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TITLE PAGE

Title

HER2 testing of gastro-oesophageal adenocarcinoma – a commentary and guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Committee

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ABSTRACT

The use of biologics targeted to the HER2 protein is the latest addition to the armamentarium used to fight advanced gastric or gastro-oesophageal junction carcinoma. The decision to treat with the biologic trastuzumab is completely dependent upon HER2 testing of tumour tissue. In 2017, the College of American Pathologists, American Society for Clinical Pathology and the American Society of Clinical Oncology jointly published guidelines for HER2 testing and clinical decision making in gastro-oesophageal adenocarcinoma. The Association of Clinical Pathologists Molecular Pathology and Diagnostics Committee has issued the following document as a commentary of these guidelines and, in parallel, to provide guidance on HER2 testing in NHS pathology departments within the UK. This guidance covers issues related to case selection, pre-analytical aspects, analysis and interpretation of such HER2 testing.

KEY WORDS

Adenocarcinoma, Stomach, Oesophagus, HER2, immunohistochemistry, in-situ hybridisation, guidance.

INTRODUCTION

A ‘biologic’ is a recombinant monoclonal antibody directed against a specific antigen. The use of biologics is becoming increasingly commonplace in the management of both cancers and inflammatory diseases. A biologic (trastuzumab, also known as Herceptin) targeting the human epidermal growth factor receptor 2 (HER2) has been shown to improve outcomes in patients with advanced gastric or gastro-oesophageal junction (GOJ) adenocarcinoma.[1] Furthermore, a technology appraisal guidance (TA208) issued by the National Institute for Health and Care Excellence (NICE) in 2010, has supported the use of trastuzumab, in combination with cisplatin and capecitabine (or 5-fluorouracil), in patients with HER2 positive metastatic adenocarcinoma of the stomach or GOJ, who have not received prior treatment for the metastatic disease.[2]

The clinical use of trastuzumab is dependent on demonstration of either aberrant HER2 expression (by immunohistochemistry, IHC) or HER2 gene amplification (by In-Situ Hybridisation, ISH) in the patient’s tumour. Guidance documents on HER2 testing of upper gastrointestinal tumours have been published.[3 4] Most recently, the College of American Pathologists (CAP), the American Society for Clinical Pathology and the American Society of Clinical Oncology jointly published a guidance document entitled “HER2 testing and clinical decision making in gastroesophageal adenocarcinoma”.[5] These guidelines (henceforth referred to as the ‘CAP-guidelines’ for brevity) included a list of recommendations. The following document - issued by the Association of Clinical Pathologists Molecular Pathology and Diagnostics Committee (http://www.pathologists.org.uk/page.aspx?id=130) - is a commentary, particularly of

these CAP-guidelines, and, in addition, provides guidance on HER2 testing of gastric and GOJ adenocarcinoma, in the context of the NHS in the United Kingdom.

The guidance will focus on the technical aspects of HER2 testing together with its interpretation and reporting, rather than clinical decision making. As such, this document is structured to follow the specimen pathway of HER2 testing and uses a similar format to that of guidance previously issued by this Committee.[6] At each step of this pathway, any relevant and corresponding recommendation from the CAP-guidelines will be discussed.

CASE SELECTION FOR TESTING

Appropriate case selection is essential for successful and cost-effective application of a test. The CAP-guidelines are introduced with a statement that gastro-oesophageal adenocarcinoma represents the 8th (oesophageal) and 5th (stomach) most common cancers worldwide, and then cite previous literature based on adenocarcinoma of the stomach or GOJ.[5] Similarly, the NICE TA208 document specifically states that trastuzumab be considered for metastatic adenocarcinoma of the stomach or GOJ.[2] It is noted that neither document[2 5] nor the original ToGA trial publication[1] precisely defines a GOJ tumour and its distinction from adenocarcinoma of the oesophagus. This may be a moot point in that oesophageal adenocarcinoma frequently arises on a background of Barrett's metaplasia and is almost always located in the distal oesophagus, which would usually meet the definition of a Siewert type I GOJ tumour (i.e. with an epicentre which is no more than 5 cm proximal to the GOJ[7]). The

Committee is not aware of UK multidisciplinary teams which exclude oesophageal adenocarcinoma patients from trastuzumab therapy purely based on the anatomical location of their tumours. However, for clarity, the following document and its recommendations pertain to oesophageal, GOJ and gastric adenocarcinomas as a whole. As such, all three tumour types will be collectively referred to by the term ‘gastro-oesophageal adenocarcinoma’ hereafter.

