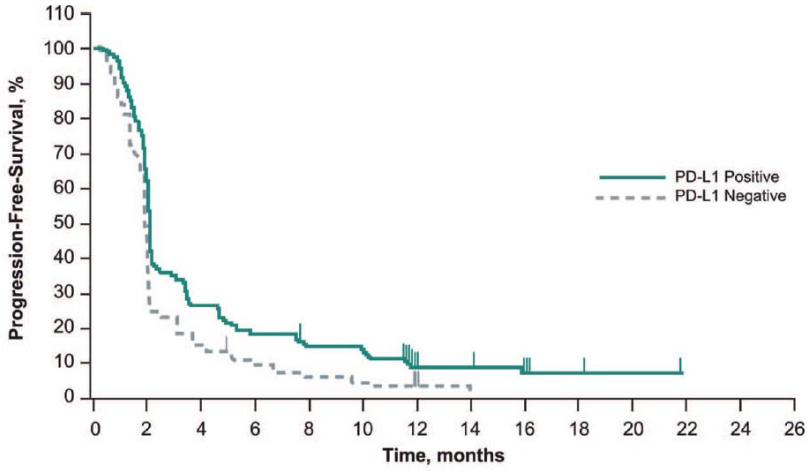
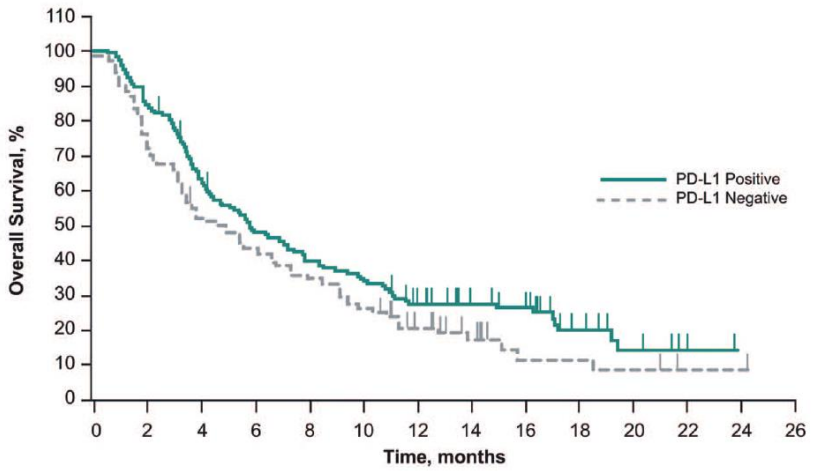
Table 4. Objective Response Rate (ORR) From KEYNOTE-059 by Combined Positive Score (CPS) and Tumor Proportion Score (TPS; N = 257)

	Total (N = 257), No. (%)	Response	No Response	ORR, %	Odds Ratio
CPS ≥1	148 (57.6)	24	124	16.2	2.8
CPS <1	109 (42.4)	7	102	6.4	
TPS ≥1	32 (12.5)	5	27	15.6	1.4
TPS <1	225 (87.5)	26	199	11.6	





Kulangara et al. Arch Pathol Lab Med 2019

Systematic review

Meta-analysis of microsatellite instability in relation to clinicopathological characteristics and overall survival in gastric cancer

K. Polom[1,6], L. Marano[2], D. Marrelli[1], R. De Luca[3], G. Roviello[4,5], V. Savelli[1], P. Tan[7] and F. Roviello[1]

Medizinische Universität Graz

Reference	Relative weight (%)	Hazard ratio
An *et al.*[30]	6·42	1·26 (0·89, 1·78)
Bae *et al.*[25]	5·49	1·80 (1·10, 2·94)
Chuiaravalli *et al.*[53]	4·44	0·64 (0·33, 1·25)
Cristescu *et al.*[4]	6·16	0·58 (0·39, 0·86)
Czopek *et al.*[54]	4·63	0·62 (0·33, 1·17)
Falchetti *et al.*[55]	4·96	0·52 (0·29, 0·93)
Fang *et al.*[36]	4·59	0·51 (0·27, 0·97)
Gazvoda *et al.*[56]	2·32	0·53 (0·16, 1·73)
Giampieri *et al.*[57]	6·14	0·24 (0·16, 0·36)
Inada *et al.*[37]	5·31	0·74 (0·44, 1·25)
Kim *et al.*[10]	4·64	0·74 (0·39, 1·39)
Kim *et al.*[5]	3·35	0·56 (0·23, 1·36)
Marrelli *et al.*[8]	6·17	0·62 (0·42, 0·91)
Oki *et al.*[14]	4·89	1·06 (0·59, 1·91)
Oliveira *et al.*[60]	5·31	0·62 (0·37, 1·04)

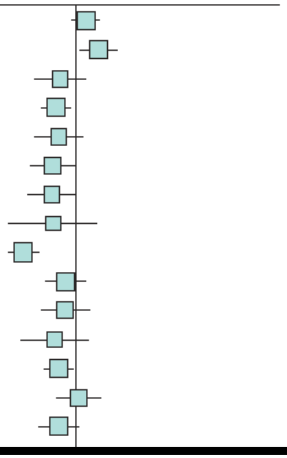


Table 1 Summary of meta-analyses investigating relationship between microsatellite instability status and demographic and clinicopathological characteristics

	No. of studies	Pooled odds ratio	*P*	Heterogeneity I^2 (%)	Heterogeneity *P*	Effect model
Sex (F *versus* M)	35	1·57 (1·31, 1·89)	< 0·001	54·1	< 0·001	Random
Age (≥ 65 *versus* < 65 years)	48	1·58 (2·20, 1·13)	< 0·001	0	< 0·001	Fixed
Laurén classification (intestinal *versus* diffuse/mixed)	34	2·23 (1·94, 2·57)	< 0·001	29·9	0·05	Fixed
Tumour location (upper *versus* middle/lower)	36	0·38 (0·32, 0·44)	< 0·001	43·9	< 0·05	Fixed
Lymph node metastasis (yes *versus* no)	35	0·70 (0·57, 0·86)	< 0·001	61·0	< 0·001	Random
TNM stage (I–II *versus* III/IV)	8	1·77 (1·47, 2·13)	< 0·001	0	< 0·001	Fixed

Values in parentheses are 95 per cent confidence intervals.

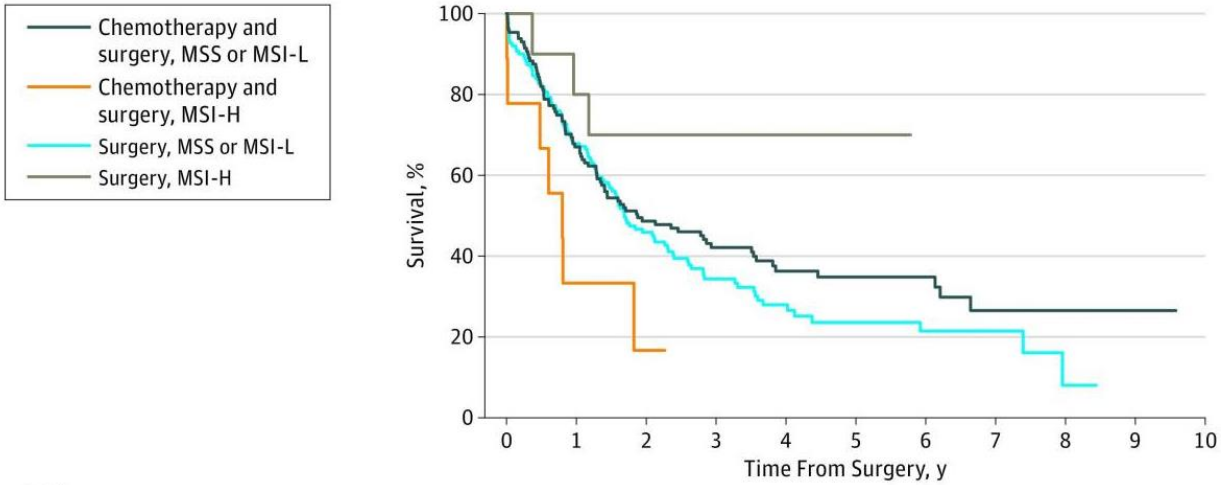
Polom et al. Br J Surg 2018

JAMA Oncology | Original Investigation

Mismatch Repair Deficiency, Microsatellite Instability, and Survival

An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial

Elizabeth C. Smyth, MB, BCh, MSc; Andrew Wotherspoon, MD; Clare Peckitt, MSc; David Gonzalez, PhD; Sanna Hulkki-Wilson, BSc, MSc; Zakaria Eltahir, PhD; Matteo Fassan, MD, PhD; Massimo Rugge, MD, FACG; Nicola Valeri, MD, PhD; Alicia Okines, MD; Madeleine Hewish, MD, PhD; William Allum, MD; Sally Stenning, MSc; Matthew Nankivell, MSc; Ruth Langley, MD, PhD; David Cunningham, MD, FMedSci



Correlation of MMRD With MSI Status

A total of 254 patients had MSI and MMR results available. Of these, 15 of 17 MSI-H tumors had MMRD detected. Thirteen of 15 MLH1-negative tumors (86.7%) with available MSI results had MSI-H tumors compared with 4 of 239 MLH1-positive tumors (1.7%). This finding results in a sensitivity of MLH1 deficiency testing for MSI prognosis of 76.5% (95% CI, 50.1%-93.2%) and a specificity of 99.2% (95% CI, 97.0%-99.9%). All patients with absent MSH2 and MSH6 had MSI-H tumors. Twelve of 16 patients (75.0%) with absent PMS2 and MSI results had MSI-H tumors compared with 4 of 236 patients (1.7%) with PMS2-positive tumors. Overall concordance between MSI-H and MMRD status was 97.6%

