Imaging HER2, the target of trastuzumab (Herceptin), in breast cancer

By Tim A D Smith
Aberdeen Biomedical Imaging Centre, School of Medical Sciences (Biomedical Physics Building), University of Aberdeen

HER2 (or erbB-2) is a member of the type 1 transmembrane tyrosine kinase receptor family, each of which consists of an extracellular domain attached via a transmembrane region to an intracellular domain with tyrosine kinase activity. Other family members are HER1 (epidermal growth factor receptor (EGFR)), HER3 and HER4. Signalling through the HER family is crucial for many vital cellular events, including cell differentiation, survival and proliferation.

Activation of intracellular signalling pathways downstream of HER, which include PI3/Akt, occurs via dimerisation of two HER molecules. Dimer formation is brought about by the interaction of the external domains of two HER molecules which can be identical (homodimers) or consist of two different HER molecules (heterodimers), eg HER2:HER3. Dimerisation brings together the intracellular domains of the two molecules and enables cross-phosphorylation of the tyrosine kinase units to occur. Unlike HER1, HER3 and HER4 which require activation by ligands, HER2 is constitutively activated and ready to form dimers. Aberrant signalling through HER2 due to HER2 gene amplification is found in about 25% of invasive breast carcinomas and is associated with poor prognosis and aggressive tumour behaviour.

Trastuzumab (Herceptin) is indicated in the treatment of breast cancer patients with HER2-overexpressing tumours. HER2-overexpression is currently determined on the primary tumour by either fluorescence in-situ hybridisation (FISH) to measure HER2 gene amplification or by direct measurement of HER2 protein using immunohistochemistry. However, detection of HER2-over-expression is compromised by heterogeneity of cell surface receptor expression within different parts of a breast tumour. Further, in a significant number of cases there is disparity between HER2 status in primary tumours and in their metastasis, calling into question the practice of basing the decision to treat breast cancer patients with trastuzumab on primary tumour HER2 status.

Medical imaging potentially facilitates the detection of HER2 receptor expression in-vivo on both the primary tumour and detectable secondary tumours thus bringing about a more accurate indication of a patient’s probable response to treatment with trastuzumab.

There are a number of imaging modalities that can be applied to detect cell surface receptor expression in-vivo including magnetic resonance imaging (MRI), single photon emission tomography (SPECT), positron emission tomography (PET) and ultrasound. Molecular imaging procedures require the administration of a targeted tracer that homes in on the molecule of interest. The tracer includes a beacon, the nature of which is dependent on the imaging modality employed. A PET tracer will include a positron emitting nuclide. Commonly for large tracers such as antibodies these include positron emitting nuclides of metals such as $^{64}$Cu ($t_{1/2}=12.8$h) or $^{89}$Zr ($t_{1/2}=78.4$h). SPECT tracers are commonly labelled with $^{99m}$Tc ($t_{1/2}=6$h) or $^{111}$In ($t_{1/2}=2.8$d). Labels for magnetic imaging probes are paramagnetic in nature causing localised perturbations in water proton relaxation parameters.

To obtain low background levels, tracer molecules that have not bound to the target molecules of interest at the time of imaging must have cleared appreciably from the bloodstream. Short-lived isotopes, such as $^{99m}$Tc ($t_{1/2}=2.8$h), can only be used to directly label small targeting molecules such as peptides or fragment antibodies which are cleared rapidly. Whole antibodies, such as trastuzumab, take several days to clear so must be labelled with longer lived isotopes. However, a recent innovation, the anti-HER2-affibody, has been applied to HER2-imaging. Monovalent Affibodies have a molecular weight (Mw) of only 7,000 Da (cf trastuzumab – Mw of 150,000 Da) so undergo rapid blood clearance. They also have a very high affinity for HER2.

A considerable number of recent studies (see: Smith TAD 2010) have reported the development and preclinical testing of tracers derived from whole and fragment versions of trastuzumab and anti-HER2-affibodies labelled with the radionuclides $^{111}$In or $^{99m}$Tc for SPECT, with $^{99m}$Tc, $^{68}$Ga, $^{89}$Zr or $^{124}$I PET or with paramagnetic imaging probes for MRI. Labelled affibodies have proved particularly promising in tumours derived from HER2-overexpressing tumour cells grown in mice (xenografts) in which tumour-to-blood ratios as high as 190 have been achieved four hours after injection. The first clinical study (Allan et al 1993), reported in 1993 by a group at the Institute of Cancer Research, carried out using a radiolabelled molecule to target HER-2 in vivo was $^{99m}$Tc-labelled ICR12, a rat antiherb2 antibody for MRI. Labelled affibodies have proved particularly promising in tumours derived from HER2-overexpressing tumour cells grown in mice (xenografts) in which tumour-to-blood ratios as high as 190 have been achieved four hours after injection.

Performing HER2 imaging studies during treatment to determine the levels of HER2 expressing tumour tissue can be confounded when using labelled trastuzumab by the potential for signal dilution due to the presence of residual levels of therapeutic trastuzumab. However, anti-HER2 antibodies bind to a different epitope to that of trastuzumab so accurate cell surface levels of HER2 can be determined during the course of treatment. While tracers based on anti-
HER2-affibodies also have the advantage of rapid blood clearance facilitating same-day administration and imaging, the pharmacodynamics and pharmacokinetics of radiolabelled trastuzumab will more closely resemble that of the drug itself, so providing better guidance in the determination of patient loading levels of trastuzumab to optimise biodistribution.

Baum et al in 2010 reported on the application of anti-HER2-affibody labelled with either $^{111}$In or with $^{68}$Ga, for SPECT and PET imaging respectively, to detect metastasis in three patients with breast cancer. Dynamic imaging using $^{111}$In-ABY-002 detected four metastatic lesions in Patient 1. One of these metastatic lesions was biopsied and confirmed to be HER2 positive with a score of 3+ (scale: minimum=0, maximum =3+). In Patient 2, FDG-PET had confirmed the presence of a liver and potentially a lymph node metastasis but neither the SPECT nor PET using ABY-002 could unambiguously detect HER2 positive metastasis. However, in Patient 3 all five metastatic lesions detected using FDG-PET demonstrated uptake of both $^{111}$In- and $^{68}$Ga-labelled ABY-002. The latter tracer also detected a further lesion in this patient that had not been detected using FDG-PET.

The few clinical studies that have examined the performance of HER2-targeted tracers have produced mixed but some encouraging results. However, the number of patients recruited in most of these studies is low and there are still factors that remain to be established, including the ability of these tracers to detect all sizeable HER2-overexpressing tumour masses, the most useful targeting moiety and the optimal time between administration and imaging for tumour detection.

Finally, there are other factors that should be taken into account when basing clinical decisions on HER2 imaging data. Imaging averages out the amount of tumour-associated tracer over the region of interest as a whole. Breast tumours generally exhibit a high degree of heterogeneity in tumour cellularity so it is conceivable that breast cancers with a low proportion of HER2-overexpressing tumour cells may appear on an image as only exhibiting relatively low levels of HER2 and may not be selected for treatment with trastuzumab. Further, although the possession of an intact HER2 is an essential prerequisite for cellular sensitivity to trastuzumab, mutations to components of the intracellular signalling pathways downstream of HER2, most notably PTEN, can induce trastuzumab resistance independent of HER2 status.

References