It remains controversial whether molecular testing of tumours, particularly in the setting of personalised medicine, is best performed as a reflex (i.e. on all cases) or whether such testing should be performed at request/on demand. In view of how trastuzumab is licensed for use in the UK (only for patients with metastatic disease) and in the setting of the NHS, HER2 testing of gastro-oesophageal adenocarcinoma is generally performed on demand. Requests are almost exclusively made by a multidisciplinary team or an oncologist. This practice is in accordance with the CAP-guideline that “in patients with advanced GEA who are potential candidates for HER2-targeted therapy, the treating clinician should request HER2 testing on tumor tissue”.[5] However, there is an argument that reflex HER2 testing of gastro-oesophageal adenocarcinomas would prevent delays to the start of treatment with trastuzumab, and that resource saved and improved outcomes achieved by preventing these delays would compensate for the extra cost of reflex testing. Whether HER2 testing is performed as a reflex or on demand procedure should be decided at a local level following consideration of these health economic factors.

Neoplasm Type

Both the NICE TA208 document[2] and the CAP-guidelines[5] specify that HER2 testing should only be performed on adenocarcinoma. The morphological distinction between high grade dysplasia and intramucosal adenocarcinoma can be particularly challenging.[8] The Committee is aware of the practice of treatment decisions based on the scoring of high grade dysplasia alone when adenocarcinoma cannot be confidently diagnosed in the tested tissue. However, the CAP-guidelines note that “false positivity” can be seen in areas of high grade dysplasia and recommend that these areas should not be scored (in the same way in-situ disease is not scored for HER2 expression in breast cancer).[5] In one study of 13 cases of paired gastric dysplasia and carcinoma, seven showed discordant HER2 protein expression between the dysplasia and carcinoma; four of these seven cases showed expression in the dysplasia but not the carcinoma.[9] Therefore and in order to prevent over-treatment, it is recommended that HER2 scoring is only performed on tumour foci which unequivocally demonstrate carcinoma.

Another neoplasm type for which HER2 testing remains controversial is poorly differentiated carcinoma. The CAP-guidelines acknowledge that HER2 data on variants of adenocarcinoma (for example adenosquamous) are limited.[5] Based on the probability of cancer of the stomach and GOJ being of glandular origin, it is suggested that if a carcinoma shows only a focal glandular component or, after full work-up, shows no demonstrable line of differentiation, it is acceptable to test for HER2 expression and agree a treatment decision with the patient.

Specimen Type

Primary tumour versus metastatic deposit

In the case of metastasis or recurrence, it would be ideal to HER2 test the metastatic or recurrent tumour as tumours may evolve with time. However, a diagnosis of metastasis/recurrence is frequently made on clinical or radiological grounds without definitive tissue diagnosis. The CAP-guidelines, based on studies comparing primary tumour with lymph node metastases and primary tumour with both synchronous and metachronous liver metastases, recommend that HER2 testing can be performed on either primary or metastatic carcinoma.[5] This recommendation is generally supported though a nuanced approach should be considered in the following scenario. It is known that chemotherapy and radiotherapy can exert selection pressures on a tumour.[10] Thus, if a patient had received neoadjuvant and/or adjuvant therapy prior to recurrence, it is recommended that HER2 testing is performed on tissue sampled after the therapy and, when such tissue is not available and where clinically reasonable, efforts are made to obtain tissue from the recurrence for the purposes of HER2 testing.

Biopsy versus resection

The CAP-guidelines recommend that HER2 expression can be tested on either biopsy or resection specimens as review of admittedly limited published literature suggests a "fair degree of concordance".[5] However, within NHS practice, it is recommended that, when the choice is available, HER2 testing is preferentially performed on resection tissue in order to obviate the effects of intratumoral heterogeneity. The confounding

effects of heterogeneity are acknowledged by the CAP guidelines which recommend that, if only biopsies are available for testing, a minimum of 5 biopsy fragments and optimally 6-8 should be obtained for biomarker testing.[5] While this is a worthy aspiration, it is unlikely that this will always be achieved in the NHS setting, and therefore this minimum number of biopsies should not be used to preclude a biopsy specimen from HER2 testing. Finally, this Committee endorses the CAP-guidelines proposal that cytology specimens (particularly cell blocks of fine needle aspiration specimens) are acceptable for HER2 testing if no other tissue is available.[5]

