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Molecular imaging of HER2-positive breast cancer - a step toward an individualized “Image and Treat” strategy

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Abstract

Purpose of review—HER2 overexpression is correlated with aggressive tumor behavior and poor clinical outcome. Therefore, HER2 has become an important prognostic and predictive factor, as well as a target for molecular therapies. The article reviews recent advances in molecular imaging of HER2 that could facilitate individual approaches to targeted therapy of HER2-positive breast cancers.

Recent findings—Because of the heterogeneity of breast cancer and possible discordance in HER2 status between primary tumors and distant metastases, assessment of HER2 expression by non-invasive imaging may become an important complement to immunohistochemistry or fluorescence in situ hybridization analyses of biopsied tissue. Monoclonal antibodies such as trastuzumab and pertuzumab, or small scaffold proteins such as Affibody molecules are used as HER2-targeting agents. For imaging purposes, these agents are labelled with positron-, gamma-emitting radionuclides, optical dyes, or paramagnetic contrast molecules for PET, SPECT, optical, and magnetic resonance imaging, respectively. HER2-specific molecular probes combined with modern imaging techniques, providing information on HER2 expression not only in primary tumors but also in distant metastases not amenable to biopsy may reduce problems with false negative results and, thereby, influence patient management by selecting patients that would benefit from HER2-targeted therapies.

Summary—The new “Image and Treat” strategy, involving assessment of target presence and distribution in an individual patient followed by optimized, target-specific drug delivery, may potentially improve efficacy of cancer treatment while reducing side effects.

Keywords

Breast cancer; HER2; Molecular imaging; PET; SPECT; MRI; Optical imaging

Introduction

Human epidermal growth factor receptor 2, known as HER2, HER2neu, ErbB2 or c-erbB2, is a member of the epidermal growth factor receptor (EGFR) family that also includes HER1 (EGFR, ErbB1), HER2 (ErbB2), and HER4 (ErbB4) (1). As other members of this family, HER2 is a transmembrane glycoprotein consisting of three domains: extracellular (N-terminal), transmembrane (single alpha-helix), and intracellular (tyrosine kinase). Several ligands have been identified for EGFR family receptors, including epidermal growth factor

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(EGF) and transforming growth factor alpha (TGFalpha), that induce receptor dimerization required for tyrosine autophosphorylation and triggering downstream signaling. HER2 is the only receptor that does not have known natural ligands but it forms heterodimers with other members of EGFR family leading to activation of signaling pathways involved in cell growth, differentiation, survival, adhesion, and migration.

While normal epithelial cells express very low levels of HER2 protein on the cell surface, HER2 has been found to be over-expressed in a number of cancers including breast, ovarian, salivary gland, stomach, kidney, colon, prostate, urinary, and non-small cell lung cancer (2–5). Amplification of the HER2/neu gene and/or overexpression of the protein have been identified in approximately 20% of invasive breast cancers (6, 7). HER2 overexpression promotes proliferation, motility and survival rate of cancer cells and has been associated with resistance of cancers to therapeutic interventions including hormone therapy, radiation, and certain types of chemotherapy, leading to poor outcomes. Therefore, HER2 has become an attractive target for breast cancer therapy (6, 7). Several approaches, including inhibition of HER2-triggered signal transduction by humanized antibodies and small molecules targeting catalytic activity of receptors, have been tested in clinical trials and new anti-HER2 strategies are being actively developed (6–8).

The efficacy of trastuzumab, the first monoclonal antibody approved by FDA for treatment of HER2-positive breast cancers, depends on the degree of HER2 expression in the primary tumor, and its approval was made possible thanks to the proper selection of patients based on the HER2 status of the primary tumor (6, 9). Presently, there are three types of ex-vivo tests used to assess HER2 expression level in breast cancers: immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) (10), and chromogen in situ hybridization (CISH) (11). However, several recent studies indicated serious problems with their implementation in clinical practice resulting in error rates exceeding 20% (12–14). Furthermore, the invasive nature of the biopsies limits the amount of samples, if any, that could be obtained from different sites of metastatic disease and imposes severe restrictions on the number of time points at which the HER2 status could be tested.