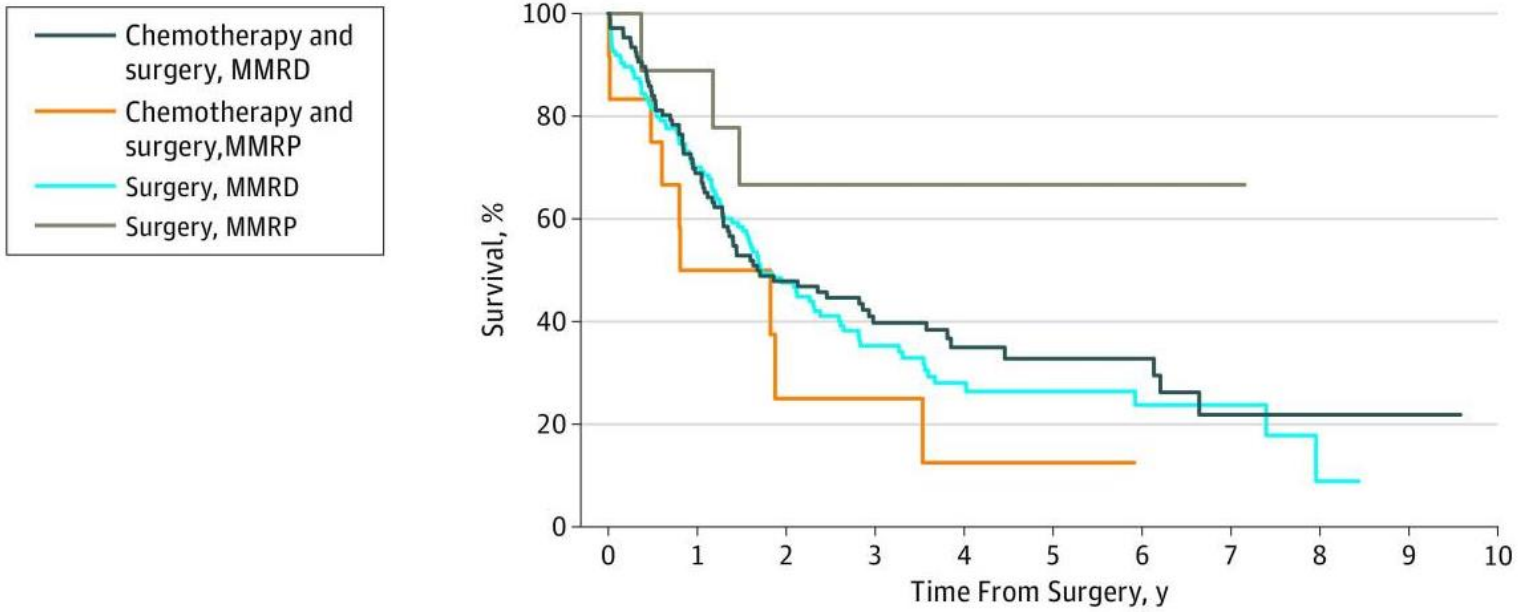
Smyth et al. JAMA Oncol 2017

JAMA Oncology | Original Investigation

Mismatch Repair Deficiency, Microsatellite Instability, and Survival

An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial

Elizabeth C. Smyth, MB, BCh, MSc; Andrew Wotherspoon, MD; Clare Peckitt, MSc; David Gonzalez, PhD; Sanna Hulkki-Wilson, BSc, MSc; Zakaria Eltahir, PhD; Matteo Fassan, MD, PhD; Massimo Rugge, MD, FACG; Nicola Valeri, MD, PhD; Alicia Okines, MD; Madeleine Hewish, MD, PhD; William Allum, MD; Sally Stenning, MSc; Matthew Nankivell, MSc; Ruth Langley, MD, PhD; David Cunningham, MD, FMedSci



Conclusions and Relevance

In the MAGIC trial, MMRD and high MSI were associated with a positive prognostic effect in patients treated with surgery alone and a differentially negative prognostic effect in patients treated with chemotherapy. If independently validated, MSI or MMRD determined by preoperative biopsies could be used to select patients for perioperative chemotherapy.

Smyth et al. JAMA Oncol 2017

Medizinische Universität Graz

Microsatellite Instability and Programmed Cell Death-Ligand 1 Expression in Stage II/III Gastric Cancer

Post Hoc Analysis of the CLASSIC Randomized Controlled study

Yoon Young Choi, MD,† Hyunki Kim, MD, PhD,‡ Su-Jin Shin, MD, PhD,§ Ha Yan Kim, MS,¶ Jinae Lee, PhD,¶ Han-Kwang Yang, MD, PhD,|| Woo Ho Kim, MD, PhD,** Young-Woo Kim, MD, PhD,†† Myeong-Cherl Kook, MD, PhD,†† Young Kyu Park, MD, PhD,‡‡ Hyung-Ho Kim, MD, PhD,§§ Hye Seung Lee, MD, PhD,¶¶ Kyung Hee Lee, MD, PhD,|||| Mi Jin Gu, MD, PhD,*** Seung Ho Choi, MD, PhD,††† SoonWon Hong, MD, PhD,‡‡‡ Jong Won Kim, MD, PhD,§§§ Woo Jin Hyung, MD, PhD,* Sung Hoon Noh, MD, PhD,*¶¶¶ and Jae-Ho Cheong, MD, PhD*†¶¶¶||||||*

TABLE 2. Associations Between Microsatellite Instability Status and Programmed Cell Death Ligand 1 Expression in Tumor Cells and Stromal Immune Cells

	MSI Status			sPD-L1 Status		
	MSS (n = 543)	MSI-H (n = 39)	*P*	Negative (n = 417)	Positive (n = 165)	*P*
tPD-L1 status						<0.001*
Negative (n = 566)	530 (97.6)				150 (90.9)	
Positive (n = 16)	13 (2.4)				15 (9.1)	
sPD-L1 status						
Negative (n = 417)	400 (73.7)					
Positive (n = 165)	143 (26.3)					

*Fisher exact test.

TABLE 3. Disease-free Survival Analyzed Using a Multivariable Cox Proportional-hazards Model

	HR (95% CI)	*P*
Treatment		<0.001
Surgery only	Reference	
Surgery + CTx	0.618 (0.472–0.809)	
Age, yr	1.016 (1.004–1.028)	0.009
Tumor grade		0.037
Intestinal	Reference	
Nonintestinal	1.385 (1.019–1.881)	
pTNM stage *		<0.001
II	Reference	
III	2.092 (1.568–2.79)	
MSI status		0.008
MSS	Reference	
MSI-H	0.301 (0.123–0.736)	
sPD-L1 status		0.044
Negative	Reference	
Positive	0.714 (0.514–0.991)	

CTx indicates adjuvant chemotherapy (capecitabine plus oxaliplatin).
*According to TNM 6th edition.

Choi et al. Ann Surg 2018 [Epub ahead of print]

Medizinische Universität Graz

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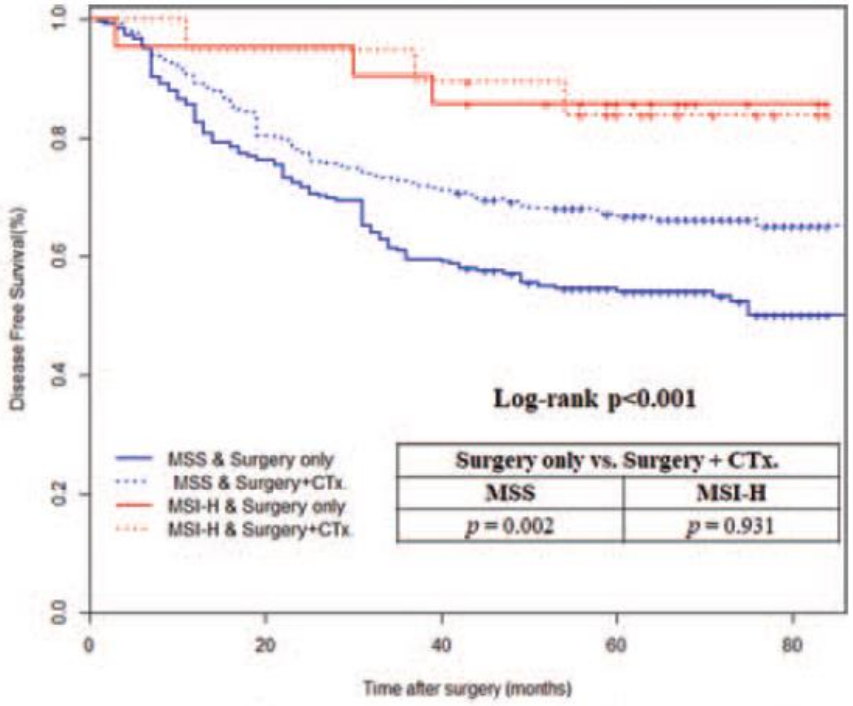

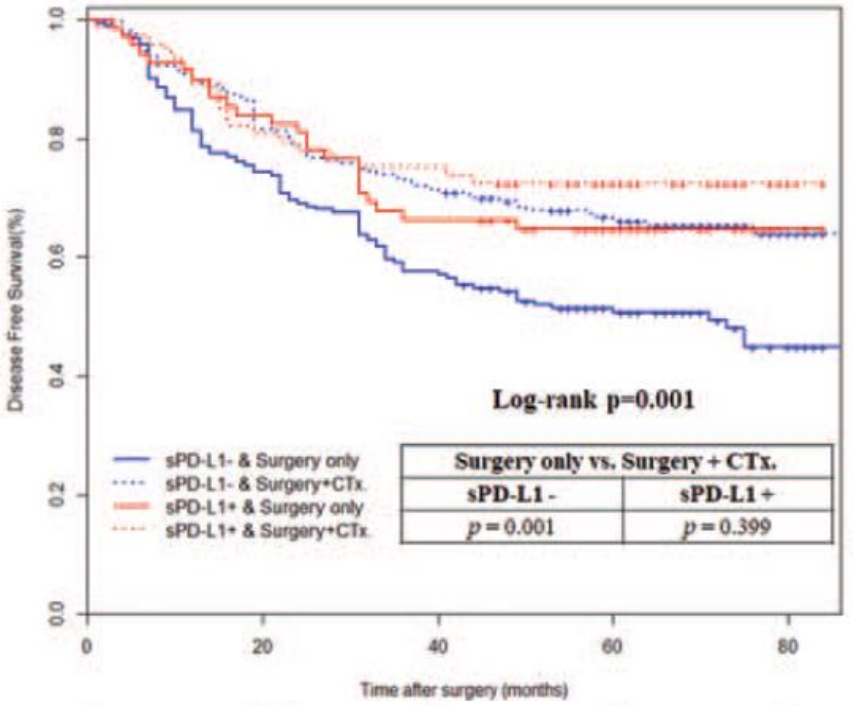

Choi et al. Ann Surg 2018 [Epub ahead of print]

