

Supplementary Text 3: Protocol for macroscopic examination and sampling of *CDHI* mutation-related gastrectomy specimens

Total gastric mucosa embedding and mapping is the gold standard for pathology examination and is pivotal to determine the stage of cancer and additionally to better understand the phenotype and biology of *CDHI* mutation-related gastric cancer. However, experience in the examination of prophylactic gastrectomies for HDGC is quite limited in many pathology departments due to the rarity of these surgical specimens. Additionally, the routine workload may be incompatible with performing the detailed examination of hundreds of sections typically obtained after totally embedding these stomachs. Actually, total gastric mapping requires approximately 120-270 blocks (a higher number of blocks has even been described in some studies²³), with up to three slices per block resulting in an average of 9.6m of mucosa to examine per gastrectomy.^{16, 24} As an approximation, total gastric mucosa embedding consumes ten-fold the resources compared to a conventional gastrectomy specimen. The resources are mainly in staff time required for mapped macroscopic dissection, laboratory embedding and cutting, and pathologist time required to examine the slides and map microscopic lesions to the macroscopic photo. To address these shortcomings, a three level protocol for pathological examination of gastrectomy specimens, depending on availability of resources, is herein proposed (Supplementary Table 4).

Macroscopic examination and sampling of prophylactic gastrectomies should follow specific protocols. Begin with painting the margins or removing the margins before fixation. Then dissect the omentum and retrieve lymph nodes. Fresh gastrectomy specimens should be opened along the greater curve and pinned onto a cork board. A life size specimen photo should be used as a template to identify the exact location of the tissue blocks. The collection of fresh tissue samples from any macroscopic lesion and normal looking mucosa should be considered for research purposes. Overnight fixation in buffered formalin is recommended before sampling for routine histopathology, including any macroscopically abnormal areas such as pale lesions. Sections of the margins should be taken and labelled. The remainder of the stomach should be sectioned according to the level selected for pathological examination depending on availability of resources (see Supplementary Table 4). Regardless of the selected protocol, each section (2 cm x 0.3 cm, full thickness) is blocked (paraffin-embedded). The location of each section should be marked on the map of the stomach. Any macroscopic lesions identified should be precisely localised within the map. An alternative for pathologists experienced in the method is to use an adaptive version of the Swiss roll technique.²⁵ With this technique, the gastrectomy should only be fixed briefly, for 2-3 hours, after which the mucosa is dissected from the submucosa and muscle layers. Another technique is to use giant histological sections with the whole-mount technique, also called large-format histology. This method will save time and blocks, as each stomach is represented in approximately 25 blocks. The histological examination should be made using a checklist focusing on the items listed in Supplementary Table 3.

Regardless of the level selected for pathological examination depending on availability of resources, the minimum examination of a macroscopically normal gastrectomy should include: 1. Proximal and distal margins to confirm all the gastric mucosa has been resected. 2. All lymph nodes should be sampled as per a usual gastrectomy. 3. Photograph. 4. Mapped sampling from all zones: antrum, transitional zone (*incisura angularis*), body, and fundus. 5. If no foci of carcinoma are found, then to go back to the specimen and take more blocks. If no foci of SRCC are found, the gastrectomy should not be reported as negative for carcinoma, but as 'no carcinoma found in xx% of the mucosa examined'.

The pathology of HDGC and HLBC is unique and expertise is needed to provide high quality diagnosis, both in biopsies and in resection specimens. In order to increase the experience of pathologists and the accuracy of the diagnosis, it would be useful to build a free, online open-access digital slide bank of the different types of lesions observed in the setting of *CDHI*-related cancer. The use of (scanned) slides to be submitted for evaluation by experienced pathologists in the field should be seriously considered.

In the event of a lack of pathologist experience in dealing with these cases, or restricted time available due to the pathologist's workload and laboratory resources, the entire formalin-fixed gastrectomy or mastectomy specimen can be sent to an experienced pathology laboratory. An alternative option is to totally embed the stomach or breast, perform H&E and PAS-D stain on all blocks and send the slides and blocks to an experienced centre for specialist pathology reporting. If these alternative strategies are not feasible, and it is not possible to totally embed the gastric (or breast specimen), this should be communicated to clinicians and the patient.

