## **Ancillary studies** (Core and Non-core)

For gastric carcinomas, where there is a suspicion based on morphology, of neuroendocrine differentiation, including gastric neuroendocrine carcinomas (NECs) and mixed neuroendocrine-non-neuroendocrine carcinomas, the reporting of neuroendocrine marker expression and Ki-67 proliferation index are core elements. These elements are non-core for other types of gastric carcinomas.

Gastric neuroendocrine neoplasms are classified into neuroendocrine tumours (NETs), NECs and mixed neuroendocrine–non-neuroendocrine neoplasm (MiNENs). NETs are graded 1-3 using the mitotic count and Ki-67 proliferation index.[1] However, pure NETs are not considered within the scope of this dataset.[2] Most NECs show marked cytological atypia, brisk mitotic activity, and are subclassified into small cell and large cell subtypes.[1] NECs are considered high grade by definition, typically with a Ki-67 proliferation index >55%.[3] MiNENs are usually composed of a poorly differentiated NEC component and an adenocarcinoma component. If a pure or mixed NEC is suspected on morphology, immunohistochemistry (IHC) is required to confirm neuroendocrine differentiation, usually applying synaptophysin and chromogranin A as a minimum.[1]

The National Comprehensive Cancer Network guidelines recommend assessment of HER2 expression using IHC, followed up by assessment of *HER2* amplification using in situ hybridization (ISH) when ISH is equivocal, for patients with inoperable locally advanced, recurrent and metastatic gastric/OGJ adenocarcinoma for whom therapy with trastuzumab is considered.[4] For HER2 IHC in resection specimens, both intensity and percentage of immunoreactive cancer cells is assessed with scores ranging from 0 to 3+ (Table 7). ISH is used if IHC is equivocal (2+). IHC 3+ or ISH showing *HER2* amplification (including IHC 2+ with *HER2* amplification by ISH) is considered HER2 positive. The HER2 IHC report should include the IHC score and primary antibody used. The *HER2* ISH report should include the result (amplified or not amplified), number of invasive cancer cells counted, and which assay used (dual-probe versus single-probe assay). The HER2 scoring system by Hofmann et al (2008) can be used to evaluate HER2 expression in gastric cancers.[5]

**Table 7: Criteria used in the ToGA trial for scoring HER2 expression by immunohistochemistry (IHC) in gastric and oesophagogastric junction adenocarcinoma.[5]**

| HER2 IHC Score | HER2 IHC pattern in surgical specimen | HER2 Expression assessment |
|---|---|---|
| 0 | No reactivity or membranous reactivity in <10% of cancer cells | Negative |
| 1+ | Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are reactive only in part of their membrane | Negative |
| 2+ | Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells | Equivocal (perform in situ hybridisation (ISH)) |
| 3+ | Strong complete, basolateral or lateral membranous reactivity in ≥10% of cancer cells | Positive |

Microsatellite instability (MSI)/mismatch repair deficiency (dMMR) status and PD-L1 expression have been used as predictive biomarkers for checkpoint inhibitor therapy since the United States (US) Food and Drug Administration (FDA) approved pembrolizumab for the treatment of patients with MSI-high (MSI-H) or PD-L1 positive unresectable or metastatic gastric cancers.[6] While MSI status has been highly predictive of response to PD-1 pathway blockage in several clinical trials, the value of PD-L1 expression in selecting patients for checkpoint inhibitors in oesophageal and gastric cancer needs to be further investigated.

Approximately 40% of gastric/oesophageal cancers express PD-L1 based on the combined positive score (CPS). Unlike other malignancies (i.e., non-small cell lung cancer), PD-L1 expression in gastric/oesophageal cancers is mainly observed in immune cells. The CPS, which takes into account PD-L1 expression by both tumour cells and tumour-associated immune cells, was developed and refined for scoring gastric and oesophageal cancers.[7] CPS is calculated by dividing the total number of PD-L1 positive cells (including tumour and immune cells) by the total number of viable tumour cells. A CPS ≥1 as determined by an FDA-approved companion diagnostic test (the Dako PD-L1 IHC 22C3 PharmDx Assay) is currently used to classify a tumour as PD-L1 positive. A low overall response rate (ORR) has been reported when using a CPS cutoff of <1.[8] Practices may differ in other countries. Studies are ongoing to investigate whether the ORR can be improved by using a different cutoff.

DNA mismatch repair defect can be determined by either polymerase chain reaction (PCR)-based MSI testing or by IHC stains for MLH1, MSH2, MSH6 and PMS2. Mismatch repair (MMR) IHC may be reported using the template outlined in Table 8.[9] MSI-high/dMMR is seen in 8-25% of gastric cancer. While some of MSI-high/dMMR gastric cancers result from hypermethylation of *MLH1* promotor, others develop in association with Lynch syndrome, which is caused by germline mutations in one of the mismatch repair genes, namely *MLH1*, *MSH2*, *MSH6* and *PMS2* or rarely *EPCAM*. Germline mutational analyses can be performed if there is a suspicion of Lynch syndrome.

**Table 8: College of American Pathologists template for reporting mismatch repair protein immunohistochemistry results.**[9]

| **Immunohistochemistry results for mismatch repair (MMR) proteins** | |
|---|---|
| MLH1 | |
| | Intact nuclear expression |
| | Loss of nuclear expression |
| | Cannot be determined (explain) |
| MSH2 | |
| | Intact nuclear expression |
| | Loss of nuclear expression |
| | Cannot be determined (explain) |
| MSH6 | |
| | Intact nuclear expression |
| | Loss of nuclear expression |
| | Cannot be determined (explain) |
| PMS2 | |
| | Intact nuclear expression |
| | Loss of nuclear expression |
| | Cannot be determined (explain) |
| Background non-neoplastic tissue/internal control shows intact nuclear expression | |
| **MMR interpretation** | |
| No loss of nuclear expression of MMR proteins: No evidence of deficient mismatch repair (low probability of MSI-H) | |
| Loss of nuclear expression of one or more MMR proteins: deficient mismatch repair | |

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Epstein-Barr virus associated gastric cancers (EBVaGC) are associated with a better prognosis.[10] In addition, EBVaGCs are more likely associated with overexpression of PD-L1 and PD-L2. A recent study suggested that EBVaGC could be a marker for efficacy of immunotherapy.[8] EBVaGC accounts for approximately 10% of all gastric cancers, most of which are located in the proximal stomach.[11] Histologically, EBVaGC can be sub-classified into: 1) poorly differentiated carcinoma with abundant tumour-infiltrating lymphocytes (gastric (adeno)carcinoma with lymphoid stroma); 2) tubular adenocarcinoma with prominent lymphoid follicles and active germinal centres (also termed carcinoma with Crohn disease-like lymphoid reaction); and 3) conventional-type adenocarcinoma with scant lymphocytic infiltrate.[10] Although EBVaGC can be poorly differentiated, EBVaGC is a distinct subtype with a low risk of lymph node metastasis.[12] Epstein-Barr encoded region (EBER) ISH is widely used to detect EBVaGC.

Other molecular testing includes targeted next generation sequencing. This testing is usually only performed to identify other actionable targets.

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