

HER-2/*neu* measurement: a model for the future of individualised medicine

by Fiona Howe

In many parts of Europe and the USA, only patients whose tissue samples have tested positive for the human epidermal growth factor receptor-2 oncoprotein (HER-2/*neu*) are eligible to receive Herceptin treatment. However, patients that test negative at the time when the tissue biopsy was taken may become HER-2/*neu* positive as they progress to metastatic disease. A new automated serum immunoassay which detects the circulating extra-cellular domain of HER-2/*neu* has been developed. This assay enables clinicians to follow patient progress more closely, optimising treatment regimens to suit the individual patient.

The human epidermal growth factor receptor-2 oncogene, also referred to as HER-2/*neu*, is capable of driving cell proliferation in breast cancer patients. The extra-cellular domain (ECD) of HER-2/*neu* oncoprotein is shed from cancer cells into the blood of around 18% of patients suffering from primary breast cancer and over 50% of those suffering from metastatic breast cancer [1].

Assessment of a patient's HER-2/*neu* status plays a vital role in guiding therapy with Herceptin - a humanised monoclonal antibody raised against HER-2/*neu* which binds to the ECD, inhibiting proliferating tumour cells that over-express this oncoprotein. Measurement of this over-expression can now be accomplished using both histological and biochemical techniques.

The traditional approach

Two methodologies have traditionally been used in histopathology laboratories for HER-2/*neu* status assessment: immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH). IHC is used to detect HER-2/*neu* protein levels in biopsy samples, whereby the degree of tissue staining is subjectively scored on a range from 0 (negative) to 3+. Greater accuracy may be achieved by using the semi-quantitative FISH method which detects the amount of genetic material present in the tissue, but requires a high degree of technical expertise.

Use of histological techniques to determine a patient's HER-2/*neu* status has two distinct disadvantages. Firstly, there is the impracticality of continuously taking biopsies for disease monitoring purposes, particularly when metastases occur in inaccessible areas. This is compounded by the problem of patients whose original tissue biopsies were HER-2/*neu* negative and who subsequently go on to over-express the HER-2/*neu* oncoprotein when metastases develop. Under current clinical guidelines which apply in many parts of Europe and the USA, only those patients whose tissues have tested positive for HER-2/*neu* are eligible for treatment with Herceptin. Therefore, there is a population of women with breast cancer who have HER-2/*neu* positive tumours but who are not eligible for the benefits of Herceptin therapy.

New dynamic serum test

A new diagnostic alternative now exists in the form of an immunoassay which is targeted towards the circulating ECD of HER-2/*neu* in blood specimens. This automated immunoassay, currently approved by the US Food and Drug Administration (FDA) for follow-up testing and treatment monitoring in patients with metastatic breast cancer, overcomes the disadvantages of the histological techniques, and serum specimens can be run routinely on the ADVIA Centaur Immunoassay System from Bayer Diagnostics.

The ability to assess a patient's HER-2/*neu* status from blood samples offers clear advantages over tissue testing. In a sense, tissue testing provides old news about a tumour that may have been taken away years ago, and the HER-2/*neu* status can change from the primary to the metastatic setting. Tissue testing only provides information about the HER-2/*neu* status when the tissue sample was

taken, whereas the serum assay is a dynamic test which can be performed at any time before or after surgery.

The new serum test is an enzyme-linked sandwich immunoassay which can be used for the detection and quantitation of HER-2/*neu* in serum using colourimetric means. One monoclonal antibody (Mab NB-3) is used as a capture reagent, while the other (Mab TA-1 labelled with biotin) serves as a detector [2]. Work on development of the assay began in 1986 as part of a discovery research project which revealed that antibodies to the *neu* oncogene in rats could be used to cure mice of cancers. This concept led to the development of trastuzumab (now marketed by Roche Pharmaceuticals as Herceptin), whilst also providing the basis for this new diagnostic method.

Herceptin currently has the distinction of being one of the few drugs in the world whose prescription is dependent on the results of a diagnostic test. However, Bayer is working in close collaboration with several other pharmaceutical companies which are in the process of developing other anti-HER-2/*neu* therapies, not just for use in metastatic breast cancer, but also in several cancers including those of the bladder, colon and kidney cells. Herceptin is targeted against tumours which over-express HER-2/*neu*, while other drugs will counter the proliferation of cells where the level of HER-2/*neu* expression is much lower. The therapy is most successful when used in combination with various chemotherapies.



Figure 1. A new serum HER-2/*neu* assay is available on the ADVIA Centaur Immunoassay System.

Precise quantitation

In order to establish a normal range cut-off point for the new assay, serum samples from 199 apparently healthy females were tested. The upper limit of normal for this group, defined as the 95th percentile of the observed results, was 12.7 ng/mL, with a 90% confidence interval of 12.1 to 13.7 ng/ml. The cut-off was then set at 15 ng/ml.