Supplementary Table 3: Checklist for reporting of prophylactic gastrectomy specimens

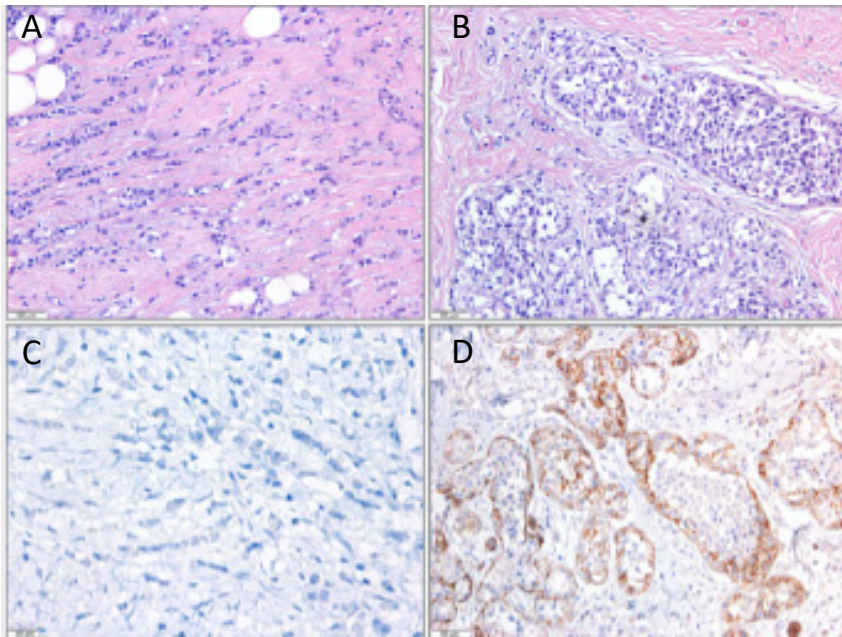
<p>(1) Features of \geqpT1b carcinoma(s)</p>	<p>Growth pattern (diffuse infiltration versus localized tumour) Anatomic location (cardia, fundus, body, transitional zone, antrum) Measurements Histological type according to WHO 2019 and Laurén's classifications Lymphatic, venous and neural invasion (present or absent) TNM stage</p>
<p>(2) Features of intraepithelial precursor lesions and intramucosal (pT1a) signet ring cell carcinoma</p>	<p>Number of lesions Anatomic location (cardia, fundus, body, transitional zone, antrum) Measurements Aggressive features: pleomorphism, loss of mucin, spindle cells, small cells, mitoses Stromal reaction related to lesions: desmoplasia; lymphocytic, eosinophilic or granulomatous inflammatory reaction Surgical margin status (proximal oesophageal, distal duodenal mucosa, including donuts), to confirm there is no residual gastric mucosa and no tumour at margins. Lymph node status</p>
<p>(3) Non-neoplastic mucosa: changes more commonly seen in this condition</p>	<p>Tufting/ hyperplastic mucosal changes Surface epithelial vacuolisation Globoid change</p>
<p>(4) Other findings in surrounding mucosa</p>	<p>Inflammation (acute, chronic, erosion, ulceration) <i>Helicobacter pylori</i> Intraepithelial lymphocytes Lymphoid infiltrates Glandular atrophy Intestinal metaplasia Adenomatous dysplasia Hyperplastic polyps Fundic gland polyps</p>

Supplementary Table 4. Levels of pathological examination depending on availability of resources



Levels of pathological examination depending on availability of resources			
Level	Level 1 Minimum required	Level 2 [level 1 plus...]	Level 3 [Level 2 plus...]
Morphologic	<ul style="list-style-type: none"> • Pin out and photograph • Sample margins and lymph nodes • Sample tissue from all gastric zones • Map blocks to photo • Examine all slides 	<ul style="list-style-type: none"> • Embed all mucosa, process to paraffin blocks. • Cut a subset of blocks, sampling all gastric zones • Examine sampled slides. 	<ul style="list-style-type: none"> • Cut all blocks. Examine all slides.
Repeat	<ul style="list-style-type: none"> • Sample tissue from all zones • Map blocks • Examine all slides 	<ul style="list-style-type: none"> • Cut a subset of blocks, sampling all zones • Examine sampled slides. 	
Stop	When invasive carcinoma is found or up to arbitrary limit for example 50 blocks	When invasive carcinoma is found, or up to arbitrary limit for example 50 slides.	When all mucosa is examined.
Report	Multiple of foci of pT stage carcinoma in xx% of mucosa examined microscopically	Number of foci of pT stage carcinoma in xx% of mucosa examined microscopically	Number of foci of pT stage carcinoma, all mucosa examined microscopically
Blocks	~ 20-50*	~ 120-270	~ 120-270
Slides	~ 20-50*	~ 20-50*	~ 120-270

A three level protocol is suggested where Level 1 is the minimum examination to obtain sufficient data (margins, carcinoma stage, lymph node status) necessary for patient care. Level 2 represents a compromise between clinical reporting and preserving tissue for future research, and Level 3 is total gastric embedding and mapping. *The upper limit number of blocks and slides required to find foci of stage pT1a carcinoma, or pTis (signet ring cell carcinoma *in situ*) is variable.



Supplementary Fig. 3. Lobular breast cancer. (A) Invasive lobular breast cancer. (B) Lobular carcinoma *in situ*. Loss of E-cadherin immunoreactivity is shown both in the invasive (C) and *in situ* (D) components.