PRE-ANALYTICAL ASPECTS

Optimal pre-analytical preparation of tissue is crucial to achieving a high standard of subsequent molecular testing.[11] The CAP-guidelines evaluated published data on the effects of delayed tissue fixation (cold ischaemia) on both immunohistochemistry for HER2 expression and ISH for HER2 amplification. For optimal downstream analysis, it is recommended that biopsies or resection specimens are immersed in fixative within one hour of sampling/resection.[5] Within an NHS setting, most (if not all) endoscopic biopsies will be fixed immediately after sampling. Achieving this target for upper GI surgical resections will be less likely especially if fresh tissue is required for other purposes such as research. Sampling of fresh tissue is usually undertaken in the Pathology department. Cold ischaemia may be exacerbated by delays in transport from surgical theatre to the Pathology department and, once the specimen has arrived, delays in sampling and formalin fixation. If there are likely to be delays in either transport or sampling, specimens should be refrigerated in order to reduce the rate of

cellular degradation. Finally, samples submitted fresh should be clearly documented as such and, if possible, the actual cold ischaemia time should be indicated in the final histopathology report.

Different fixative types can potentially affect subsequent molecular testing[11] although this was not discussed in the CAP-guidelines. It has been reported that, compared with 10% neutral buffered formalin (NBF), fixation in 20% NBF or non-buffered formalin reduces intensity of HER2 immunostaining.[12] Therefore, only 10% NBF is recommended for gastro-oesophageal adenocarcinoma specimens (biopsies and resection specimens) which may potentially undergo HER2 testing. Where other fixatives are used or have been used on tissue which is then HER2 immunostained, caution should be exercised when interpreting this HER2 immunostaining.

Both under-fixation (insufficient time in fixative) and over-fixation (excessive time in fixative) can affect IHC and ISH. When using 10% NBF, the CAP-guidelines recommend a fixation period of 6 – 72 hours. Most biopsies are unlikely to remain in fixative for more than 24 hours and given the small size of the tissue fragments, under-fixation is not likely to be a problem. For resection specimens, assuming there is an adequate volume of fixative, a minimum of 24 hours fixation is recommended.

A previous study of breast carcinoma (in patient tissue specimens or as xenografts of a cell line) showed that, of three decalcifying treatments tested, only hydrochloric acid (and neither EDTA nor formic acid) had a deleterious effect on HER2 ISH.[13] By

contrast and as was noted in the CAP guidelines, the exact effects of decalcifying treatments on HER2 immunohistochemistry are unknown. Although it is rare, it is possible that for such HER2 testing may be requested on tissue which needs treatment with decalcifying agents (such as bony metastases). Given the studies demonstrating concordance between primary tumours and metastases, it is recommended that HER2 immunohistochemistry is performed on earlier biopsy/resection material if this had been processed without decalcification.

The CAP-guidelines did not directly address the effect of tissue block age on subsequent HER2 testing or whether pre-cut sections are suitable for such testing. However, with regards to the former and in the experience of Committee members, it is highly unusual for HER2 testing of oesophageal and gastric adenocarcinoma to be requested on tissue blocks of greater than 5 years of age. Work on breast carcinoma has shown that HER2 protein antigenicity is reduced in pre-cut and stored tissue sections.[14] While there are no known published, equivalent data for gastro-oesophageal adenocarcinoma, this document recommends that only freshly cut sections be used for HER2 IHC and ISH of gastro-oesophageal adenocarcinoma.