It is clear that the success of HER2-targeted therapies depends on accurate characterization of HER2 expression. Therefore, the HER2 status should be assessed in all patients with breast cancer to identify HER2-positive tumors that would qualify the patient for treatment with trastuzumab and for participation in clinical trials with novel HER2-directed therapies (15, 16). Unfortunately, HER2-positive breast cancer remains a heterogeneous disease, and possible discordance in HER2 status between primary tumors and distant metastases (17, 18) might lead to unpredictable long term responses to anti-HER2 therapy and variable outcomes (19). Thus, a more accurate method for assessment of HER2 status of both primary tumor and distant metastases is needed.

Modern molecular imaging may provide a complementary, noninvasive option to obtain real-time information that not only could facilitate selection of patients for HER2-targeted therapy but also could assess the immediate response to therapeutic interventions. This information would allow adjustment of the dose and treatment schedule for individual patients, based on the actual status of the HER2 receptors. Providing reliable data on HER2 expression, both in primary lesions and in distant metastases, and elimination of laborious testing procedures amiable to human error and bias, may also reduce the number of false-negative or false-positive results from the currently used ex vivo methods.

The new strategy, involving assessment of target presence and distribution in an individual patient followed by optimized, target-specific drug delivery combined with early monitoring of tumor response, may significantly improve efficacy of cancer treatment while reducing

side effects. This review presents recent advances in molecular imaging of HER2 for imaging of HER2-positive breast cancers that may bring such individualized strategy closer to clinical routine. It focuses on work published after 2008, as listed in Table 1. A comprehensive analysis of the reports on this subject from previous years can be found in a review by Tolmachev (20).

Positron emission tomography (PET)

High sensitivity, high spatial resolution, and proven quantification abilities, makes PET the modality of choice for applying molecular imaging in the clinical setting. Antibody-based radioligands for *in vivo* imaging of HER2 by PET have been developed by several groups. However, the clinical application of antibodies (MW=150 kDa) to molecular imaging is limited because of their large size, resulting in low tumor penetration and slow clearance. Often, several days are needed to obtain reasonable tumor-to-blood ratios, making most short-lived PET radionuclides inapplicable. Recently, rather exotic positron emitters with relatively long half-life were used to allow imaging at times required for clearance of unbound antibodies from circulation. For example, Dirjkes *et al.* labeled trastuzumab with a positron emitting ^{89}Zr (21). Using the resulting radioconjugate, Oude Munnink *et al.* (22) were able to assess changes in HER2 expression in SKBR3 xenografts resulting from treatment with the HSP90 inhibitor NVP-AUY922. Long half-life of ^{89}Zr allowed them to observe the radioactivity accumulation in the tumors 144 h post injection. The same tracer was then tested in a clinical trial carried out to optimize dosage and time of administration of the ^{89}Zr -trastuzumab for PET imaging of HER2-positive lesions (23). In this study, fourteen patients with HER2-positive metastatic breast cancer received 37 MBq of ^{89}Zr -trastuzumab at one of three doses (10 or 50 mg for those who were trastuzumab-naïve and 10 mg for those who were already on trastuzumab treatment). The patients underwent at least two PET scans between days 2 and 5. The results showed that administration of ^{89}Zr -trastuzumab at appropriate doses allows visualization and quantification of uptake in HER2-positive lesions in patients with metastatic breast cancer by PET. However, the best time for assessment of ^{89}Zr -trastuzumab uptake by tumors was only 4–5 days after the injection.

