

## BREAST CANCER AND HER-2/NEU

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### 9.1 Introduction

Breast cancer is the most common malignancy in women with 180,000 new cases and 45,000 occurring annually in the USA. The lifetime risk for a woman for developing breast cancer is 1 in 8, with a greater risk in the presence of a family history of breast or ovarian cancer. Over the past 10 years the mortality rate from breast cancer has been slowly decreasing, probably due to a combination of earlier detection from breast screening programmes and improvements in available treatments. Treatment of breast cancer uses a combination of surgery, radiotherapy, hormonal manipulation and adjuvant chemotherapy. Hormone therapy is most effective in the post-menopausal patient whose tumour is positive for oestrogen receptors (ER+). Adjuvant chemotherapy is most effective in pre-menopausal women, with anthracycline-based therapy probably superior to most other types. Surgical treatment of breast cancer relies on removal of the tumour with as wide an excision margin as possible, which may necessitate mastectomy. Auxiliary sampling and dissection aids in the staging of the disease and may help prevent spread.

Diagnosis of breast cancer is based on history and examination, radiology and cytological/histological examination of needle aspirates or biopsies. There are no biomarkers at present that help in the diagnosis, with the possible exception of the carbohydrate antigen marker CA15-3. Detection of gene mutations in the BRCA1 or BRCA2 genes indicate an increased risk of developing familial breast cancer and some women opt for prophylactic mastectomy if found to be positive for the mutations. There are, however, markers that are important in assessing prognosis. Classical histological prognostic factors include the type of tumour, grade, size, vascularization and the lymph node status. The Nottingham Prognostic Index (NPI) incorporates tumour grade (1-3), size (0.2 x size in mm) and lymph node involvement (number of nodes involved and score: 0=1, <4=2, >4=3). An NPI <3.4 is associated with a five year survival rate of 89%, whereas a NPI >5.6 has a five year survival rate of only 29%. Molecular factors that have been shown to provide prognostic information include oestrogen receptors, DNA ploidy, proliferation growth factors (e.g. EGFR), oncogenes (HER-2/neu, ras etc.) and tumour suppressor genes (p53). Recent development of a drug that targets the oncogene HER-2/neu has

heralded a new era of biomarkers with the potential to direct therapy.

### 9.2 HER-2/neu

HER-2 refers to human epidermal growth factor receptor 2 and is also known as neu, HER-2/neu and c-erbB-2. Other members of the type I growth factor family include EGFR (erbB-1), HER-3 and HER-4. The HER-2 gene is located on chromosome 17 (17q 11-q12) and encodes a transmembrane glycoprotein, p185. The intracellular domain of the protein has tyrosine kinase activity promoting cell growth. The extracellular domain of the protein (97-115 kDa) is shed into the circulation and is measurable in plasma/serum samples. The target ligand for HER-2/neu has not been identified, but activation has been demonstrated following binding of growth factor to adjacent receptors with subsequent dimerization.

Autophosphorylation and gene activation then occurs. HER-2/neu is expressed in many normal cells with up to 100,000 copies per cell. Over-expression occurs by gene amplification or by increased gene copies and in some tumours may be increased up to 40-fold. Cancers in which HER-2/neu over-expression has been reported include: breast, ovarian, endometrial, pancreatic, gastric and salivary gland. Most studies to date, however, have focused on HER-2/neu over-expression in breast cancer.

### 9.3 HER-2/neu and breast cancer

Many studies have now determined the proportion of women with advanced breast cancer whose tumours are over-expressing HER-2/neu and although there is a wide spread of results the consensus is that 30-40% of such tumours are HER-2/neu positive. The figure is much lower in studies that included primary breast cancer, although the proportion may be as high as 40-60% in ductal carcinomas. Tumour cells over-expressing HER-2/neu have been shown to have a higher cell proliferation rate, are associated positively with mitotic activity and negatively with estrogen receptor status. In vivo HER-2/neu positive tumours have been shown to metastasise faster. Patients with breast cancers over-expressing the HER-2/neu oncogene respond differently to endocrine and chemotherapy, have decreased disease-free intervals and overall survival times than do those who are HER-2/neu negative and are candidates for Herceptin therapy. In vitro studies have shown that cells transfected with HER-2/neu acquire resistance to tamoxifen, cisplatin, 5-fluorouracil and carboplatin, chemotherapy agents commonly used in the treatment of breast cancer.