With regards to cytological specimens, the CAP-guidelines acknowledge the potential effects of different fixatives (e.g. alcohol versus formalin fixation) on HER2 testing.[5] The guidelines therefore stress the need for proper evaluation of such testing on cytological specimens in individual laboratories before such testing is used to guide the management of clinical patients. This is endorsed, as with any other example of

immunohistochemistry applied to cytological samples in the context of personalised medicine.[15]

ANALYTICAL ASPECTS

Assay choice

The CAP-guidelines recommend assessment of HER2 expression by IHC as the test of choice, with ISH used only when IHC yields a 2+ score. Further, the European Medicines Agency stipulates that trastuzumab “should only be used in patients with metastatic gastric cancer whose tumours have HER2 overexpression as defined by IHC2+ and a confirmatory SISH or FISH result, or by an IHC3+ result.”[16] This document further proposes that if there is any equivocation between a 1+ and 2+ score, the latter should be reported and ISH performed. There are published data showing that *HER2* gene amplification may occur without expression of HER2 protein.[17] However, because there is no documented benefit of treatment with trastuzumab in the absence of tumour HER2 protein expression, the above recommended sequence of testing remains justified.

The CAP-guidelines do not recommend any specific antibody or specifically comment on immunohistochemical protocols (with regards to, for example, antigen retrieval) or staining platforms for HER2 testing.[5] However, the CAP-guidelines do recognise previous reports that the 4B5 antibody may produce stronger staining than other antibodies.[5] It is also worth noting that this antibody usually decorates physiological foveolar epithelium (often with a weaker membranous and stronger cytoplasmic and/or

nuclear pattern). Finally, it is recognised that several commercial HER2 antibodies are only available in pre-diluted preparations and that many are marketed with recommended protocols and/or are tied to specific staining platforms.

There are various ISH methods (for example, FISH, CISH and D-DISH) available for the study of gastro-oesophageal adenocarcinoma. The CAP-guidelines recognise that brightfield ISH techniques may be advantageous over FISH because they can be performed on automated platforms, do not require fluorescence microscopy and permit easier identification of neoplastic nuclei.[5] However, as with immunohistochemistry, no specific ISH method is considered as being superior for use in the NHS setting. HER2 ISH can be performed with single probe (HER2 only) or dual probe (HER2 and centromere 17, CEP17) systems. This Committee recommends only the use of dual probe systems for ISH of gastro-oesophageal adenocarcinoma.

This Committee endorses the CAP-guidelines that whichever assay chosen, it is appropriately validated and continuously monitored through participation in quality assurance schemes.[5] It is questionable whether technical details (e.g. which antibody and which ISH technique were used) in a HER2 report are of interest and use to the patient's clinical team. However, it is still recommended that such details are retained in the report to facilitate quality and process-related audits performed by the laboratory itself and/or external assessors.

Assay validation

There is variation in the proposed numbers of cases which should be validated by a laboratory before it offers clinical HER2 testing. Recommendation 2.1 of the CAP-guidelines refers to the CAP laboratory accreditation program for HER2 validation of breast carcinomas which proposes validation using 20 positive and 20 negative specimens for an FDA-approved test and 40 positive and 40 negative cases for a laboratory developed test. By contrast, the updated UK recommendations for HER2 assessment in breast cancer recommends validation by dual HER2 immunohistochemistry and ISH of a minimum number of 100 cases of breast carcinoma.[18] This Committee is not aware of any published data defining and justifying the minimum number of cases needed to validate HER2 testing of gastro-oesophageal adenocarcinoma. The most clinically significant events are under-calling of 2+ scores as 1+ (leading to potential under-treatment) and over-calling of 2+ scores as 3+ (leading to potential over-treatment). While the following numbers are arbitrary, it is sensible to include the entire range of HER2 immunoscores as part of the validation exercise. Thus, it is recommended that such validation be performed on gastro-oesophageal adenocarcinoma tissue with a minimum of 20 reference cases in each category of 0/1+, 2+ and 3+. All the HER2 testing techniques (IHC and/or ISH) that the laboratory plans to offer clinically should be applied to these reference cases.

Test failure

A ‘false negative’ immunohistochemical score (i.e. a HER2 expressing carcinoma which does not immunostain for the protein) may occur due to pre-analytical and analytical failure. Regarding the former, the 4B5 antibody is useful because positive staining of

foveolar epithelium and intestinal metaplasia serves as an internal positive control. Technical failures can be reduced by using a positive control with each batch of HER2 immunostaining. Ideally, the positive control tissue should be mounted on the same slide as the clinical case tissue (see below for types of control tissue). Whether it is mounted on the slide or a separate slide, assessing the positive control tissue (particularly the 0 and 2+ samples) before the patient tissue, is crucial to exclude the possibility of over-staining and to therefore prevent a ‘false positive’ immunohistochemical score.