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Microsatellite Instability and Programmed Cell Death-Ligand 1 Expression in Stage II/III Gastric Cancer

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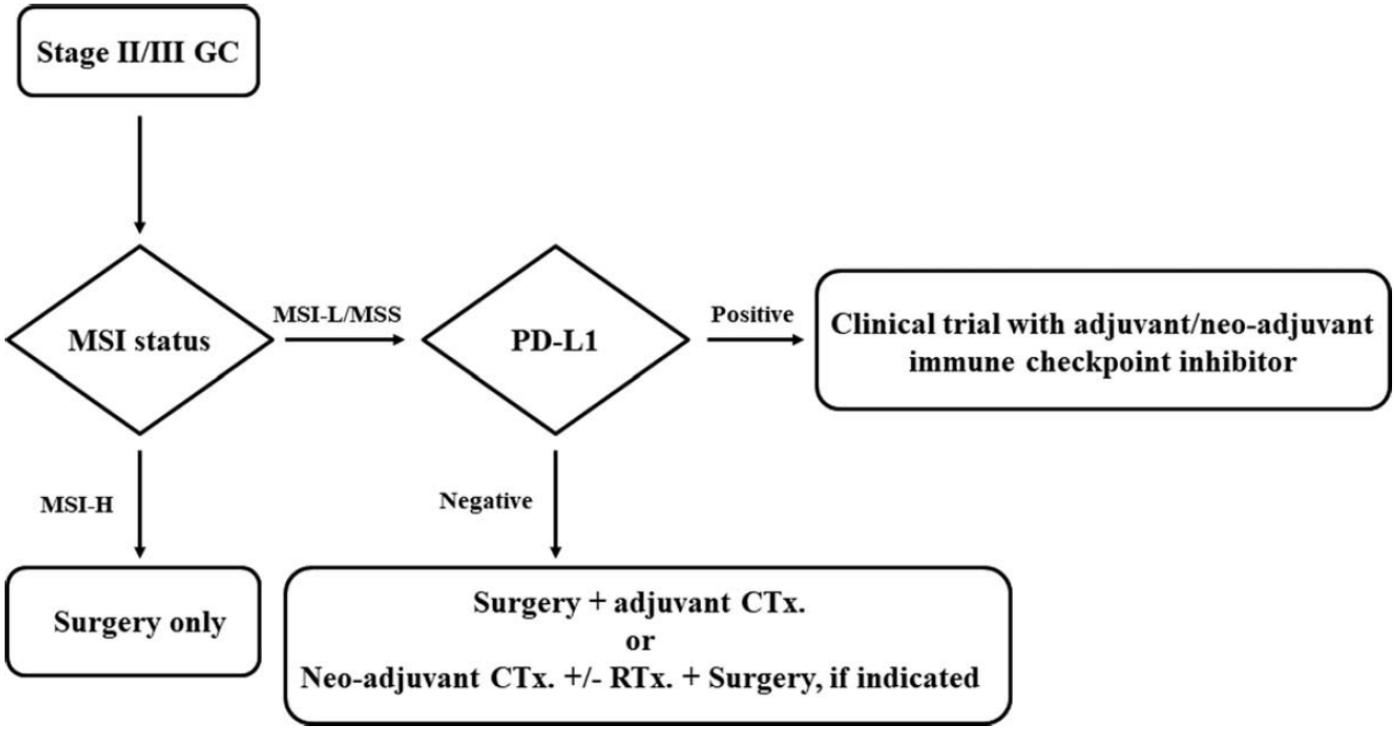

FIGURE 4. A suggested treatment algorithm for stage II/III gastric cancer according to MSI status and PD-L1 expression. CTx indicates chemotherapy; GC, gastric cancer; RTx, radiotherapy.

Choi et al. Ann Surg 2018 [Epub ahead of print]

Agenda

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- Classification of gastric cancer
  - Histology
  - Molecular pathology
- Predictive pathology
  - HER2
  - PD-L1
  - Microsatellite instability
- **Sporadic *vs* hereditary gastric cancer**
- Take home messages

Gastric Cancer in Hereditary Cancer Syndromes

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Syndrome	Genetic alteration
Lynch syndrome (HNPCC)	Mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*)
Li-Fraumeni syndrome	*TP53*
Familial adenomatous polyposis (FAP)	*APC*
Hamartomatous polyposis syndromes ▪ Peutz-Jeghers syndrome ▪ Juvenile polyposis	 ▪ *STK1 (LKB1)* ▪ *SMAD4 / BMPR1A*

Familial / Hereditary Gastric Cancer

Medizinische Universität Graz

Syndrome	Genetic alteration
Hereditary Diffuse Gastric Cancer (HDGC)	*CDH1* (mainly)
Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS)	*APC* promoter
Familial Intestinal Gastric Cancer (FIGC)	Unknown

Hereditary Diffuse Gastric Cancer (HDGC)

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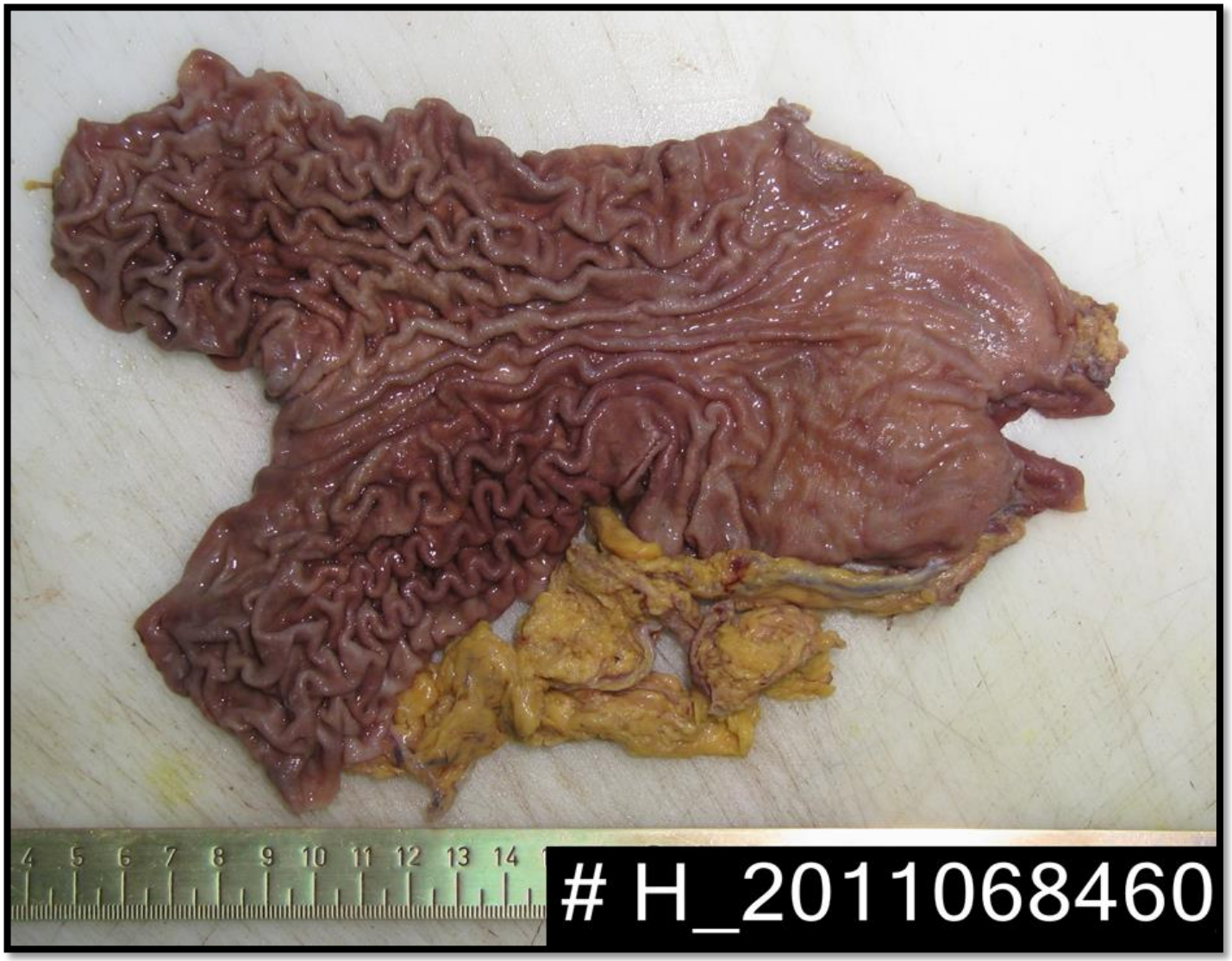

Hereditary Diffuse Gastric Cancer (HDGC)

