On 23 August 2011, expert GI oncologists, pathologists and gastroenterologists from Switzerland met in Berne for a multidisciplinary team discussion on:

Recommendations for HER2 testing in gastric and gastroesophageal carcinoma

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Gastric carcinoma (GC) is the second oncological cause of death worldwide, with a higher prevalence in Asia than in Western countries (GLOBOCAN 2008). In Europe the incidence in 2008 was 165,000 new cases with 133,000 deaths (GLOBOCAN 2008). In Switzerland 793 new cases were registered in 2008, with 528 deaths (GLOBOCAN 2008). In general the incidence of GC located distally is declining, while an increase of GCs located in the cardia-fundic region and at the gastroesophageal junction (GEJ) can be observed. Despite advances in treatment, the outcomes for gastric cancer remain poor. Complete surgical resection is the main treatment; however, at the time of the diagnosis, most cases are either locally advanced or metastatic and hence unresectable. Perioperative chemotherapy and adjuvant chemoradiotherapy have been successfully applied in non-metastatic disease [1,2]. However, the prognosis is poor, with a median survival between 8 and 10 months [3]. Therefore an essential need for new, more effective therapeutic options does exist.

Overexpression of epidermal growth factor receptor 2 (ERBB2, HER2) plays a key role in oncogenesis and drives the clinical course of disease for a number of different cancer types [4-6]. ERBB2 (HER2) is now a well-established therapeutic target in breast cancer (BC) and a tailored approach to patient selection is required, based on HER2 overexpression/amplification [7]. The first description of HER2 overexpression in gastric cancer was published in 1986 [8]. Data reported in the literature for HER2 positivity in GC rates vary form 7-34% [reviewed in 9]. The expression rate varies according to different pathologic variables. Intestinal-type GC and GC located near the GEJ are more likely to show HER2 overexpression [10]. The recently published data from the ToGA (**T**rastuzumab for HER2-p**o**sitive metastatic **GA**stric cancer) trial, a randomised, controlled phase III study, showed that adding trastuzumab (T) to a chemotherapy regimen (capecitabine or 5-fluorouracil plus cisplatin, XP/FP) significantly improved overall survival (OS) compared to chemotherapy alone [11]. The patients who benefited particularly from trastuzumab in terms of OS were those whose tumour presented in immunohistochemical (IHC) analysis an HER2 status of IHC 3+ or IHC 2+ and a positive result in the FISH test (fluorescence in-situ hybridisation). The median survival time of these patients (T+XP/FP) was 4.2 months longer than that of patients in the control group (XP/FP). On this basis, trastuzumab has been authorised for the treatment of **HER2-positive**, metastatic cancer of the stomach and GEJ. According to the Swissmedic registration [12], patients with metastatic disease whose tumors are immunohistochemistry 3+ or immunohistochemistry 2+/fluorescence in situ hybridization-positive or immunohistochemistry 2+/silver in situ hybridization-positive are eligible for trastuzumab therapy (Fig. 1).

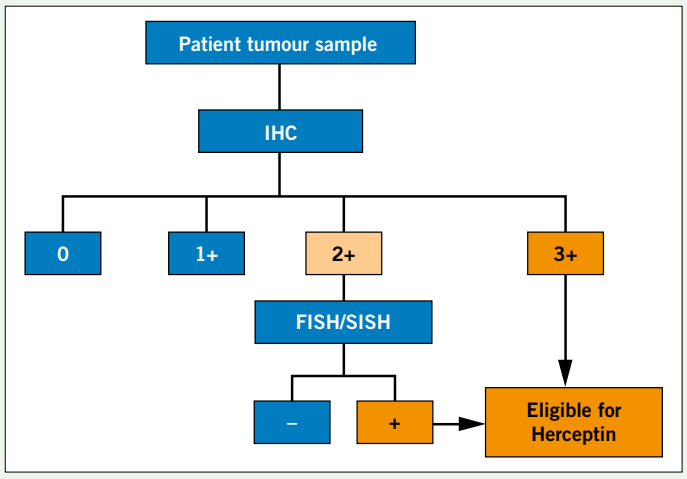

Fig. 1: HER2 testing algorithm in metastatic gastric and gastroesophageal junction cancers [based on 12]

The crucial point is of course the definition of HER2 overexpression/positivity. In daily life, HER2 testing presents numerous difficulties because of its complexity.

For these reasons, on 23 August 2011, expert GI oncologists, pathologists and gastroenterologists from Switzerland met in Berne for a multidisciplinary team discussion on recommendation for the diagnosis and treatment of HER2-positive metastatic GC and GEJ carcinoma.

