Title: Evaluation of the ErbB2 / HER2 antibody panel and reproducibility of a HER2 testing algorithm specific for p53-abnormal endometrial cancers.

Background

ErbB2 Receptor Tyrosine Kinase 2 is a protein coding gene, a member of the epidermal growth factor receptor family of receptor tyrosine kinases [1]. It is generally referred to as HER2. The increased number of copies of this gene has been reported in many cancers, including breast, ovarian, oesophageal and stomach carcinomas [1]. Approximately 12-25% of invasive breast carcinomas demonstrate an increased expression of HER2 [2]. HER2-positive tumours are treated differently from other molecular subtypes. For example, HER2-positive breast carcinomas show sensitivity to targeted drugs such as Herceptin® (Trastuzumab) and Neratinib® (Nerlynx) [3]. The status of HER2 is usually determined by immunohistochemistry (IHC) testing on tumour biopsy tissue samples. Currently, validated HER2 scoring algorithms are available for breast and gastrointestinal tumours, but not for other tumours which express high levels of HER2 [4]. For instance, a subset of high-grade endometrial carcinomas (EC) was reported to express elevated levels of HER2 and therefore, can be treated with targeted therapies [4]. Several research groups have produced the IHC scoring protocols for high-grade ECs, but they have not yet been validated [4, 5]. In particular, Buza has introduced a level of clarity into the HER2 scoring for EC and given guidelines into what types of samples should be subjected to HER2 IHC [4], whereas Vermij et al. investigated p53-abnormal ECs and found a strong correlation with the HER2 positivity [5].

We have performed preliminary testing using p53-abnormal and p53 wild-types biopsy samples with available CB11 clone (Leica/Bond-3), and currently validated for the use in breast carcinomas on our Bond-3 system. From our results, the main observations were that the interobserver correlation scoring was very low due to some background cytoplasmic staining, often quite strongly expressed, and inconsistent stanning between 2+ and 3+ rated samples, which also poorly correlated with the Fluorescence in situ hybridisation testing results. This could be due to the fact that the clone CB11 detects the internal domain of the ErbB2 oncoprotein. However, we observed a higher number of consistencies with stronger staining and better reproducibility in p53-abnormal EC cases, as previously been reported [5]. We tested two scoring algorithms with the 30% and 10% cut-off recently proposed by Buza and Vermij, respectively [4, 5]. Moreover, we tested a selection of samples using PATHWAY® rabbit monoclonal antibody clone 4B5 (Roche/Ventana), which demonstrated a much better optimal staining compared to CB11 clone.

Following aforementioned preliminary work, we are proposing to investigate the reproducibility and uniformity of HER2 IHC scoring algorithms on high-grade p53-abnormal ECs using Leica Bond-3 and Ventana diagnostic HER2 systems, and an antibody panel, in order to evaluate which is the best ErbB2 / HER2 antibody for our IHC p53-abnormal EC protocol for establishment of routine HER2 testing on ECs biopsies in the near future.

This is an educational service development project between the University of Portsmouth and St. Richards Hospital Cellular Pathology Department. It will be carried out by a student undertaking an apprentice degree in Biomedical Sciences under the supervision by the laboratory and academic staff.

Hypothesis: EC specific HER2 IHC scoring method is highly reproducible on EC biopsies using Bond-3 platform and the best antibody clone. A screening strategy based on IHC scoring on all cases with subsequent FISH testing on IHC 2+/3+ cases has perfect test accuracy for identifying HER2-positive ECs.

Methods

12 test samples containing p53-abnormal high-grade EC previously scored between 0-3+ will be tested on in-house Leica Bond-3 IHC system at Cellular Pathology St Richard's Hospital, Chichester, using a recombinant primary rabbit monoclonal anti-human ErbB2 / HER2 antibody panel (EP1045Y, EPR19547-12, SP3, SP101, CAL27), available from Abcam (ab252204), and CBE-356 mouse monoclonal antibody (clone 10A7), available from Leica (NCL-L-CBE-356), and EP3 rabbit monoclonal antibody, available from Bio SB (BSB 2514).

PATHWAY® rabbit monoclonal antibody (clone 4B5), available from Roche (790-2991) will be used at the 'sister-site' hospital laboratory in Brighton. Correlation of IHC results with the DDISH testing results will be evaluated.

Scoring of IHC staining using recommended algorithms by Buza [4] and Vermij et al. [5]. Observers: 2 gynae pathologists and 2 biomedical scientists. Results will be assessed to determine a high pass rate, a number of optimal results, sensitivity and specificity, clone and interobserver correlation.

Expected Results

*Development of validated protocols for staining and scoring of HER2 in ECs; *Selection of the best antibody to boost the reproducibility and interobserver agreements of HER2 IHC in EC; *Understanding if both IHC and ISH should be routinely performed on ECs; *Implementation of HER2 testing for all p53-abnormal EC into the routine laboratory work.

References

1. GeneCards. The Human Gene Database. ERBB2 Gene - Erb-B2 Receptor Tyrosine Kinase 2. Weizmann Institute of Science, 2023. [Accessed on 4th May 2023]. Available from: https://www.genecards.org/cgi-bin/carddisp.pl?gene=ERBB2

2. Memon, R, et al. Discordance Between Immunohistochemistry and In Situ Hybridization to Detect HER2 Overexpression/Gene Amplification in Breast Cancer in the Modern Age: A Single Institution Experience and Pooled Literature Review Study. *Clin Breast Cancer.* 2021;22 (1):e123–e133. DOI:10.1016/j.clbc.2021.05.004

3. National Institute for Health and Care Excellence (NICE). Breast cancer. All NICE products on breast cancer. Includes any guidance, advice and quality standards. NICE, 2023. [Accessed on 4th May 2023]. Available from: https://www.nice.org.uk/guidance/conditions-and-diseases/cancer/breast-cancer

4. Buza, N. HER2 Testing in Endometrial Serous Carcinoma Time for Standardized Pathology Practice to Meet the Clinical Demand. *Arch Pathol Lab Med.* 2021;145:687–691; DOI:10.5858/arpa.2020-0207-RA

5. Vermij, L, et al. Performance of a HER2 testing algorithm specific for p53-abnormal endometrial cancer. *Histopathology.* 2021;79:533–543. DOI:10.1111/his.14381