To improve the imaging performance, alternative ligands have been developed and extensively studied over the last few years. Among them are antibody fragments and engineered variants such as F(ab')₂, F(ab'), single-chain Fv, diabodies, and minibodies. However, there are no recent reports on the application of these molecules for monitoring of HER2 expression in breast cancer. Instead a new class of relatively small (6.5 kDa) proteins called Affibody molecules (24, 25) has been used to image HER2-positive tumors. These very stable and highly water-soluble α -helical proteins are based on a 58-amino-acid Z-domain scaffold, derived from the B domain of staphylococcal surface protein A. They can be readily expressed in bacterial systems or produced by peptide synthesis, and the lack of sulfite bridges and high stability facilitate the conjugation chemistry. The small size, resulting in rapid blood clearance, good tumor penetration, and high binding affinity to selected targets, makes Affibody molecules ideal candidates for imaging purposes. In addition, HER2-specific Affibody molecules bind with low nanomolar affinity to a HER2 epitope distinct from those involved in binding of trastuzumab or pertuzumab, which enables their application to monitor potential changes in HER2 expression during therapeutic interventions involving these antibodies.

A recent work by Orlova *et al.* (26) provide direct comparison of ^{124}I -labeled Affibody molecules and trastuzumab. The authors showed that, although both radioiodinated Z_{HER2:342} and trastuzumab specifically targeted HER2-expressing xenografts *in vivo*, the tumor-to-organ ratios were substantially higher for ^{124}I -Z_{HER2:342} due to the more rapid clearance of radioactivity from blood and normal organs.

Fast clearance of Affibody-based tracers from the blood allow their labeling with another positron emitter - ^{68}Ga . It can be easily obtained from commercially available $^{68}\text{Ge}/^{68}\text{Ga}$ generators, and the labeling procedure of DOTA functionalized Affibody molecule, ABY-002, is efficient and straightforward. Recently, Tolmachev *et al.* labeled ABY-002 Affibody molecules with ^{68}Ga and compared the resulting tracer with previously well characterized ^{111}In -ABY-002 (27). The authors showed that ABY-002 could be efficiently labeled with ^{68}Ga . While both radiotracers accumulated specifically in HER2-positive xenografts, ^{68}Ga -ABY-002 cleared more rapidly from blood and healthy tissues (except kidneys) than ^{111}In -ABY-002, leading to high tumor to organ ratios as soon as 2 h after injection.

First clinical studies using synthetic ^{111}In - or ^{68}Ga -labeled ABY-002 for molecular imaging of HER2-expressing malignant tumors in breast cancer patients have been carried out by Baum *et al.* (28). In this preliminary work, three patients with recurrent metastatic breast cancer and known lesions identified by CT or ^{18}F -FDG PET/CT received approximately 80–90 μg of radiolabeled ABY-002. Two patients received both ^{111}In -ABY-002 and ^{68}Ga -ABY-002, and 1 patient received ^{68}Ga -ABY-002 only. No adverse effects were observed. Analysis of the blood kinetics revealed rapid blood clearance of radiolabeled ABY-002 in humans with a first half-life of 4–11 min for ^{111}In -ABY-002 and 10–14 min for ^{68}Ga -ABY-002. These results were in agreement with preclinical data on mice. The rapid kinetics enabled high-contrast SPECT or PET with radiolabeled ABY-002 to be performed in humans as soon as 2–3 h after injection. High uptake of radiolabeled ABY-002 was seen in the metastases, the kidneys, and the liver. Most of the previously identified lesions with ^{18}F -FDG PET/CT were visualized. However, two metastatic lesions, one close to the kidney and one in the liver, positive on ^{18}F -FDG PET/CT scans, could not be visualized due to high background radioactivity in these organs. These results lead to the conclusion that, although ABY-002 is much better suited for molecular imaging than antibody or antibody fragments, further clinical studies are needed to optimize the time of scan, peptide and radioactivity doses, and to assess the sensitivity and specificity of this method.

