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

For gastric neuroendocrine carcinomas, including 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), neuroendocrine carcinomas (NECs) and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs).

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

The National Comprehensive Cancer Network (NCCN) guidelines recommend assessment of HER2 expression using immunohistochemistry (IHC) or *HER2* amplification using in situ hybridization (ISH) for patients with inoperable locally advanced, recurrent and metastatic gastric/OGJ adenocarcinoma for whom therapy with trastuzumab is considered.[4] For 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 when IHC is equivocal (2+). IHC 3+ or ISH showing *HER2* amplification (ISH positive) (including IHC 2+ with ISH positivity) 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).

**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 (do ISH) |
| 3+ | Strong complete, basolateral or lateral membranous reactivity in ≥10% of cancer cells | Positive |

Microsatellite instability/mismatch repair deficiency (dMMR) status and PD-L1 expression have been used as predictive biomarkers for checkpoint inhibitor therapy since the United States Food and Drug Administration (FDA) approved pembrolizumab for the treatment of microsatellite instability 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. Unlike other malignancies (i.e., non-small cell lung cancer), PD-L1 expression in gastric/oesophageal cancers is mainly observed in immune cells. The combined positive score (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] Many studies are ongoing to investigate whether the ORR can be improved by using a different cutoff.

Microsatellite status of a tumour 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 are recommended for individuals suspicious for 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 | |

Reproduced with permission from College of American Pathologists (2018). *Template for reporting results of biomarker testing of specimens from patients with carcinoma of the colon and rectum.* College of American Pathologists.[10]

Epstein-Barr virus (EBV) positive gastric cancers are associated with a better prognosis. In addition, EBV positive tumours are more likely associated with overexpression of PD-L1 and PD-L2. A recent study suggested that EBV positive tumours could be a strong marker for efficacy of immunotherapy.[8]

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

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