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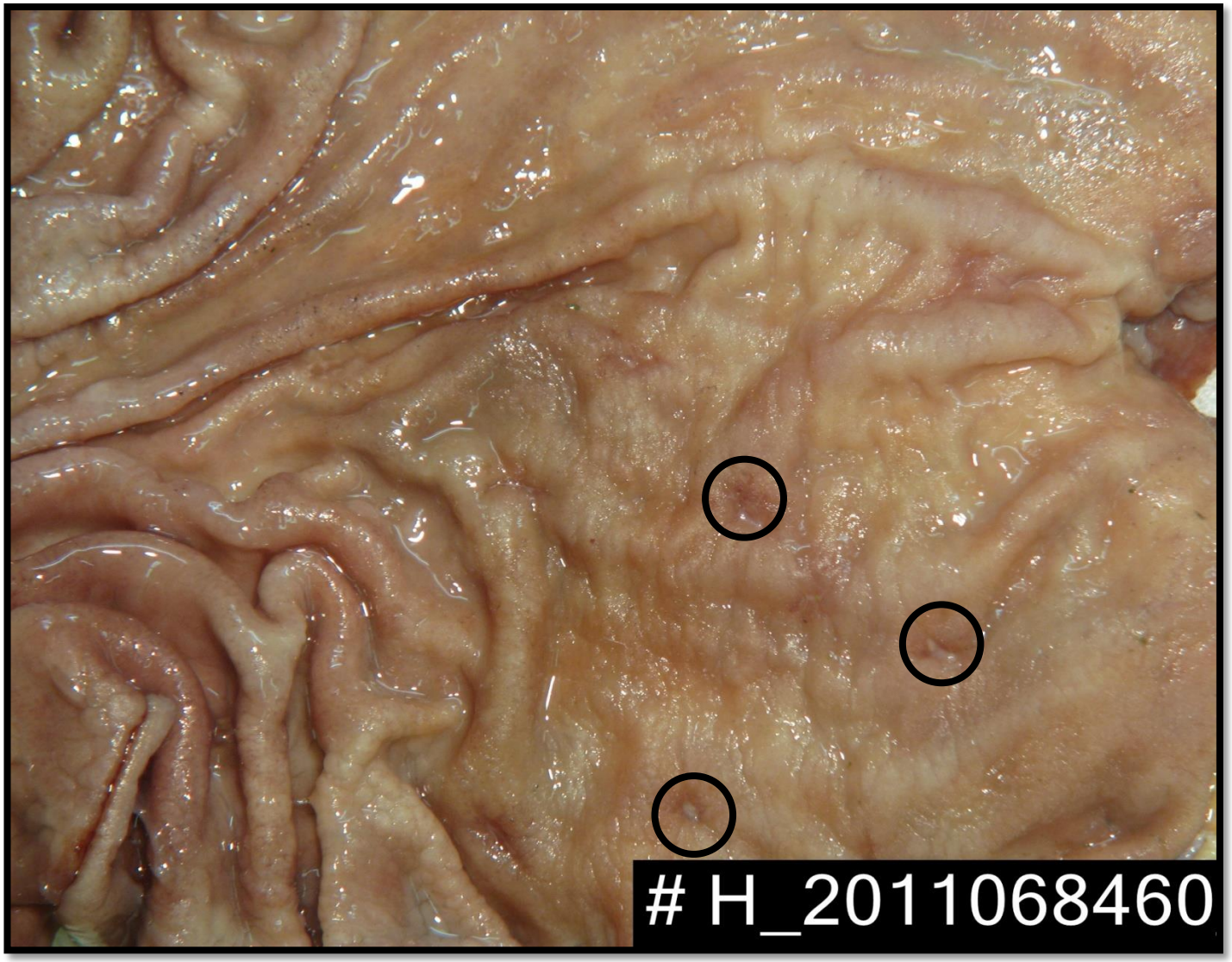
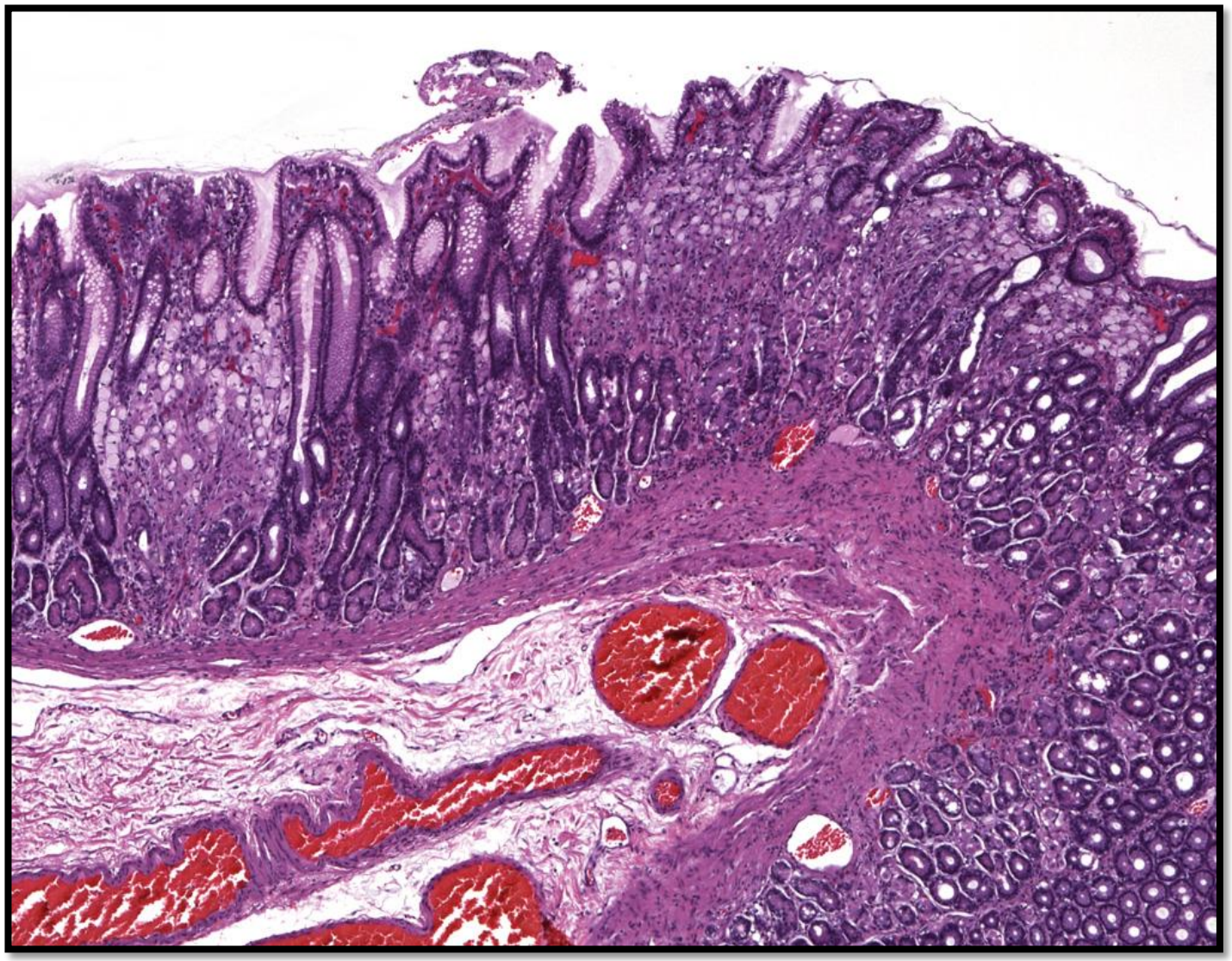


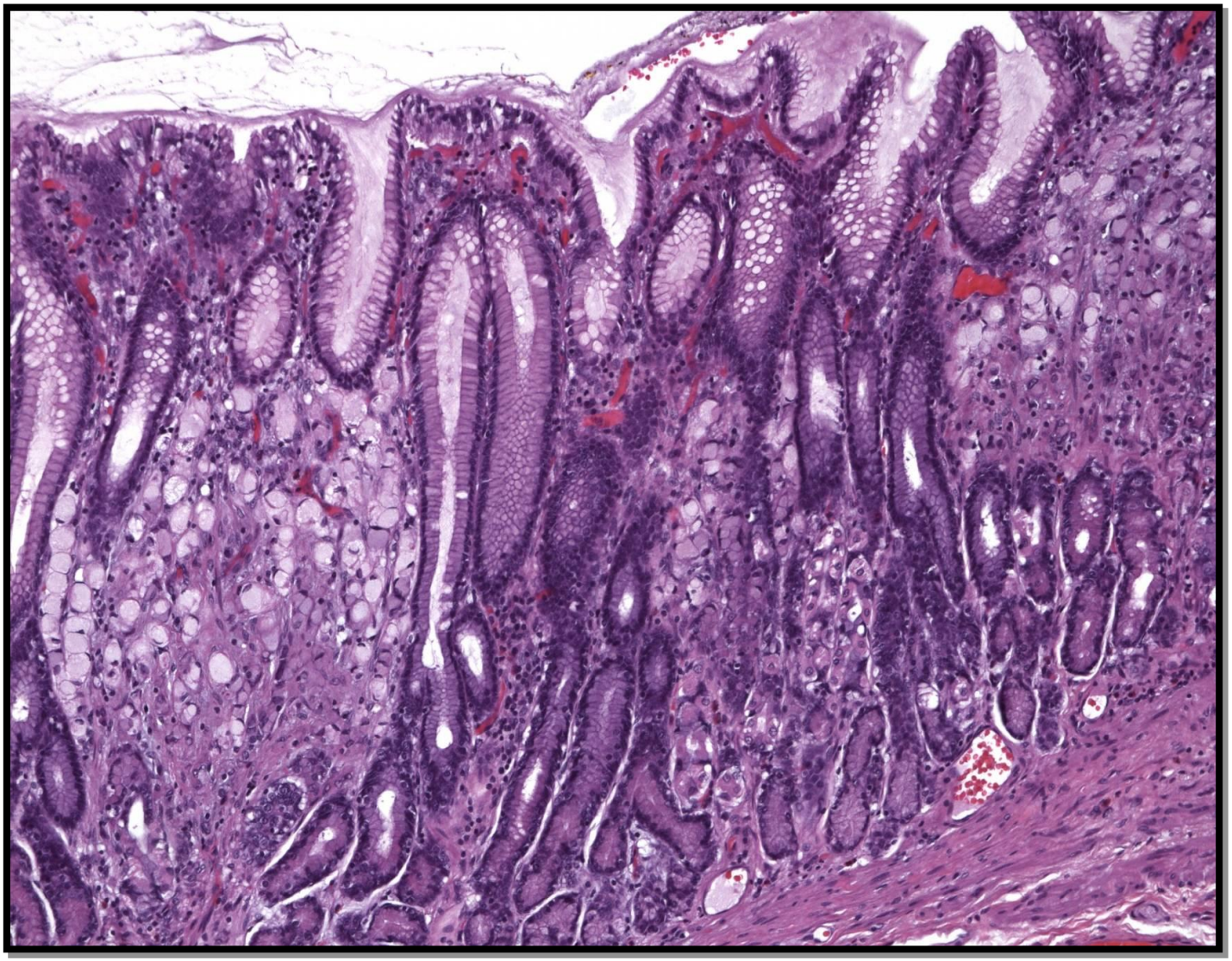
Hereditary Diffuse Gastric Cancer (HDGC)