Over 50% of women with metastatic breast cancer can have an elevated level of HER-2/*neu* at 15 ng/ml or more. Traditionally, publications have always stated

that only 20% - 30% of breast cancer patients have HER-2/*neu* positive tumours based on tissue testing. However, recent studies have indicated that the figure is really much higher than this [1]. Because of the inaccuracy of tissue testing, some of these patients are being missed and are not benefiting from the positive effects of Herceptin. It has recently been shown that 17% - 20% of women with primary cancer actually have a negative tissue test, but go on to develop an elevated HER-2/*neu* level in metastatic cancer [1, 3]. This explains the discrepancy.

Tissue testing is carried out on the primary tumour, and because of the arbitrary 10% cut-off for positive staining cells, some HER-2/*neu* positive tumours are actually being scored as negative. However, the HER-2/*neu* positive cells in the primary tumour which have been scored as negative may actually be the cells that travel to other sites and cause metastatic breast cancer later in life. This is most likely the reason that many more HER-2/*neu* positive tumours are being discovered in women with metastatic breast cancer. Incidences of women with levels of circulating ECD up to 9000 ng/ml have been reported. It appears that the higher the amount of ECD shed, the more aggressive the tumour.

Treatment decisions

Today, many doctors base their treatment of breast cancer patients on the presence or absence of the oestrogen receptor. HER-2/*neu* status is now becoming equally important for administering anti-HER-2/*neu* specific drugs such as Herceptin. Recently published data indicate that elevated pre-treatment levels of serum HER-2/*neu* predict a favourable response to Herceptin-based therapies [1]. There is also evidence to suggest that if a patient's level of HER-2/*neu* is elevated, there is a good probability that she will not respond to hormone therapy and some chemotherapy regimens [1]. Studies are now ongoing to determine the clinical value of combining Herceptin with hormone therapy in those women who appear to be hormone resistant.

The serum test can be used at any time for monitoring HER-2/*neu* ECD levels of HER-2/*neu* positive tumours, regardless of what form of therapy the patient is taking. Various studies have demonstrated that increasing HER-2/*neu* levels are associated with progressive disease, and that falling levels indicate response to therapy. There is very good correlation between rises and falls in the level of HER-2/*neu* and the actual clinical course of the disease. It is hoped that additional studies will confirm existing findings, and that use of the serum test will allow doctors to monitor disease progress regularly without the need for expensive X-rays or CAT scans. If a doctor sees that a patient is progressing, therapy may be adjusted early to increase the chances of successful treatment [4, 5]. The serum test allows clinicians to understand a patient's HER-2/*neu* status and use it as the basis for treatment decisions.

Serological determination of HER-2/*neu* in conjunction with CEA (carcinoembryonic antigen) and CA 15-3 (cancer antigen) has been shown to increase the chance of a relapse being detected before the actual clinical signs of progressive disease are apparent [1]. This approach would enable clinicians to intervene earlier with therapy while the tumour burden is lower, which should improve the patient's response to various combinations of therapy. Assays for measurement of HER-2/*neu*, CEA and CA 15-3 are all available from Bayer for automated processing on the ADVIA Centaur Immunoassay System.

The usefulness of these markers helps to emphasise the important role that clinical laboratories have to play in the management of breast cancer. However, HER-2/*neu* measurement is unique in terms of its link with Herceptin and the direct contribution it makes to therapy decisions.

HER-2/*neu* provides an excellent model for the future of individualised and personalised medicine, whereby a biomarker is used to direct therapy. Over the next 10 - 20 years it is expected that a number of new diagnostic and therapeutic combinations of this kind will be introduced. Having the right test for the right therapy will be key.

References

1. Carney WP, *et al.* Potential clinical utility of serum HER-2/*neu* oncoprotein concentrations in patients with breast cancer. *Clin Chem* 2003; 49(10): 1579-1598.
2. Carney WP, *et al.* Detection and quantitation of the human *neu* oncoprotein. *J Tumor Marker Oncol* 1991; 6(2): 53-72.

3. Yeh I. Measuring HER-2/*neu* in breast cancer: IHC, FISH or ELISA? *Am J Clin Pathol* 2002; 117(Suppl.): s26-235.

4. Lipton A, *et al.* Elevated serum HER-2/*neu* level predicts decreased response to hormone therapy in metastatic breast cancer. *J Clin Oncol* 2002; 20: 1467-1472.

5. Lipton A, *et al.* Serum HER-2/*neu* and response to the aromatase inhibitor Letrozole versus Tamoxifen. *J Clin Oncol* 2003; 21: 1967-1972.

The author

Fiona Howe,
ADVIA Centaur Product Manager,
Bayer Diagnostics,
Newbury, UK
Tel.: +44 1635 566248
Email: fiona.howe.fh@bayer.co.uk