HER2 in GC: Differences in comparison with BC

Unlike breast cancer testing, IHC and ISH are used differently in gastric cancer. As primary test method IHC is recommended and, if the IHC score is 2+ (see below), this should be followed by ISH (FISH or SISH) for final clarification of the HER2 status. If the test result is IHC 3+, or IHC 2+ and ISH-positive, the tumour should be classified as HER2-positive, and the patient may benefit from treatment with trastuzumab. A FISH analysis should be performed in any case of uncertain result of the IHC test.

The definition of immunohistochemical HER2 positivity in GC differs substantially from the one in BC. The specific membrane staining in stomach cancer and GEJ cancer is often incomplete and confined to the basolateral or lateral membrane regions, producing a predominantly U-shaped staining of the tumour cells (Fig. 2). This type of staining is consi-

dered negative in BC [7]. Moreover, intratumoral heterogeneity is very frequent and marked in GC, both at the protein and the genomic level [9-11,13-15]. Also the definition of genomic amplification differs slightly in comparison with BC, as no "borderline" category has been defined [10]. These differences must be taken into account when devising the optimal HER2 testing protocol for gastric cancer.

Pre-analytic phase in HER2 testing in GC

Selecting the patients

HER2 testing is mandatory in patients who would be eligible for trastuzumab treatment; this emphasizes the role of pathologists for providing accurate and reproducible HER2 testing results. In addition, pathologists should provide oncologists with all predictive information available and IHC screening for HER2 should be considered in all GC. In tumors scoring 2+ (see below), the confirmation by ISH may be applied following consultation with the oncologist in charge. Both GC and GEJ cancers should be tested for HER2.

Sample types and fixation

Both endoscopic biopsies (**at least 5 and ideally 8 fragments** sampling the cancer should be collected by the endoscopist) and surgical resections are suitable for HER2 testing by IHC and ISH (for type of tests and scoring see below). Cytological specimens are **not** recommended. Fixation should always be done in 10% neutral-buffered formalin at room temperature and should be started immediately (no more than 30 minutes after excision for surgical specimens) and with an adequate volume of fixative [16]. The optimum fixation time for forceps biopsies is 6-24 hours, for surgical specimens 24-48 hours [17].

HER2 testing

In general, recent sections are recommended, both for IHC and for ISH. The paraffin block must be representative of the tumor, avoiding hypocellularity, necrosis, hemorrhage, autolysis and artifacts. In neoplasms with mixed histology, intestinal-type areas should be selected.

Methods

IHC

The use of certified diagnostic kits is recommended, with prior validation in the laboratory (see also below). Positive and negative controls must be included in each run. The evaluation must be performed by a pathologist.

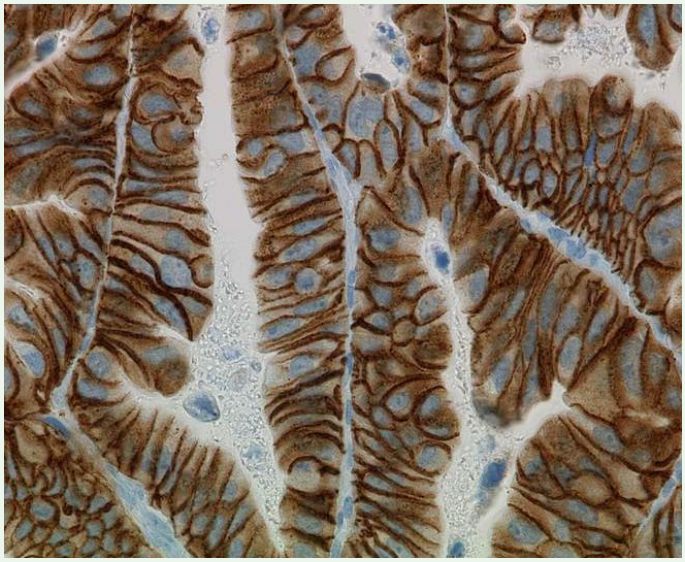

Fig. 2: Incomplete membranous immunoreactivity in a HER2-positive gastric cancer sample. (Image reproduced with courtesy of TARGOS Molecular Pathology GmbH)

IHC Scoring

The IHC scoring criteria take into account 3 main features:

1. **Heterogeneity:** Heterogeneity of the staining has been proven to be greater than in breast cancer. In resection specimens, ≥**10%** positive tumour cells showing a specific membrane staining, consider the tumor being positive, either 2+ or 3+ depending on the staining intensity (see below). In biopsy samples, the limit of 10% positive tumor cells does not apply and a group of **more than 5 tumor cells** showing a distinct HER2 membrane staining, are considered positive.
2. **Type of staining:** The tumour cells in gastric cancer do not primarily exhibit ring-like membrane staining, but rather basolateral or lateral membrane staining not involving the luminal membrane. In gastric cancer therefore, in contrast to breast cancer, even incomplete membrane staining can be diagnosed as IHC-positive if the HER2 marking is sufficiently intense. Only apical staining does not qualify for positivity
3. **Intesitiy of staining:** Evaluating the intensity of HER2 staining on the basis of differing levels of microscopic magnification has shown to increase the reproducibility for the evaluation of staining intensity [9–11]. The rule in Tab. 1 can be applied.

The overall result is based on **the highest positive scoring area** without averaging the results in multiple biopsy specimens. Specimens with IHC score 0–1+ are assessed negative, with IHC score 3+ positive. Specimens with IHC score 2+ are considered equivocal and must be further analysed by ISH. In cases of a negative endoscopic biopsy, the test should be repeated on the resection specimen when available.

In the evaluation of HER2 IHC positivity a particular attention should be given to possible artifacts, in particular to unspecific staining of hyperplastic and metaplastic as well as unspecific cytoplasmic staining. Therefore, comparison with the H&E slide is mandatory.

ISH

The interpretation of the results must be performed by a pathologist. Fluorescent ISH (FISH) or colorimetric ISH (CISH or SISH) can be used. The use of certified diagnostic kits, including a centromeric probe is highly re-

IHC score	Microscope lens	Intensity of specific membrane staining (resected tissue or biopsy)	Interpretation
0	40x	no staining visible	Negative
1+	40x	very weak / barely visible staining	Negative
2+	10x to 20x	weak to moderate staining	Borderline (IHC)
3+	2.5x to 5x	strong staining	Positive

Tab. 1: Semiquantitative determination of IHC score [based on 9–11]

ISH result	Scoring	Interpretation
Amplified	HER2/Chr.17≥2.0	Positive
Non-amplified	HER2/Chr.17<2.0	Negative
Monosomy	Chr.17 < 1.5/nucleus	Negative
Polisomy	> 3 Chr. 17/nucleus	Reevaluation of HER2/Chr. 17 value

Tab. 2. Interpretation of HER2 testing by ISH [10].

commended with prior validation in the laboratory. Positive and negative controls should be included in each run. A correlation with the H&E slide is recommended.

Counting the signals

At least 20 adjacent tumor cells in the area of greatest amplification (i.e. signal intensity) must be examined [10]. The definition of amplification is a HER2/chromosome 17 ratio ≥2.0 (Table 2). In case of a ratio between 1.8 and 2.2 additional 20 cells should be counted.

Turnaround time

Since rapid clarification of HER2 status is desirable in view of the progression rate of advanced gastric cancers, a result from HER2 testing should ideally be available within 7 days at the latest.

Quality assurance

Testing should be performed by laboratories with experience in IHC and ISH and the personnel undertaking the tests must be specifically trained [10, 13-15, 18-19]. Periodic participation in quality proficiency testing schemas is highly recommended (QUIP, UK NEQAS, Nordic, Association Française D'Assurance Qualité en Anatomie et Cytologie Pathologiques, etc.).

Reporting the HER2 results

The report should include at least the following elements (apart from unequivocal patient identification, specimen site etc):

For IHC: Antibody and method used (clone, suppliers), suitability of the sample, scoring (i.e. 0, 1+, 2+, 3+) and the mode of external quality control (name of the schema).

For ISH: Probe used (supplier), suitability of the sample, number of nuclei assessed, ratio HER2/Chr. 17 (to two decimal places) including the interpretation of the ratio assessed (i.e. amplified/not amplified/uninterpretable) and the mode of external quality control (name of the schema).

Conclusions

While various therapeutic options exist for metastatic gastric cancer/GEJ cancer, none of the currently available chemotherapy regimens has yet established itself as a standard treatment. Treatment with trastuzumab, supplementing chemotherapy (XP/FP), is beneficial in patients with HER2-positive metastatic GC and GEJ cancer, as defined in the ToGA trial. Correct performance of HER2 testing in GC is therefore crucial for the choice of therapy. HER2 testing must be standardized and performed by adequately trained personnel, following strict criteria in methodology, evaluation, interpretation and reporting.

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