Medizinische Universität Graz



Hereditary Diffuse Gastric Cancer (HDGC)

Medizinische Universität Graz



Hereditary Diffuse Gastric Cancer (HDGC)

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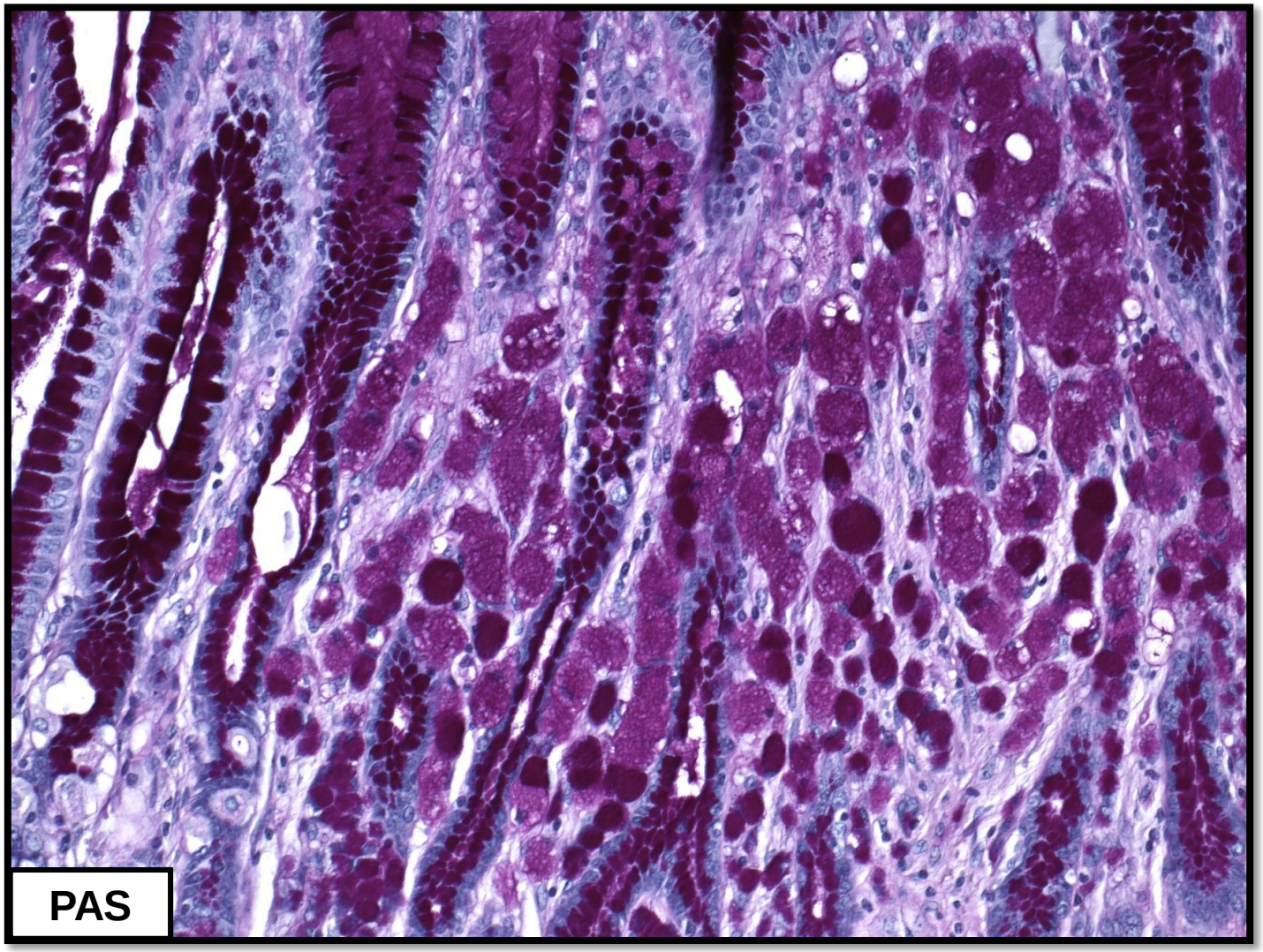

Hereditary Diffuse Gastric Cancer (HDGC)

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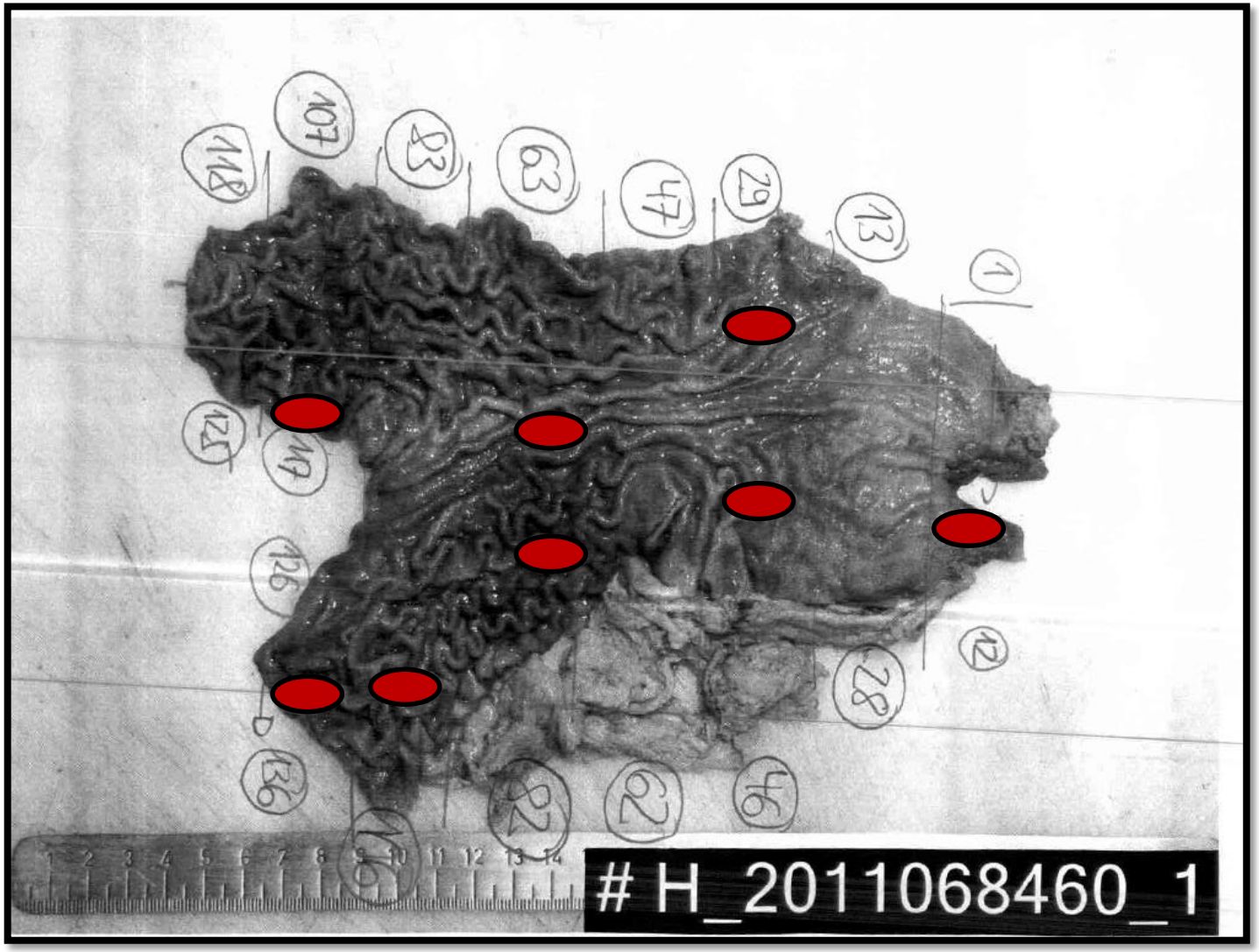


HDGC Precursor Lesions

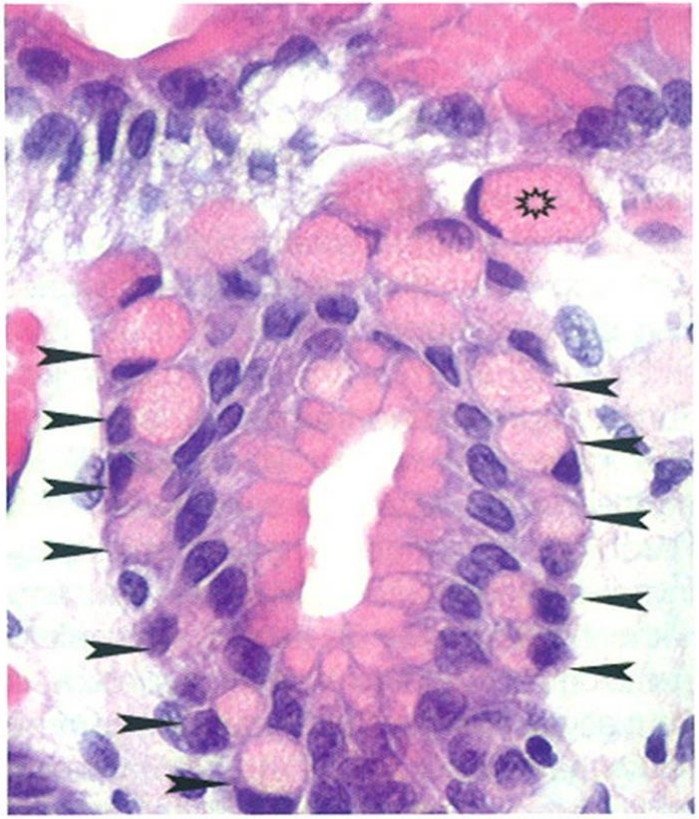


Fig. 4.24 Pagetoid spread of signet-ring cells (arrow heads) representing Tis, and invasion of the lamina propria by a signet-ring cell (empty star), indicating T1a invasive intramucosal signet-ring cell carcinoma.