For HER2 ISH and as had been highlighted in the CAP-guidelines,[5] normal signals (i.e. up to 2 copies per cell of discrete HER2 and CEP17 signals) should be detected in non-neoplastic cells (such as stromal cells) and failure to detect probe signals in these cells is an indicator of poor quality hybridisation.

POST-ANALYTICAL ASPECTS

Reporting and interpretation

The Rüschoff/Hofmann grading system for HER2 IHC and ISH[4] is universally used as the scoring method for gastro-oesophageal adenocarcinoma. Its use is recommended by the CAP-guidelines and further endorsed by this Committee. The Figure presents examples of 0, 1+, 2+ and 3+ HER2 scores of the Rüschoff/Hofmann grading system for gastro-oesophageal adenocarcinoma.[4] It is reiterated that the 4B5 antibody will decorate physiological foveolar epithelium and, therefore, this should not be mistakenly scored as positive expression. In addition, intestinal metaplasia (IM) can overexpress

HER2[19] which, in cases where the IM component is small and adjacent to or embedded within the carcinoma, can lead to interpretative confusion. It is worth repeating that only membranous staining is scored whilst both cytoplasmic and nuclear expression are ignored. Finally, for 2+ and 3+ scores, this membranous staining must be complete, basolateral or lateral whereas luminal staining by itself is considered a negative finding. In clinical practice, the following rule-of-thumb has emerged to aid grading of HER2 immunohistochemistry for gastro-oesophageal adenocarcinoma: if membranous staining can be clearly seen at x4 objective, the score is 3+; if membranous staining can only be clearly seen at x10 objective or higher, the score is 2+; if membranous staining can only be clearly seen at x20 objective or higher, the score is 1+.

By the Rüschoff/Hofmann method, the highest HER2 score of a carcinoma in a surgical resection specimen must be from a focus representing 10% or more of the carcinoma area, whereas for biopsies, the highest score is taken from a focus comprising 5 or more carcinoma cells. It is presumed that the rule for biopsies was formulated to acknowledge limited sampling of the whole tumour. In view of this, it is recommended that these rules be identically applied when metastatic tumour tissue is assessed. For example, the 10% rule would be used for a completely resected peritoneal metastasis whereas the 5+ cell rule would be used for a core biopsy of a liver metastasis.

The CAP-guidelines recommend the use of 4 micron thick paraffin sections for ISH studies.[5] Their recommendation 2.6 is that areas of invasive adenocarcinoma are

identified and those areas with strongest intensity HER2 expression be marked to assist any subsequently required ISH scoring.[5] The CAP-guidelines recommend scoring of at least 20 non-overlapping tumour cell nuclei to calculate a ratio of HER2 to CEP17 signals, and define HER2 amplification as a ratio of ≥2 and absence of amplification as a ratio of <2.[5] In the latter scenario but where there is an average CEP17 signal count of 3 or more, the CAP-guidelines (presumably in recognition of the 2013 American Society of Clinical Oncology / CAP guidelines on HER2 testing of breast carcinoma[20]) then define amplification based on average numbers of HER2 signals alone: positive if more than 6 signals; negative is less than 4 signals , and equivocal if between 4 and 6 signals.

This definition of HER2 amplification when the HER2 to CEP17 ratio is <2 remains controversial. However, to maintain alignment of guidelines internationally and in keeping with the recently updated UK breast cancer HER2 guidelines,[18] this Committee defines HER2 amplification in gastro-oesophageal adenocarcinoma as a HER2 to CEP17 ratio of ≥2 and/or an average HER2 signal count of 6 or more. If this ratio is <2, an average HER2 signal count of <4 indicates non-amplification and a count between 4 and 6 indicates an equivocal result. For equivocal cases, the following may be then be performed, as recommended by the CAP-guidelines[5]: consultation between scorer and pathologist regarding selection of malignant cells or tumour areas for scoring; selecting a different tumour block for HER2 testing; and/or using other genomic analyses or an alternative method to evaluate HER2 amplification (though see below regarding availability of molecular techniques in the NHS setting).

Report layout

As with pathology reporting of cancer resection specimens, use of templates in molecular pathology helps ensure neither important clinical nor technical information is omitted from final reports. Recommendation 2.5 of the CAP-guidelines is that HER2 test results should be reported using the College of American Pathologists template for HER2 biomarker testing.[5] Similar template reporting, with at least the data points presented in Table 1, is recommended.

