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REVIEW

Preclinical and clinical applications of specific molecular imaging for HER2-positive breast cancer

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ABSTRACT Precision medicine and personalized therapy are receiving increased attention, and molecular-subtype classification has become crucial in planning therapeutic schedules in clinical practice for patients with breast cancer. Human epidermal growth factor receptor 2 (HER2) is associated with high-grade breast tumors, high rates of lymph-node involvement, high risk of recurrence, and high resistance to general chemotherapy. Analysis of HER2 expression is highly important for doctors to identify patients who can benefit from trastuzumab therapy and monitor the response and efficacy of treatment. In recent years, significant efforts have been devoted to achieving specific and noninvasive HER2-positive breast cancer imaging *in vivo*. In this work, we reviewed existing literature on HER2 imaging in the past decade and summarized the studies from different points of view, such as imaging modalities and HER2-specific probes. We aimed to improve the understanding on the translational process in molecular imaging for HER2 breast cancer.

KEYWORDS Breast cancer; human epidermal growth factor receptor 2 (HER2); molecular imaging; probes

Introduction

Breast cancer is one of the most common malignant tumors and cause of mortality for women all over the world[1]. Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family of tyrosine kinases. The HER2/*neu* gene is a proto-oncogene located on the long arm of chromosome 17 and encodes a transmembrane receptor protein[2]. The HER2 protein comprises an intracellular domain that can regulate important aspects of the physiology and growth of cells, as well as an extracellular domain that facilitates signal transduction as a receptor. Cultured cells can be transformed into a malignant phenotype upon the overexpression of HER2[2]. HER2/*neu* amplification and/or overexpression is observed in 15%–20% of patients with breast cancer[3,4]. HER2 is a factor that indicates high-grade breast tumors, high rates of lymph node involvement, high risk of recurrence, and high resistance to general chemotherapy[2]. Patients with HER2-positive breast cancer present a worse prognosis than those without HER2 overexpression, indicating that HER2 overexpression can be a marker of aggressive malignancy. Moreover, discordance in HER2 status may exist between primary HER2-positive breast cancer and distant metastasis because of heterogeneity[5]. Trastuzumab is a humanized monoclonal antibody that is FDA-approved for therapeutic use in HER2-positive breast cancer. It is created to specifically bind to HER2 to further inhibit the growth of tumor cells via different mechanisms, including provoking apoptosis, altering downstream signal transduction, and decreasing HER2 expression[1].

Currently, immunohistochemistry (IHC) and fluorescence *in situ* hybridization are the most important pathological techniques applied in the detection of HER2 amplification and/or overexpression. These techniques have also been recommended as guidelines by the American Society of Clinical Oncology/College of American Pathologists. About 20% of HER2/*neu* tests (including biopsy and IHC) provide false negative or false positive results because of technical problems, sampling bias, heterogeneity of the tumors and other reasons, which further lead to the improper selection of treatments[6]. Therefore, non-invasive molecular imaging techniques are needed for the accurate assessment of HER2 expression in both primary tumors and distant

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metastasis *in vivo*.

With the development of molecular imaging, one can now monitor the biological and pathophysiological processes of normal and abnormal tissues *in vivo*. Molecular imaging can thus be a complementary tool in breast cancer imaging for early detection, specific diagnosis, and evaluation of therapeutic responses. Nuclear medicine imaging [including positron emission tomography (PET) and single photon tomography computed tomography (SPECT)], ultrasound, and MRI are the most popular techniques for HER2 breast cancer imaging in both preclinical and clinical practice.

Preclinical HER2-positive breast cancer imaging

Great efforts have been made to image HER2 positive breast cancer with different modalities in the last decade. Probes are the most critical part for the molecular imaging of breast cancer. Probes should be able to easily penetrate the tumor parenchyma and be far from blood and normal organs to increase the tumor-to-tissue contrast. An optimal molecular probe for HER2-positive tumor imaging should have the following characteristics: (1) The targeting component has high affinity and specificity to the extracellular domain of HER2 protein. Various HER2-targeted parts for molecular imaging tracers, such as monoclonal antibodies, antibody-based fragments, diabodies, nanobodies, non-immunoglobulin scaffolds, affibodies, peptides, and designed ankyrin-repeat proteins, have been explored for HER2-positive breast cancer diagnosis and therapy in the past few years. (2) The signaling component can elevate the contrast difference between tumors and normal tissues to make tumors visible and detectable. Signaling components are designed on the basis of different imaging modalities.

