HER2 heterogeneity in gastric/gastroesophageal cancers: from benchside to practice

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• Prognosis for gastric cancer (GC) patients still remains dismal as diagnosis is often late and (...) only about half of patients undergo curative resection. (...) GC remains one of the most common causes of cancer-related deaths, with approximately 950000 new cases/year and an estimated number of deaths close to 720000.

• Gastroesophageal junction carcinoma (GEJC) is showing a rapid rise in incidence in Western countries.

• Survival for patients with metastatic disease remains poor, with overall survival rates of 5%-20% at 5 years.

• A randomized phase III trial [Trastuzumab for Gastric Cancer (ToGA)] showed improved response rate, median progression-free survival, and overall survival when the monoclonal antibody against HER2, Trastuzumab, was added to the first-line fluoropyrimidine/platinum based treatment in HER2 positive GC/GEJC.

• Trastuzumab and chemotherapy have since become the new standard of treatment for patients with advanced, HER2 positive, GC/GEJC
1. *Sample selection with regards to cancer morphology:*

   In mixed-type carcinomas, samples with a prevalence of intestinal-type areas should be selected when performing HER2 evaluation.

2. *Choice of immunohistochemistry scoring criteria:*

   A 10% cut off was established when evaluation was performed on surgical samples whereas a single cluster of at least 5 positive cells was sufficient in endoscopic biopsies; staining intensity was maintained in three categories i.e., faint, moderate or intense.

3. *Choice of HER2 evaluation method:*

   European Medicines Agency (EMA) states IHC as the initial testing method and ISH only for equivocal score 2+ cases.
1. **Impact of pre-analytic variables:**

   Fixation should be exclusively based on the use of 10% neutralbuffered formalin and fixation time should be comprised between of 8 h - 48 h

2. **Which methodology: IHC, FISH, CISH or SISH?**

   HER2 testing in GC should be performed by IHC as the first approach

3. **HER2 Heterogeneity**

   Possibilities include neoplastic clones in which HER2 is amplified/overexpressed in an otherwise HER2 negative tumor or silencing of HER2 expression in an area of a tumor with homogeneous HER2 amplification
4. Is the minimum area of > 10% cut off value appropriate in HER2 IHC assessment?

Similarly to the modifications adopted for breast cancer HER2 testing, for which minimum area cut offs were changed from 30% to 10%, a change in the GC HER2 staining analysis protocol may be considered in the future.

5. How many GC surgical resection specimen blocks should be analyzed?

Laboratories should adopt a decisional workflow chart to maximize HER2 positive case discovery, taking into account costs and workload for pathologists. If one block is chosen, this must at least contain the largest amount of differentiated, intestinal type tumor, which is more likely to express HER2.
6. Are biopsies reliable in correctly identifying HER2 status?
   - HER2 status in inoperable patients is based on endoscopic biopsy evaluation
   - Studies have found variable concordance rates between biopsy and paired surgical resections ranging from 45.5% to 94%
   - An important question, which stems from HER2 status heterogeneity, is whether small endoscopic biopsies are reliable for HER2 assessment

One possible option is to consider ISH analysis for both IHC score 2+ and 1+ biopsy cases, while a second approach is in the repeat assessment of endoscopic biopsies, especially if IHC 1+ or 2+ at initial biopsy as suggested by the GASTHER 1 study
7. How many biopsies must be taken at endoscopy?

- National Comprehensive Cancer Network guidelines recommend more than 6 samples to be taken, but this is not evidence-based.
- Gullo et al identifying five superficial samples to be the optimal biopsy set.
- Anh et al identified 4 biopsy samples of cancer as the optimal number.

In a real life situation not all samples submitted by the endoscopist prove to contain assessable neoplastic cells at histologic examination. Furthermore, endoscopists should preferentially take biopsies in the lateral parts of the tumor as this area has been shown to be more frequently HER2 positive while the central part of the tumor should be avoided when macroscopically ulcerated.
8. Are there differences in HER2 status between primary and metastases?

The GASTHER 1 study has shown that repeat HER2 assessment in recurrent sites may be recommended in patients with advanced GC/GEJC whose initial evaluation was HER2 negative (5.7% patients were HER2 positive on biopsy of metastases) and that these patients show similar treatment benefits with Trastuzumab as patients identified as HER2 positive at initial evaluation.

In particular, liver as site of metastasis was 5.88 times more likely to show HER2 positivity on repeat biopsy than those who had HER2 reassessment in other metastatic sites.
CONCLUSIONS

1. HER2 assessment in GC/GEJC cancer is reliable once the pre-analytical variables and technical procedures are standardized

2. Endoscopic biopsies can provide reliable HER2 status assessment when a sufficient number of samples are available

3. IHC and ISH assessment are both reliable, but confirmation by ISH is mandatory in cases of equivocal IHC

4. Tumor heterogeneity is a major problem (but not insurmountable) which must be taken into account when selecting samples

5. There is relative consistency between HER2 status in the primary tumor and in distant metastases