Cheng *et al.* used a maleimide-functionalized chelator, Mal-DOTA to label another anti-HER2 Affibody molecule $\text{Z}_{\text{HER2:477}}$, either in monomeric or dimeric form with ^{64}Cu (29). The resulting radiotracers were evaluated in nude mice bearing subcutaneous SKOV3 tumors. Biodistribution experiments demonstrated that the use of monomer resulted in higher tumor accumulation and tumor/blood ratio as compared to dimer. The results were confirmed by MicroPET imaging showing ^{64}Cu -DOTA- $\text{Z}_{\text{HER2:477}}$ potential for in vivo imaging of HER2 receptor expression.

The physical characteristics of ^{18}F and its application in clinical oncology in the form of fluorodeoxyglucose, make it the most widely used and readily available positron emitter. A methodology for labeling Affibody molecules with ^{18}F has been developed at the U.S. National Institutes of Health (30). The resulting radioconjugate, N -[2-(4-[^{18}F]fluorobenzamido)ethyl]maleimide (^{18}F -FBEM)- $\text{Z}_{\text{HER2:342}}$ was then used for in vivo monitoring of HER2 expression by PET (31). In a recent study Kramer-Marek *et al.*, tested the feasibility of ^{18}F -FBEM- $\text{Z}_{\text{HER2:342}}$ Affibody molecules for quantitative assessment of HER2 downregulation after anti-HER2 therapy (32). To assess the correlation of signal observed by PET with receptor expression, the authors administered the new tracer to athymic nude mice bearing subcutaneous tumors with three different levels of HER2 expression and showed that ^{18}F - Z_{HER2} -Affibody was eliminated quickly from the blood and normal tissues, including the kidneys and liver, providing high tumor/blood and tumor/muscle ratios as soon as 1 h post injection, and the signal correlated very well with the number of receptors expressed in the xenografts. To study the down-regulation of HER2 in human breast cancer xenografts, the mice were treated with 17-DMAG, an inhibitor of

Hsp90 known to decrease the HER2 expression. The levels of HER2 expression estimated by post-treatment PET decreased by 71% and 33%, respectively, for mice bearing BT474 and MCF7/clone18 tumors. These changes were confirmed by the biodistribution studies, enzyme linked immunosorbent assay, and Western blot. The results suggest that the ^{18}F -Z_{HER2} can be used to assess HER2 expression *in vivo* by PET imaging providing means to monitor possible changes of receptor expression in response to therapeutic interventions.

Ren *et al.* proved that even smaller Affibody-derived protein scaffold obtained by truncating one alpha-helix that is not responsible for receptor recognition in the Affibody, could be used for HER2 targeting. In a recent work, they modified a 2-helix small protein, MUT-DS, with DOTA and labeled with ^{68}Ga (33). The resulting anti-HER2, 2-helix tracer was evaluated in SKOV3 xenografts. The results showed that the 2-helix DOTA-MUT-DS bound to HER2 with high affinity, was stable in mouse serum, had rapid and high tumor accumulation, and was quickly cleared from normal organs. This preliminary study indicated that synthetic 2-helix ^{68}Ga -DOTA-MUT-DS might become another promising probe for imaging HER2 expression *in vivo*.

Single Photon Emission Tomography (SPECT)

Conjugates of gamma emitting radioisotopes with HER2 targeting molecules can be used for imaging of HER2-positive tumors by SPECT. For example, McLarty *et al.* used ^{111}In -labeled trastuzumab to assess the level of HER2 expression *in vivo* (34). They also showed that the tumor response to Herceptin depends on HER2 expression. The same group used ^{111}In -labeled pertuzumab, a HER2 dimerization inhibitor that binds to an epitope different from that of trastuzumab, to study downregulation of HER2 in human breast cancer xenografts after treatment with trastuzumab (35). The authors showed that, after three-day and three-week treatment with trastuzumab, *in vivo* tumor uptake of ^{111}In -DTPA-pertuzumab decreased by 2- and 4.5-fold, respectively, as compared to PBS-treated controls. Furthermore, this decrease was associated with an almost-complete eradication of HER2-positive tumor cells. These results suggest that monitoring of possible downregulation of HER2 by SPECT imaging might be used for early assessment of tumor response to the treatment with trastuzumab. If verified by clinical trials, such a correlation between the downregulation of HER2 with the overall response to trastuzumab could provide unique opportunity to use molecular imaging for early prediction of the treatment efficacy in individual patients and, thereby, enable identification of non-responders, who should be treated by other means rather than remain on trastuzumab.