Pagetoid spread of signet-ring cells (pTis)

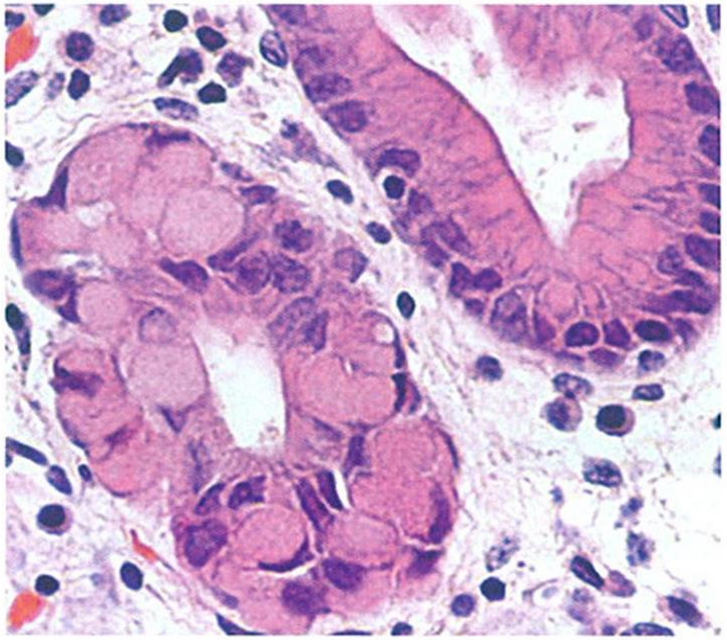


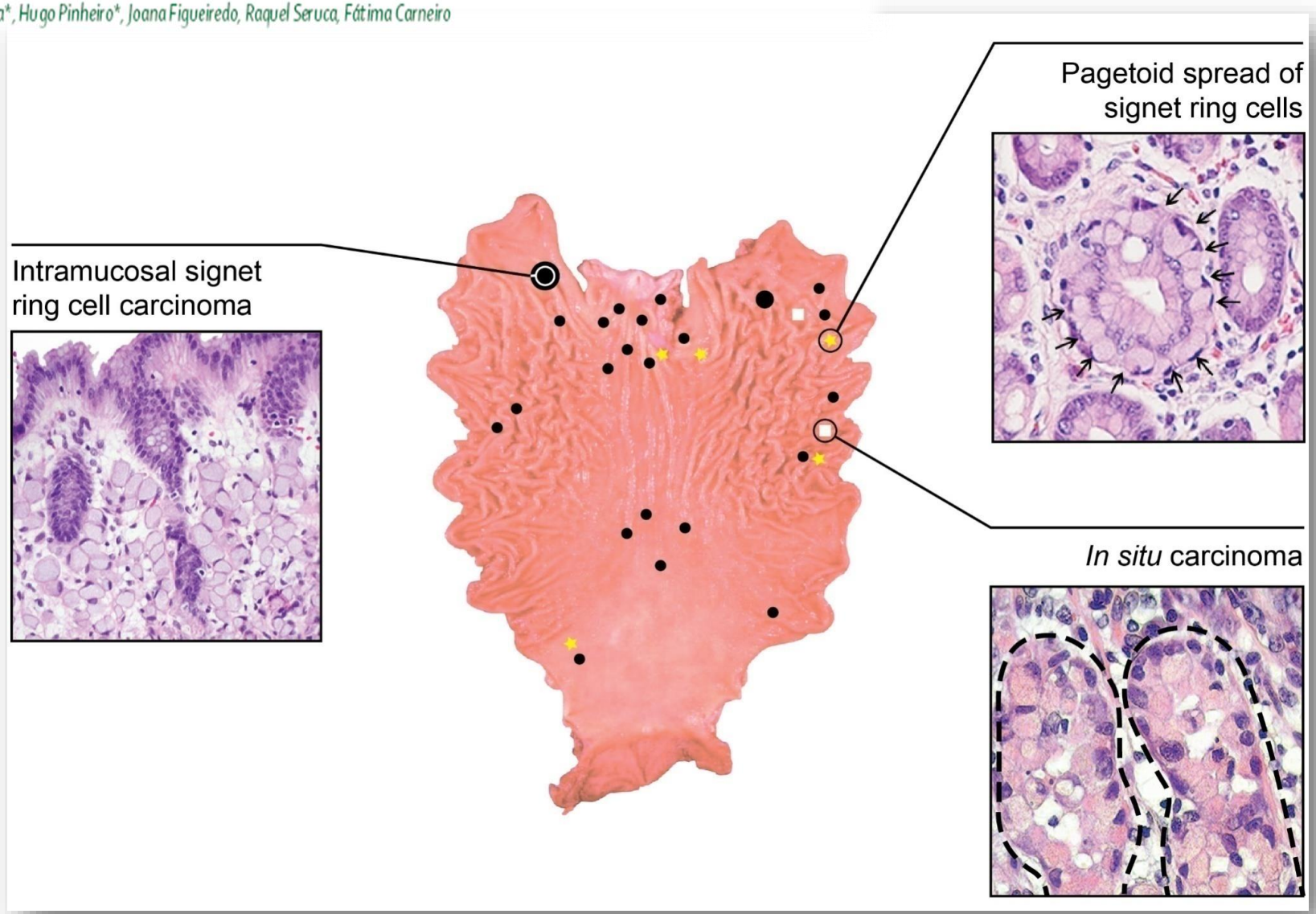
Fig. 4.23 Signet ring cell carcinoma *in situ* (Tis).

Signet-ring cell carcinoma in situ (pTis)

Carneiro F et al. WHO Classification 2010

Familial gastric cancer: genetic susceptibility, pathology, and implications for management

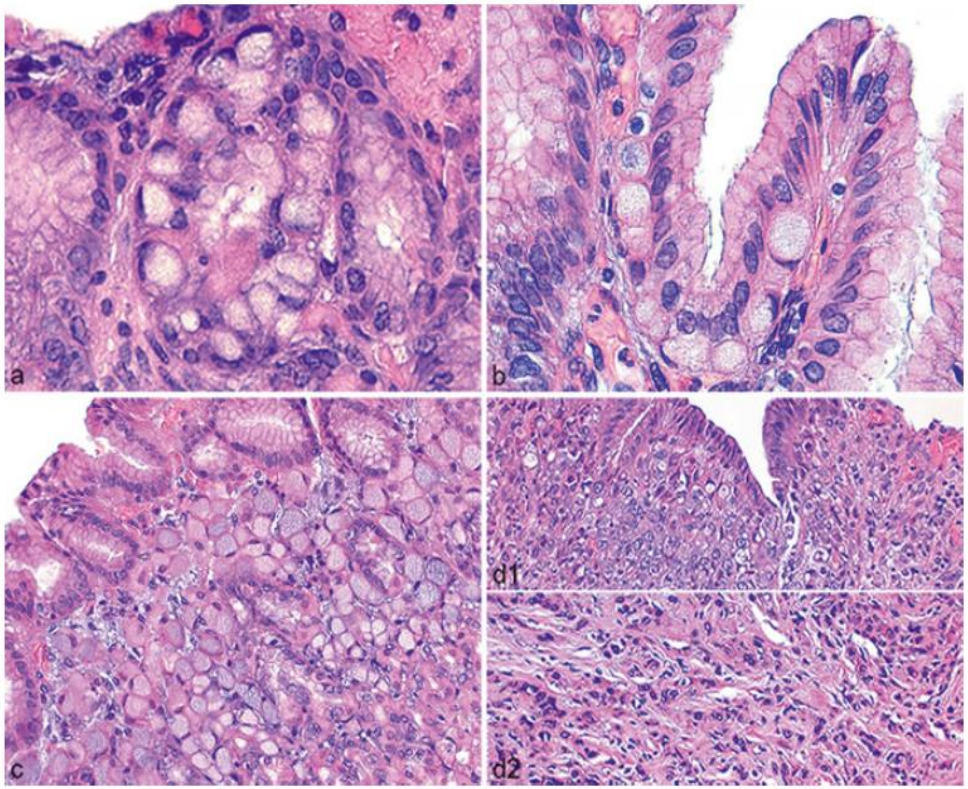
Carla Oliveira*, Hugo Pinheiro*, Joana Figueiredo, Raquel Seruca, Fátima Carneiro

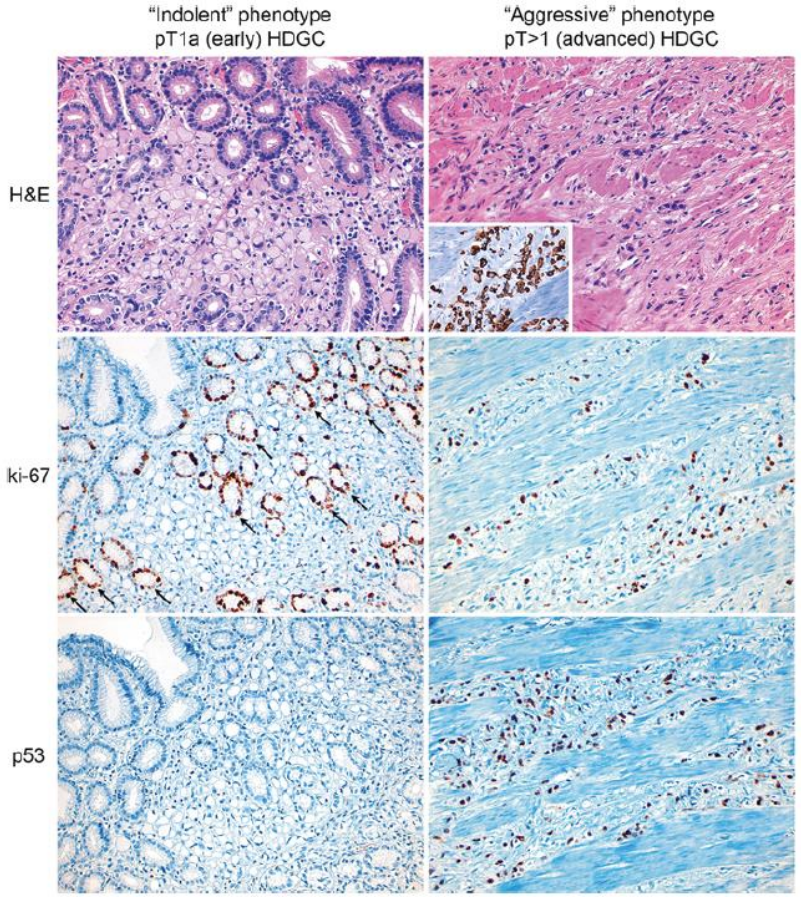


Oliveira et al. Lancet Oncol 2015

Histopathological, Molecular, and Genetic Profile of Hereditary Diffuse Gastric Cancer: Current Knowledge and Challenges for the Future

