The Royal College of Pathologists
Pathology: the science behind the cure

HER2 in colorectal cancer: guidance for pathologists receiving requests for testing

Published date: January 2024

Review date: January 2027

Background

Human epidermal growth factor receptor 2 (HER2) amplification is observed in several solid organ cancers. HER2-positive cancers are likely to respond to drugs that target the HER2 protein, e.g. trastuzumab. HER2 testing in breast and gastric cancer is considered standard of care, with NICE recommended therapies in use across the NHS. A recent meta-analysis in over 17,000 colorectal cancer (CRC) patients has shown that around 4% are HER2 positive, rising to 6% in RAS wild-type tumours.[1] UK randomised clinical trials have confirmed HER2-positive rates of 5% in metastatic RAS wild-type CRC.[2] The phase 2 MOUNTAINEER study has recently shown that HER2-positive RAS wild-type metastatic CRC patients show radiological response to treatment with tucatinib plus trastuzumab in 38% of patients (including 4% complete response).[3] Similar results were obtained in the HERACLES trial.[4] There are currently no NICE recommended treatments for metastatic HER2-amplified CRC patients, but a Health Technology Evaluation commenced in June 2023.[5]

INVESTORS IN PEOPLE®
We invest in people Standard

Methods of testing

In the MOUNTAINEER and HERACLES studies, HER2 testing was initially performed at local sites and then confirmed with central testing. Possible testing methods include immunohistochemistry (IHC), fluorescence in situ hybridisation (FISH), chromogenic in situ hybridisation (CISH) and a genomic approach, e.g. next generation sequencing (NGS) or quantitative real-time polymerase chain reaction (PCR). In both studies, HER2-positive status was defined as 3+ staining by IHC or 2+ staining on IHC with amplification by FISH/CISH. In addition, MOUNTAINEER accepted amplification by NGS and HERACLES included central testing by quantitative real-time PCR. These approaches largely mirror testing taking place across the NHS for breast and gastric cancer, with the exception that determining HER2 status by a genomic approach is not currently standard practice.

How to handle HER2 requests in CRC

For pathologists receiving requests for HER2 testing in CRC, in advance of any NICE recommended therapies, the recommendation is to attempt to deliver a limited testing service, provided that there is a specific mechanism for the patients to access anti-HER2 therapy. Some centres are likely to outsource the testing to an external laboratory but others may look to deliver testing in house. Centres should use a validated (and preferably UKAS accredited) assay. Screening all cases by FISH/CISH is unlikely to be feasible and it is anticipated that testing pathways will mirror the current process for gastric cancer. This includes assessing IHC with a 4-tier scoring system (see Table 1). Only invasive tumour cells should be scored, ignoring normal mucosa and dysplasia. Cases that are borderline by IHC should be assessed by either FISH or CISH to determine the final status.

FISH/CISH should be performed in IHC 2+ cases to assess the ratio between the number of copies of the HER2 gene and chromosome 17. The assessment should be correlated with the IHC such that the areas of greatest intensity staining are assessed.[6] The slide should be screened at x10/20 magnification to identify potentially amplified regions and then assessed at x40/60 magnification.[7] The ratio should be determined in at least 20 evaluable non-overlapping tumour cells. A ratio of ≥2 signifies a positive result. If the ratio is between 1.8 and 2.2, it is suggested that a further 20 tumour cells are assessed. If the ratio remains borderline (1.8–1.99), the HER2 gene copy number per cell should also be

assessed. Less than 4 copies signifies a negative result. Greater than or equal to 6 copies of the HER2 gene signifies a positive result. A gene copy number between 4 and 6 should be reported as “borderline not amplified”, which is regarded as negative for treatment purposes.[8]

Table 1: HER2 IHC assessment.

Testing on biopsy specimens requires positive staining in a cluster of at least 5 cells. In resection specimens it requires staining in more than 10% of the tumour cells. Adapted from reference 9.

Score	Status	Description
0	Negative	No membranous staining when assessed at x40 magnification
1+	Negative	Faint membranous staining when assessed at x40 magnification
2+	Borderline	Weak-to-moderate complete, basolateral or lateral membranous staining, which should be visible at x10 to x20 magnification
3+	Positive	Strong complete, basolateral or lateral membranous staining, which should be visible at x2.5 to x5 magnification

HER2 status by NGS has high concordance with IHC (~97%) and can capture the presence of activating HER2 mutations that may co-exist with HER2 gene amplification.[10] However, across the NHS in England, HER2 testing for CRC does not yet appear on the national genomic test directory and is therefore not commissioned as a genomic test delivered through the genomic laboratory hubs (www.england.nhs.uk/publication/national-genomic-test-directories/). It is therefore unlikely that many centres will be able to access a genomic assay for HER2 status at the present time, although this is likely to change over the coming months.

If/when the option to test by either IHC or NGS is available, it is essential that pathologists take the lead in deciding which test to perform. This decision will be based on factors such

as the amount of tissue available, the tumour percentage and the clinical urgency of the result. If a genomic approach is centrally commissioned across the NHS, IHC/FISH would remain as the 'salvage pathway' for samples that are not suitable for NGS. Pathologists play a critical role as the 'gate keepers' in all requests for molecular testing.

Summary

HER2 status in CRC should be determined by a validated (and preferably accredited) assay using either an IHC, ISH or genomic approach. The following cases are considered HER2 positive:

- definitive HER2 overexpression by IHC (score 3+)
- borderline HER2 overexpression by IHC (score 2+) with evidence of HER2 amplification by FISH or CISH
- HER2 amplification by a genomic assay, e.g. NGS or quantitative real-time PCR.

References

1. Singh H, Kang A, Bloudek L, Hsu I-L, Palanca-Wessels MC, Stecher M et al. Systematic Literature Review and Meta-Analysis of HER2 Amplification, Overexpression, and Positivity in Colorectal Cancer. *JNCI Cancer Spectrum* 2023 (In Press).
2. Richman SD, Southward K, Chambers P, Cross D, Barrett J, Hemmings G et al. HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. *J Pathol* 2016;238:562–570.
3. Strickler JH, Cercek A, Siena S, André T, Ng K, Van Cutsem E et al. Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer (MOUNTAINEER): a multicentre, open-label, phase 2 study. *Lancet Oncol* 2023;24:496–508.
4. Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a

proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016;17:738–746.

5. Tucatinib with trastuzumab for previously treated HER2-positive colorectal cancer [ID6227]. In Progress. Available at: www.nice.org.uk/guidance/indevelopment/gid-ta11228/documents
6. Bartley AN, Washington MK, Ventura CB, Ismaila N, Colasacco C, Benson 3rd AB et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med* 2016;140:1345–1363.
7. Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F et al. HER2 testing in gastric cancer: a practical approach. *Mod Pathol* 2012;25:637–650.
8. Rakha EA, Pinder SE, Bartlett JMS, Ibrahim M, Starczynski J, Carder PJ et al. Updated UK Recommendations for HER2 assessment in breast cancer. *J Clin Pathol* 2015;68:93–99.
9. Koopman T, Louwen M, Hage M, Smits MM, Imholz ALT. Pathologic diagnostics of HER2 positivity in gastroesophageal adenocarcinoma. *Am J Clin Pathol* 2015;143:257–264.
10. Edenfield WJ, Chung KY, Gatalica Z, Basuet GD. Molecular profiling of HER2-positive colorectal cancer for identification of multiple potential drug targets. *J Clin Oncol* 2014;32:e14508.