Ahlgren *et al.* used another generator-produced and the most widely used radioisotope $^{99\text{m}}\text{Tc}$ to create another Affibody-based SPECT tracer, $^{99\text{m}}\text{Tc}$ -Z_{HER2:2395}-Cys (36). The conjugate showed rapid accumulation in HER2-positive xenografts and rapid clearance from a majority of organs except for the kidneys and liver. Clear and specific visualization of HER2 expression was possible within a few hours after injection suggesting that $^{99\text{m}}\text{Tc}$ -Z_{HER2:2395}-Cys might become a promising SPECT tracer for the detection of HER2 expression in breast cancer.

Magnetic Resonance Imaging (MRI)

MRI provides the best 3D anatomic data of all the imaging modalities. Its high spatial resolution, multiplane capabilities, and excellent soft tissue contrast make it the best modality for soft tissue imaging and tumor diagnosis, especially when combined with appropriate imaging contrast. For MRI of HER2-positive tumors, Chen *et al.* developed a novel contrast agent combining superparamagnetic characteristics of dextran-modified iron oxide nanoparticles with HER2-targeting potential of trastuzumab (37). These HER2-

specific nanoparticles bound specially to HER2-positive cells and allowed visualization of HER2-positive xenografts by a 3-T MRI scanner using T2-weighted fast spin-echo sequence.

Kinishita *et al.* used biotinylated HER2-specific Affibody molecules to target streptavidin-functionalized superparamagnetic iron oxide (SPIO) to HER2-positive tumors (38). After biotinylated affibody and streptavidin SPIO were injected into mice bearing SKOV-3 xenografts mouse, the nanoparticles bound specifically to HER2, and the tumor could be clearly seen imaged on MRI using a gradient-echo sequence. The studies demonstrated that Affibody-SPIO combination can be used as HER2-specific contrast agent for *in vivo* MR molecular imaging.

Optical Imaging

There are hazards associated with highly ionizing radiation used in SPECT and PET, and magnetic fields used in MRI. The conventional imaging systems are also expensive and complicated to use. They are typically set up in dedicated laboratory facilities. Particularly PET depends on a cyclotron to produce the required short-lifetime radioisotopes. Therefore, they can usually be found only in large clinical or research facilities. By contrast, optical imaging is safe, simple to set up, and do not require dedicated facilities.

Optical imaging uses CCD (charge coupled device) cameras and evermore sophisticated mathematical modeling to detect and analyze signal originating from bioluminescent and fluorescent probes. Bioluminescent probes rely on light produced by enzymatic reactions during which chemical energy is converted to photons with wavelengths in the range 550–650 nm. The most popular source of bioluminescence is firefly luciferase using the substrate luciferin. Typically low autoluminescence background allows detection of relatively weak bioluminescence signal *in vivo*. The other type of probes use fluorescence – triggered emission of light with one wavelength, e.g. 450 – 650 nm, after a molecule absorbs a photon with a higher energy and a longer wavelength, e.g. 400 – 600 nm. Fluorescent probes, including different fluorescent proteins, molecular dyes, and nanoparticles, are more abundant and brighter than bioluminescent ones. In addition, their detection does not require the administration of a substrate. However, the relatively high level of background from autofluorescence light *in vivo* requires sophisticated detection methods.