Rachel S. van der Post, Irene Gullo, Carla Oliveira, Laura H. Tang, Heike I. Grabsch, Maria O'Donovan, Rebecca C. Fitzgerald, Han van Krieken, and Fátima Carneiro





Van der Post et al. Adv Exp Med Biol 2015

OPEN ACCESS

Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline *CDH1* mutation carriers

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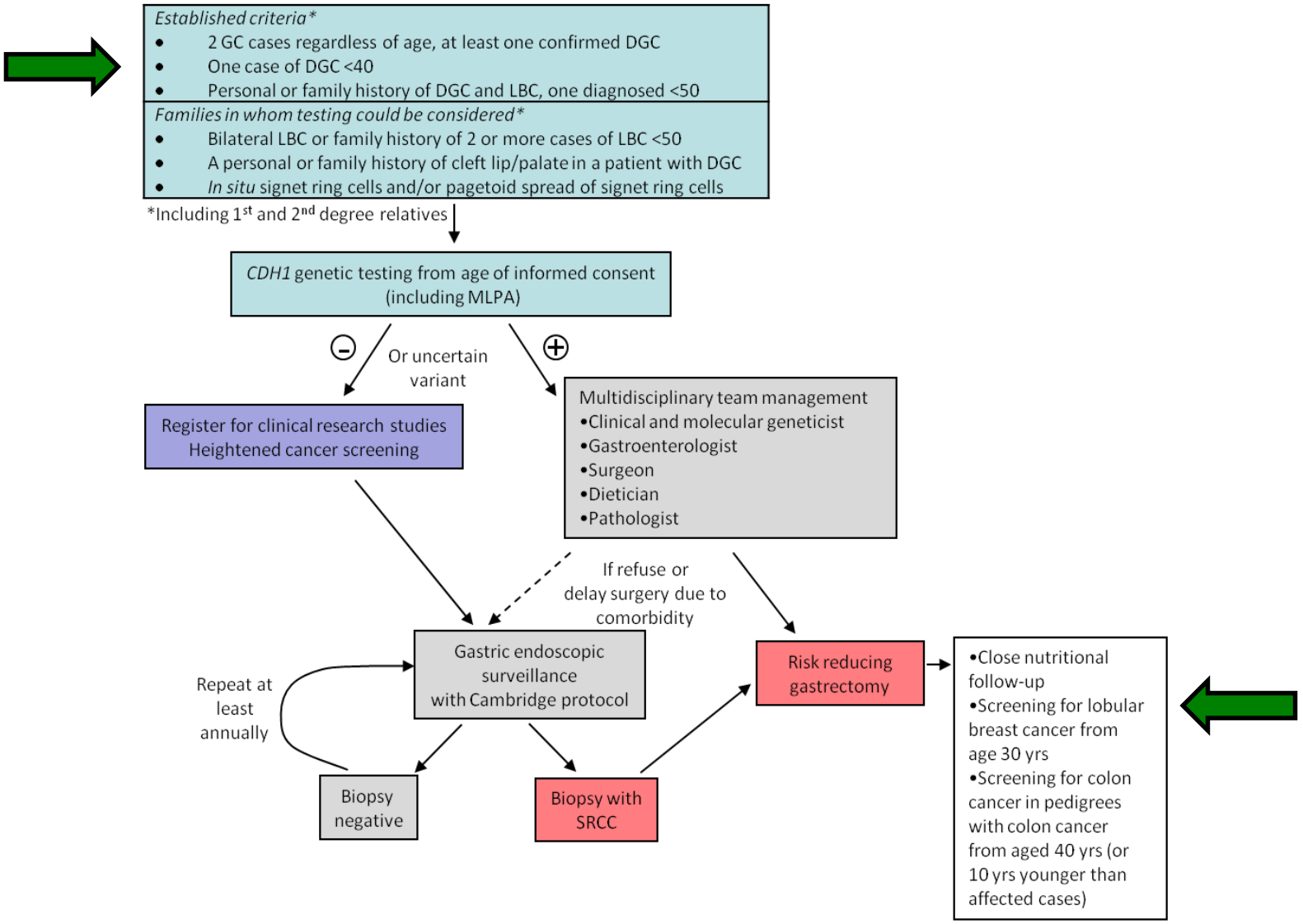
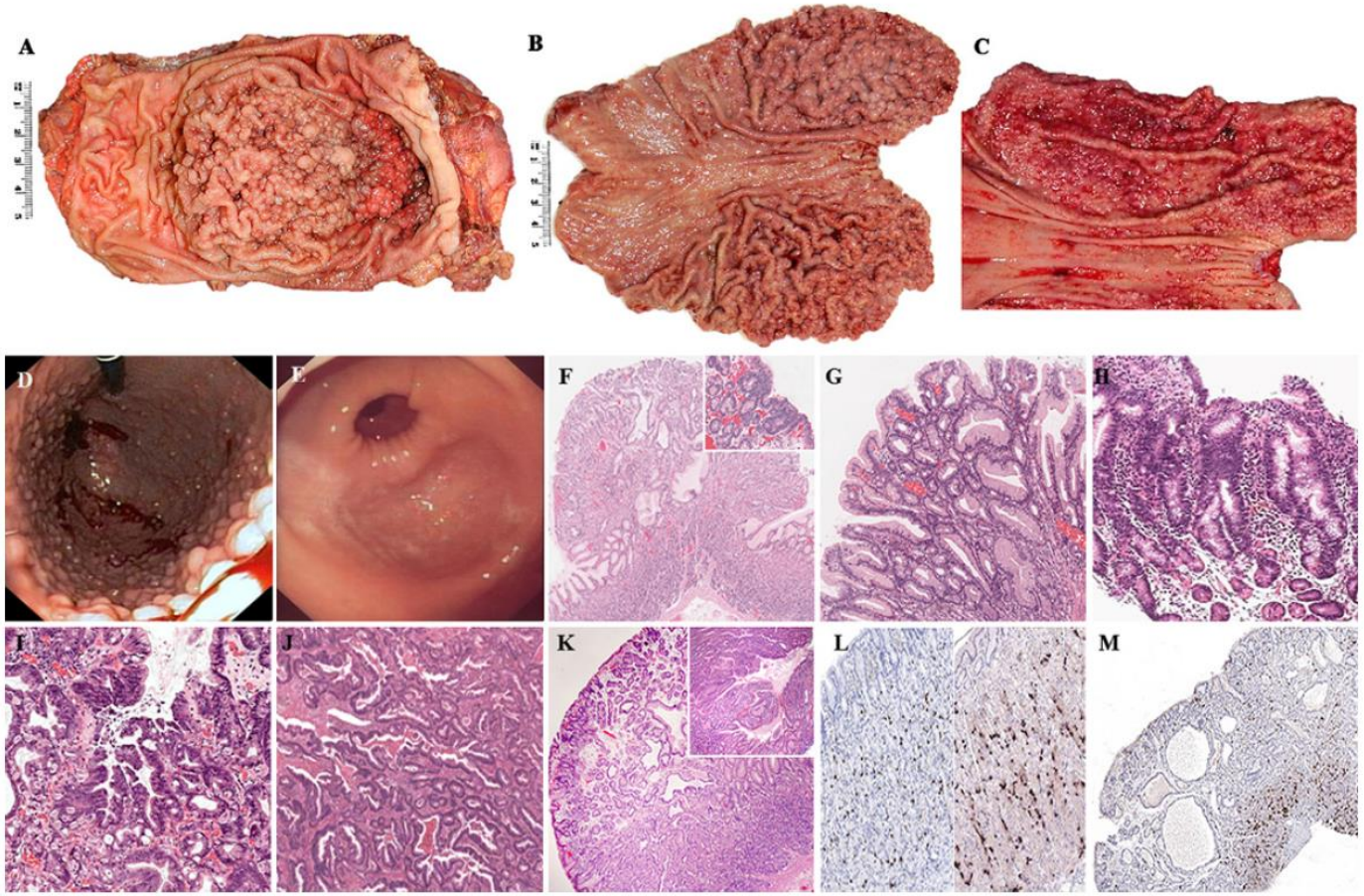


Van der Post et al. J Med Genet 2015

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome

D L Worthley,[1] K D Phillips,[2] N Wayte,[3] K A Schrader,[4] S Healey,[5] P Kaurah,[4] A Shulkes,[6] F Grimpen,[7] A Clouston,[7] D Moore,[8] D Cullen,[9] D Ormonde,[9] D Mounkley,[10] X Wen,[11] N Lindor,[12] F Carneiro,[11] D G Huntsman,[4] G Chenevix-Trench,[5] G K Suthers[2,13]



Worthley et al. Gut 2012

ARTICLE

Point Mutations in Exon 1B of *APC* Reveal Gastric Adenocarcinoma and Proximal Polyposis of the Stomach as a Familial Adenomatous Polyposis Variant