## 9.4 Herceptin

Herceptin<sup>®</sup> (trastuzumab, Roche) is a humanized antibody targeted to HER-2/neu. It has been shown to decrease the proliferation rate in cells over-expressing HER-2/neu. Patients with HER-2/neu positive tumours treated with Herceptin had a 50% improvement in response, whereas there was no response in HER-2/neu negative patients. Limited studies have shown some benefit of a combination of Herceptin and chemotherapy or taxanes. It is important, therefore, in order to maximise the benefit of using Herceptin therapy, that women whose tumours are over-expressing HER-2/neu are correctly identified.

## 9.5 Assessment of tumour HER-2/neu status

The current method for assessing the HER-2/neu status of a tumour is to use histological techniques such as immunohistochemistry (IHC) or fluorescence in situ hybridisation (FISH) on the primary tumour or metastases available for biopsy. Recently, however, immunoassays have become available to measure the extracellular domain (ECD) in the blood plasma/serum (Bayer Diagnostics).

Immunohistochemistry uses mono- or polyclonal antibodies against HER-2/neu that are visualised by a chromogenic reaction via enzyme linking. The extent of staining at the cytoplasmic membrane is assessed and scored 0-3+. Those tumours that are 3+ are considered to be HER-2/neu positive and the patient is a candidate for Herceptin therapy. Some studies have also incorporated 2+ subjects in the Herceptin treatment arm. FISH allows the detection of specific nucleic acid sequences using a single stranded DNA probe annealed to the complementary target sequence and visualised using a fluorescent tag. A second different coloured fluorescent tag attached to a DNA probe to a housekeeping gene allows expression of the results as a ratio. A ratio greater than two is considered to indicate HER-2/neu positivity.

Whilst accepted as the standard method for determining HER-2/neu positivity in breast cancer the techniques available are technically demanding, semi-quantitative and are subject to the experience of the pathologist viewing the slides. Histological assessment represents the HER-2/neu status at a single point in time – at biopsy or removal of the primary tumour – which may be years prior to recurrence. In some cases, tissue samples from metastases may not be accessible (brain or bone metastases) and it must be assumed that the HER-2/neu status of the tumour has not altered over time.

The availability of serum-based HER-2/neu assays allows “real time” assessment of HER-2/neu status with easily obtained samples. Serum Her-2/neu measurements are quantitative and repeat testing is possible for longitudinal monitoring of therapy. Herceptin does not interfere with the serum HER-2/neu assay. Measurement of serum HER-2/neu in 242 healthy women produced an upper limit of the reference range of 15 µg/L. In benign breast disease 3.3% of women had serum HER-2/neu concentrations above this cut-off, whilst in those with Stages I & II breast cancer the proportion with raised serum HER-2/neu was 3.8%, bearing out the low figures seen in primary breast cancer using histological methods. In stage III disease 18.4% were above

15 µg/L and in Stage IV disease there were 35.0% of women with raised serum HER-2/neu (Table 1).

Table 1 Serum HER-2/neu-2/neu concentrations in breast disease (µg/L)

	Healthy Women	Benign Breast Disease	Breast Cancer Stage I & II	Stage III	Stage IV
N	242	210	105	49	103
Median	9.0	9.3	9.8	10.3	12.5
Mean ± SD	9.2 ± 2.7	9.6 ± 2.7	10.7 ± 7.6	12.3 ± 5.2	75.2 ± 533
> 15 (%)	2.9	3.3	3.8	18.4	35.0

Increased concentrations of serum HER-2/neu have been associated with poor response to chemotherapy as for those where over-expression of HER-2/neu was assessed at the tissue level. There have now been over 25 studies that have demonstrated that the overall survival time or disease free survival time is shorter in women with increased serum HER-2/neu concentrations, whether treated with hormonal therapy or chemotherapy.

Serial measurement of serum HER-2/neu can be useful in monitoring response to Herceptin therapy. A falling concentration indicates an adequate response; whilst a steady or rising value may indicate inadequate dosage. The half-life of Herceptin in the circulation appears to be dependent on the concentration of the HER-2/neu ECD in the bloodstream. Where the plasma HER-2/neu concentration is low Herceptin has a half-life of 10 days whereas in the presence of high plasma HER-2/neu concentrations the half-life can be as short as 2 days. The measurement of serum HER-2/neu can, therefore, be used as a guide to the required dosage of Herceptin to achieve therapeutic concentrations in vivo.

## References

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