Due to its minimal invasiveness, optical imaging presents an attractive option for serial imaging of tumors and monitoring of possible changes of receptor expression during the course of treatment. Reduced fluorescence background and enhanced tissue penetration by near-infrared (NIR) light, with wavelength in the range 700 – 1000 nm, allows detection of targets located at the depth of several centimeters in the tissues. In addition, optical imaging can be superior in target-specific imaging by employing target-specific signal activation systems (39). Over the past years, many groups reported successful *in vivo* NIR fluorescence imaging (40–44).

In recent studies, Ogawa *et al.* combined the advantages of SPECT and optical imaging using both ^{111}In and indocyanine green (ICG), which is activated upon internalization into the target cells, to label panitumumab (anti-HER1) and trastuzumab (45). The results of multimodality imaging following injection of dual labeled, radio-optical probes showed that, while the biodistribution profile of the injected antibody was provided by nuclear imaging, only the target specific tumor was visualized by optical imaging. Thereby, simultaneous visualization, characterization, and measurement of biological processes could be obtained in a single imaging session.

Although most of the studies using optical beacons are qualitative, quantitative methods necessary for adequate monitoring of the receptor status are beginning to emerge. Recently,

Chernomordik *et al.* work investigated whether temporal changes of the signal detected in tumor xenografts following injection of an Affibody-based, HER2-specific fluorescence contrast agent (46) could be used to monitor the *in vivo* status of the receptors (47). Subcutaneous tumor xenografts, expressing different levels of HER2, were imaged with a NIR fluorescence small-animal imaging system at several times post-injection of the probe. The compartmental ligands-receptor model used to calculate HER2 expression from imaging data showed that initial slope, characterizing the temporal dependence of the fluorescence intensity detected in the tumor, linearly depends on HER2 expression, as measured *ex vivo* by an ELISA assay for the same tumor. This work suggests that optical imaging, combined with mathematical modeling, may allow noninvasive monitoring of HER2 expression *in vivo*.

Conclusion

Novel cancer therapies focus on specific molecular markers present in malignant tumors. HER2 is overexpressed in a significant fraction of breast cancers, and increased HER2 expression is correlated with a poor outcome and prognosis. The assessment of HER2 status in breast cancer patients is an essential step in the diagnostic workup, and selection of treatment strategy. However, there are problems with accuracy of the current *ex-vivo* tests used to assess HER2 in clinical practice. Several non-invasive imaging techniques, including PET/CT, SPECT, MRI and optical imaging, have recently demonstrated promising results in non-invasive assessment of HER2 status *in vivo* (Table 1). Because of the specific advantages and limitations of individual imaging modalities, their combination in a multimodality imaging approach may result in the next break through in the *in vivo* applications of molecular imaging to facilitate diagnosis and treatment of breast cancers.

These advances in HER2 imaging may be a step toward a new “Image and Treat” strategy in breast cancer patients. This individualized approach to cancer treatment will rely on multimodality imaging to support treating clinicians in selecting a proper therapy and to monitor its efficacy at early stage in order to adjust the treatment accordingly. The non-invasive character of molecular imaging will allow repeating the test serially in individual patient and, thereby, provide “real-time” data on early tumor response that could be used to optimize the dosage of the effective drugs or discontinue ineffective treatment before the tumor recurs. Therefore, application of the “Image and Treat” strategy will most likely result in better outcome and lower mobility of cancer treatment. The major problem with implementation of the proposed “Image and Treat” strategy to clinical practice is the limited number of clinical trials testing the predictive and prognostic value of HER2 expression assessed by molecular imaging. It might be due to the lack of interest of pharmaceuticals companies in molecular imaging agents. This situation might be changed if the drug regulatory agencies require combination of molecular targeting agents with appropriate imaging probes to verify their efficacy.

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Table 1

Summary of the recent studies (all xenografts were grown in mice).