TABLE 1

HER2 testing may be performed on primary or metastatic carcinoma tissue, and on biopsy or resection specimen tissue, or on a cytology specimen if no other tissue sample is available.

It is acceptable to HER2 test a carcinoma which shows no demonstrable line of differentiation or shows only a focal glandular component.

10% neutral buffered formalin should be used to fix gastro-oesophageal adenocarcinoma specimens (biopsies and resection specimens) which may potentially undergo HER2 testing.

Freshly cut tissue sections should be used for HER2 IHC and ISH.

No specific antibody clone or ISH technique can be recommended but the assay(s) chosen should be appropriately validated and continuously monitored through participation in external quality assurance schemes.

Validation should be completed with a minimum of 20 cases of 0/1+ scores, 20 cases of 2+ score and 20 cases of 3+ score, using all the HER2 techniques (IHC and/or ISH) that the laboratory plans to offer clinically.

A positive control should be run with each batch of HER2 IHC.

The positive control used for HER2 IHC should include, as a minimum, examples of 0, 2+ and 3+ immunoscores.

HER2 IHC and ISH should be scored using the Rüschoff/Hofmann method.

If ISH yields a HER2/CEP17 ratio of less than 2.0, HER2 amplification is then defined by the average number of HER2 signals: positive if more than 6 signals; negative is less than 4 signals, and equivocal if between 4 and 6 signals. .

If only immunohistochemistry is required and is performed in house, >90% of cases should be reported within 5 days of receipt or collection of the tissue block. If both immunohistochemistry and ISH are required and are performed in-house, then >90% of cases should be reported within 10 days of receipt or collection of the tissue block.

If specimens are outsourced for HER2 testing, 90% of these specimens should be sent to the testing laboratories within 3 working days of tissue processing.

QUALITY CONTROL AND ASSURANCE

The quality of HER2 testing is dependent on two major components: the technical quality of the assays, and the laboratory's ability to correctly interpret the results of the assays. The two components are effectively independent of one another but are equally crucial to reaching a correct clinical outcome from HER2 testing. For example, even the best-trained and most experienced assessor cannot report clinically useful findings from a substandard HER2 immunostudy, and equally, incorrect interpretation of a high quality HER2 immunostudy can misdirect clinical management. Therefore, quality control programs must consider both components of HER2 testing and ideally be able to assess each component separately.

Internal

As with any molecular test for cancer patients,[21] internal controls should be run with each round of HER2 testing. The CAP-guidelines suggest that HER2 expressing breast carcinoma tissue may be initially used at positive controls but should be changed to actual gastro-oesophageal adenocarcinoma tissue when possible.[5] Also, gastric carcinoma cell lines showing HER2 expression can be used as positive controls if actual carcinoma specimens are not available.[5] This Committee endorses these recommendations and further extends them with the proposal that the controls should show, as a minimum, 0, 2+ and 3+ immunoscores. This applies both to gastro-oesophageal adenocarcinoma tissue (which might therefore be assembled as a tissue microarray) and cell line blocks.

As stated above, background non-neoplastic cells can be used as internal controls for ISH studies.

External

It is recommended that any laboratory offering clinical HER2 testing of gastro-oesophageal adenocarcinoma should participate in an appropriate external quality assurance (EQA) scheme. This is consistent with recommendation 2.7 of the CAP-guidelines that laboratories should incorporate HER2 testing methods into the overall laboratory quality improvement program.[5] In the UK for example, UK NEQAS ICC and ISH run an annual EQA scheme for HER2 immunohistochemistry and ISH of gastro-oesophageal adenocarcinomas.

Audits

The CAP-guidelines recommend that laboratories consider tracking their own statistics of HER2 results including inter-observer reproducibility between pathologists and histological subtypes.[5] These recommendations are endorsed. Previous studies have shown that 21 to 33% of intestinal-type carcinomas tested and 4 to 12% of diffuse-type carcinomas tested show HER2 positivity (defined as a 3+ immunoscore and/or HER2 amplification).[22-25] These numbers are sufficiently consistent to formulate and recommend a specific audit standard that at least 20% of intestinal-type carcinomas tested and at least 4% of diffuse-type carcinomas tested show HER2 positivity as defined above.