Jun Li,[1] Susan L. Woods,[2] Sue Healey,[1] Jonathan Beesley,[1] Xiaoqing Chen,[1] Jason S. Lee,[1] Haran Sivakumaran,[1] Nicci Wayte,[1] Katia Nones,[1] Joshua J. Waterfall,[3] John Pearson,[1,4] Anne-Marie Patch,[1] Janine Senz,[5] Manuel A. Ferreira,[1] Pardeep Kaurah,[6] Robertson Mackenzie,[7] Alireza Heravi-Moussavi,[8] Samantha Hansford,[5] Tamsin R.M. Lannagan,[2] Amanda B. Spurdle,[1] Peter T. Simpson,[9,10] Leonard da Silva,[9,10] Sunil R. Lakhani,[9,10,11] Andrew D. Clouston,[12,13] Mark Bettington,[10,13,14] Florian Grimpen,[15] Rita A. Busuttil,[16,17,18] Natasha Di Costanzo,[16] Alex Boussioutas,[16,17,18,19] Marie Jeanjean,[20] George Chong,[21] Aurélie Fabre,[22,23,24] Sylviane Olschwang,[22,23,24] Geoffrey J. Faulkner,[25] Evangelos Bellos,[4,26] Lachlan Coin,[4] Kevin Rioux,[27] Oliver F. Bathe,[28,29] Xiaogang Wen,[30,31] Hilary C. Martin,[32] Deborah W. Neklason,[33] Sean R. Davis,[3] Robert L. Walker,[3] Kathleen A. Calzone,[3] Itzhak Avital,[34] Theo Heller,[35] Christopher Koh,[35] Marbin Pineda,[3] Udo Rudloff,[36] Martha Quezado,[37] Pavel N. Pichurin,[38] Peter J. Hulick,[39]

(Author list continued on next page)

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is an autosomal-dominant cancer-predisposition syndrome with a significant risk of gastric, but not colorectal, adenocarcinoma. We mapped the gene to 5q22 and found loss of the wild-type allele on 5q in fundic gland polyps from affected individuals. Whole-exome and -genome sequencing failed to find causal mutations but, through Sanger sequencing, we identified point mutations in *APC* promoter 1B that co-segregated with disease in all six families. The mutations reduced binding of the YY1 transcription factor and impaired activity of the *APC* promoter 1B in luciferase assays. Analysis of blood and saliva from carriers showed allelic imbalance of *APC*, suggesting that these mutations lead to decreased allele-specific expression in vivo. Similar mutations in *APC* promoter 1B occur in rare families with familial adenomatous polyposis (FAP). Promoter 1A is methylated in GAPPS and sporadic FGPs and in normal stomach, which suggests that 1B transcripts are more important than 1A in gastric mucosa. This might explain why all known GAPPS-affected families carry promoter 1B point mutations but only rare FAP-affected families carry similar mutations, the colonic cells usually being protected by the expression of the 1A isoform. Gastric polyposis and cancer have been previously described in some FAP-affected individuals with large deletions around promoter 1B. Our finding that GAPPS is caused by point mutations in the same promoter suggests that families with mutations affecting the promoter 1B are at risk of gastric adenocarcinoma, regardless of whether or not colorectal polyps are present.

Li et al. Am J Hum Genet 2016

Neoplastic Lesions of Gastric Adenocarcinoma and Proximal Polyposis Syndrome (GAPPS) Are Gastric Phenotype

Willem B. de Boer, MBBS, FRCPA, Hooi Ee, MBBS, FRACP, PhD,† and Marian P. Kumarasinghe, MBBS, MD, FRCPA**

Medizinische Universität Graz

Fundic Gland Polyps (10/15 Patients)

Well developed FGP-like lesions, defined as "microcysts lined by fundic epithelium including oxyphilic cells," were seen in 10 of 14 patients with polyposis (Fig. 2) but not in the biopsy of the 1 case of adenocarcinoma.

Hyperproliferative Aberrant Pits (12/15 Patients)

The most frequent pathology was disorganized proliferation of specialized/oxyntic glands high up in the mucosa involving the attenuated foveolar region around the gastric pits, forming a polypoid lesion (Figs. 3A, B). This imparted the appearance of upward migration of the tubular neck and the hyperproliferative nature was highlighted by heightened Ki-67 activity (Fig. 3C) giving the impression of an expanded proliferative zone. The surface epithelium was normal.

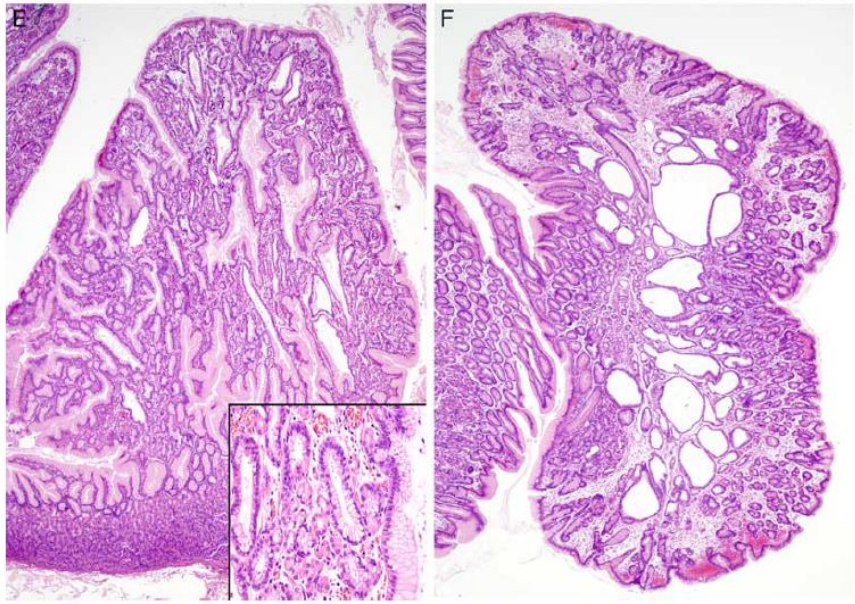


TABLE 2. Immunohistochemical Profile and Proliferation Index in Polypoid Lesions Associated With GAPPS

	Number	MUC2	MUC5AC	MUC6	CDX2	CD10	Beta Catenin	P53	Ki-67*
HPAP†	>150	Negative	Positive	Positive deep	Variable positive	Negative	Negative	Positive	Increased
FGP‡	>40	Negative	Positive	Negative	Variable positive	Negative	Negative	Negative	Normal
Adenoma	3	Negative	Positive	Positive focal	Variable positive	Negative	Negative	Variable positive	Increased
Dysplasia	7	Negative	Positive	Negative	Variable positive	Negative	Negative	Positive	Increased
Carcinoma	1	Negative	Positive	Negative	Variable positive	Negative	Negative	Positive	Increased

*Increased Ki-67 activity in the lesional area compared with the proliferative zone of the adjacent tubular neck region.
†Hyperproliferative aberrant pits.
‡Fundic gland polyps.

De Boer et al. Am J Surg Pathol 2018

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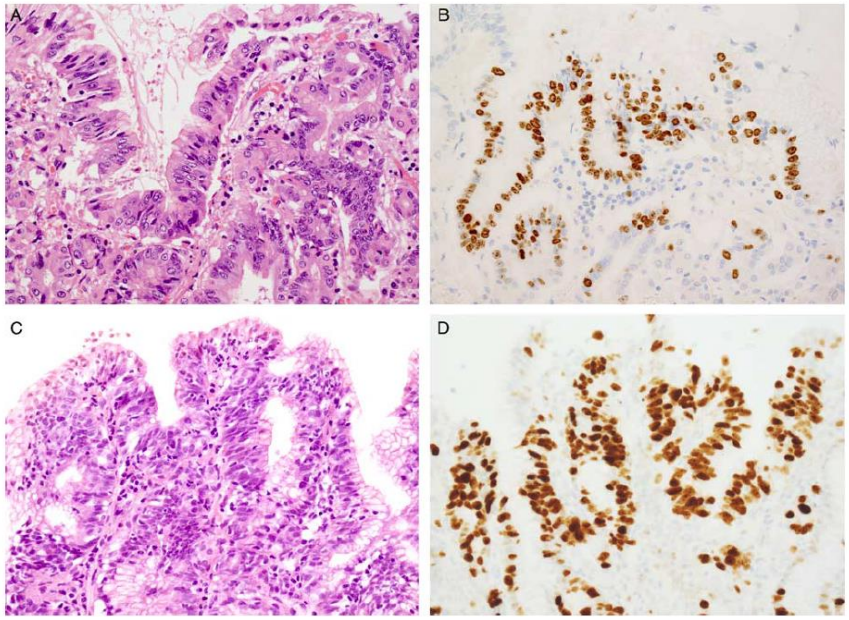

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De Boer et al. Am J Surg Pathol 2018

Take Home Messages I

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- **Poorly cohesive carcinoma (PCC)** comprises different phenotypes (signet-ring cell vs. PCC-NOS) with different molecular background and behaviour
- **Gastric carcinoma with lymphoid stroma** is related to EBV infection or microsatellite instability (MSI-H). Tumours harbour cytotoxic T-cells and often show PD-L1 positivity (compare below)
- Different types of clinically relevant **molecular subtypes** have been described (TCGA, ACRG etc). Assessment can be done by immunohistochemistry and *in situ* hybridization
- **HER2** assessment needs to be done following international Guidelines (Rüschoff et al.); select the best differentiated areas (intestinal morphology) for testing

Take Home Messages II

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- EBV-positive or microsatellite instable (MSI-H) gastric cancers are potential candidates for **tumor immunotherapy** as they harbor the highest PDL-1 positivity rates
- Potential responders may also be identified among EBV-negative and MSS/MSI-L cancers (intestinal>diffuse): **PDL-1 assessment** needs to be done applying the CPS Score
- **Neoadjuvant chemotherapy** may have a negative effect on patients with MSI-H gastric cancer (post hoc analysis from MAGIC trial) → MMR/MSI testing on pretreatment biopsies
- Gastric cancer may occur within distinct **hereditary cancer syndromes** (e.g. Lynch syndrome, Peutz-Jeghers syndrome) and also distinct **hereditary gastric cancer syndromes**, including HDGC and GAPPS

Thank you very much for your kind attention!

Cord Langner MD
Institute of Pathology
Medical University of Graz / Austria
cord.langner@medunigraz.at
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European Society of Pathology