Imaging modality	Probe	Tumor Model or Clinical Trial	Major Findings	Ref.
PET	^{89}Zr -trastuzumab	SKBR3 xenografts	Detection of the changes in HER2 expression following treatment with DMAG	22
PET	^{89}Zr -trastuzumab	Clinical trial	Quantification of the uptake is possible, optimally 4–5 days post injection	23
PET	^{124}I -Z _{HER2:342} ^{124}I -trastuzumab	NCI-N87 xenografts	Using ^{124}I -Z _{HER2:342} resulted in higher tumor-to-organ ratios as compare to ^{124}I -trastuzumab	26
PET	^{111}In -ABY-002 ^{68}Ga -ABY-002	SKBR3 xenografts	Rapid clearance of ^{68}Ga -ABY-002 blood and healthy tissues (except kidneys) resulted in high tumor to organ ratios as soon as 2 h after injection	27
PET SPECT	^{111}In -ABY-002 ^{68}Ga -ABY-002	Clinical trial	High-contrast SPECT or PET images of HER2-positive tumors can be obtained in humans as soon as 2–3 h after injection	28
PET	^{64}Cu -DOTA-Z _{HER2:477} (^{64}Cu -DOTA-Z _{HER2:477}) ₂	SKOV3 xenografts	Monomer resulted in higher tumor accumulation and tumor/blood ratio as compared to dimer	29
PET	(^{18}F -FBEM)-Z _{HER2:342}	SKOV3 xenografts	The radioactivity was eliminated quickly from the blood and normal tissues, including the kidneys and liver, providing high tumor/blood and tumor/muscle ratios as soon as 1 h post injection	31
PET	^{18}F -FBEM-Z _{HER2:342}	BT474, MD-MBA-361, MCF7, and U251 xenografts	HER2 expression and its changes caused by therapeutic intervention can be monitored by PET	32
PET	^{68}Ga -DOTA-MUT-DS	SKOV3 xenografts	The probe bound to HER2 with high affinity, was stable in mouse serum, had rapid and high tumor accumulation, and was quickly cleared from normal organs	33
SPECT	^{111}In -DTPA-trastuzumab	MDA-MB-231, MDA-MB-361, SKBR-3, BT-20, BT474, BY474het, MCF7/HER2-18 xenografts	Tumor response to Herceptin depends on HER2 expression	34
SPECT	^{111}In -DTPA-pertuzumab	MDA-MB-361 xenografts	Decrease of ^{111}In -DTPA-pertuzumab uptake following treatment with trastuzumab associated eradication of HER2-positive tumor cells	35
SPECT	$^{99\text{m}}\text{Tc}$ -Z _{HER2:2395} -CyS	LS174T, SKOV-3 xenografts	Rapid accumulation in HER2-positive xenografts and rapid clearance from a majority of organs (except for the kidneys and liver) allowed clear visualization of HER2 expression within a few hours after injection	36
MRI	Trastuzumab-conjugated, dextran-modified iron oxide nanoparticles	BT-474, SKBR-3, MDA-MB-231, MCF-7 xenografts	The HER2-specific nanoparticles bound specially to HER2-positive cells and allowed visualization of HER2-positive xenografts by a 3-T MRI scanner using T2-weighted fast spin-echo sequence	37
MRI	Affibody-SPIO nanoparticles	SKOV-3 xenografts	The nanoparticles bound specifically to HER2, and the tumor could be clearly seen imaged on MRI using a gradient-echo sequence	38

Imaging modality	Probe	Tumor Model or Clinical Trial	Major Findings	Ref.
Optical Imaging	¹¹¹ In and indocyanine green-labeled trastuzumab	MDA-MB-468, A431, 3T3/HER2 xenografts	Multimodality imaging following injection of dual labeled, radio-optical probes provides data on biodistribution profile of the injected antibody by nuclear imaging and allows the target specific tumor visualized by optical imaging.	45
Optical Imaging	AlexaFluor-labeled (ABD)-(ZHER2:342)2-Cys Affibody molecules	BT474, MD-MBA-361, MCF7, and U251 xenografts	Initial slope of the curve characterizing the temporal dependence of the fluorescence intensity detected in the tumor depends linearly on HER2 expression	47