Turnaround times

Minimising the turnaround time of molecular testing for personalised medicine is clinically critical.[21] The CAP-guidelines recommend a benchmark of 90% of reports being available within 10 working days from the date of specimen acquisition.[5] In the NHS setting, immunohistochemistry is regarded as a relatively rapid test. Therefore, it is recommended that if only immunohistochemistry is required and is performed in house, >90% of cases are reported within 5 days of receipt or collection of the tissue block. If both immunohistochemistry and ISH are required and are performed in-house, then >90% of cases should be reported within 10 days of receipt or collection of the tissue.

Like in the CAP-guidelines, it is acknowledged that some laboratories may out-source tissue for HER2 testing. The proposal of the CAP-guidelines that 90% of these specimens are sent to the testing laboratories within 3 working days of tissue processing,[5] is supported.

FUTURE DIRECTIONS AND DEVELOPMENTS

There is still controversy regarding the optimal model for IHC for the purposes of personalised medicine. Should it be every laboratory that can perform IHC, or just by selected laboratories that can treat these tests as bona fide molecular tests from a quality point of view?[26] At this point, a comparison with the breast HER2 IHC paradigm is pertinent. It is clear that mistakes had been made with HER2 testing of breast cancer, and there currently remain countries that do not allow Herceptin

treatment for this cancer unless the positive IHC result is confirmed by an ISH method run by a centralised laboratory. Some may therefore argue that, in Western countries where gastro-oesophageal adenocarcinoma is not as common as in Asia, HER2 testing should be consolidated in centres with a minimum number of tests per year, to ensure adequate quality assurance and control and accurate and consistent diagnostic interpretation.

There continue to be attempts to fully automate the reading/scoring of HER2 immunohistochemistry and ISH. As yet, however, no particular computer based system has been fully validated for clinical use and therefore no such system can be recommended in this document. Recommendation 2.8 of the CAP-guidelines states that there is insufficient evidence to recommend for or against genomic testing in gastro-oesophageal adenocarcinoma patients at the time of writing.[5] The tests discussed in these guidelines include multiplex ligation-dependent probe amplification and droplet digital PCR. The guidelines do state the possibility of genomic testing being useful for cases where standard IHC and/or ISH are not able to provide a conclusive finding, particularly when there is borderline amplification.[5] However, in the NHS setting, most if not all of these technologies, are not readily available to routine diagnostic laboratories.

Finally, there is increasing interest in the use of next generation sequencing (NGS) to directly assess for *HER2* gene amplification, therefore bypassing the need for HER2 immunohistochemistry. This use of NGS has not yet been clinically validated. Such

validation will particularly need to consider the issue of tissue heterogeneity of *HER2* amplification and of *HER2* amplification without increased protein expression.

CONCLUSION

The recommendations of the CAP-guidelines are welcomed and generally endorsed by this document. This document has, in addition, included guidance on tissue fixation and on specific audit standards. At the current time, the main recommendations for HER2 testing of gastro-oesophageal adenocarcinoma, at least in the UK, are presented in Table 2. Future developments that will be monitored include the use of genomic tests for HER2 and especially next generation sequencing.

TABLE 2

Cellular pathology laboratory number (if referred case, source cellular pathology laboratory number)
Type of specimen tested (e.g. endoscopic biopsies, surgical resection specimen, peritoneal biopsy, liver biopsy, cytology cell block, etc.)
Adenocarcinoma Lauren type (i.e. intestinal, diffuse, or mixed)
If HER2 IHC performed:
Antibody clone used
HER2 immunoscore
If HER2 ISH performed:
Technique used (e.g. FISH, CISH, D-DISH)
Probes used
Average HER2 signal count
Average CEP17 signal count
HER2/CEP17 ratio

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TABLE AND FIGURE LEGENDS

Table 1

Main recommendations for HER2 testing of gastro-oesophageal adenocarcinoma to guide trastuzumab therapy.

Table 2

Minimum dataset for reporting of HER2 IHC and/or ISH of gastro-oesophageal adenocarcinoma.

Figure

Examples of the Rüschoff/Hofmann grading system for HER2 immunohistochemistry of gastro-oesophageal adenocarcinoma: a) 3+; b) 2+; c) 1+ and d) 0 immunoscores.