Nuclear medicine imaging

Nuclear imaging, including PET and SPECT, is the first molecular imaging modality to be applied for cancer in clinical practice. It requires the intravenous administration of radio-labeled materials to generate images. Combining PET or SPECT with computed tomography (CT) or MRI can provide both functional and anatomical information with high resolution. ^{18}F-fluorodeoxyglucose (FDG) and ^{99m}Tc are the most common tracers that are used for PET and SPECT imaging, respectively, but they lack specificity to breast cancer. These agents are designed on the basis of imaging equipment.

PET

The radionuclides that are usually used for HER2 imaging on PET are ^{18}F, ^{64}Cu, ^{68}Ga, ^{89}Zr, and ^{124}I. The comparison of the radionuclides of ^{68}Ga and ^{111}In with the same affibody and chelator shows that ^{68}Ga-labeled affibodies are easier to label and are cleared more quickly from the blood and other healthy tissues, except for kidneys, than ^{111}In-labeled affibodies. ^{68}Ga-labeled affibodies also show good targeting ability and provide good tumor-to-blood and tumor-to-organ ratios within 2 h after injection in favor of clinical imaging[7]. A recent study conjugated the anti-HER2 affibody with ^{44}Sc via a 1, 4, 7, 10-tetraazacylododecane-1, 4, 7, 1-tetraacetic acid (DOTA) chelator for HER2 imaging and compared it with ^{68}Ga-labeled affibody, demonstrating the high specificity of the ^{44}Sc-labeled affibody. The distributions of radioactivity at 3 h after injection for both affibodies were found to be similar, but the blood clearance and tumor-to-blood ratio of the ^{44}Sc-labeled affibody were both lower than those of the ^{68}Ga-labeled one[8]. ^{18}F, with a half-life of 110 min, exhibits ideal nuclear physical characteristics for PET imaging in both experimental and clinical applications. Compared with other metal radioisotopes, ^{18}F is relatively difficult to be introduced into other molecules because its short half-life (<2 h) requires time-efficient synthesis. The direct introduction of ^{18}F usually involves harsh conditions. Efforts have been made to identify new ways to insert ^{18}F-fluorine into organic molecules. Recent studies employed ^{18}F as a radionuclide to label anti-HER2 nanobodies and generated high contrast PET images with high tumor-to-blood and tumor-to-muscle ratios[9-11]. The results demonstrated the feasibility of utilizing ^{18}F-labeled anti-HER2 nanobodies to evaluate HER2 expressing cancers, except for cases of high renal retention, which can be a common problem of ^{18}F-labeled anti-HER2 nanobodies. Studies even used the short-lived ^{11}C ($t_{1/2}$=20.4 min) to label a HER2 binding affibody molecule with the sel-tagging technique and compared the results with ^{68}Ga-labeled affibodies. These studies found that both ^{11}C-labeled and ^{68}Ga-labeled HER2 affibodies can be cleared rapidly from the blood and that the former shows significantly low kidney retention and overall absorbed dose. ^{11}C-labeled affibodies show excellent tumor-targeting capability and can thus successfully be used for rapid and repeated PET imaging for HER2-positive tumors[12].

The radio-labeled trastuzumab is the representative of monoclonal antibodies that are widely used for imaging HER2-positive breast cancers *in vivo* in clinical and preclinical studies[13-18]. However, some imaging problems are unavoidable because of their large size (MW=150 kDa), and

these problems include long biodistribution time, slow tumor penetration and blood clearance, and low tumor-to-tissue ratio, all of which limit the application of antibodies in molecular imaging. Affibody molecules are a new class of relatively stable small scaffold proteins (6.5 kDa). They are usually 3-alpha-helice proteins based on a 58-amino-acid Z-domain. As a result of their small size, affibodies have shown high binding affinity to targets, rapid blood clearance, and good tumor penetration, making them ideal candidates for molecular imaging. A study comparing the affibody- and antibody-based PET agents for HER2 imaging revealed that both radio-labeled affibodies and antibodies can specifically bind to HER2 and clearly display HER2 expression in xenografts. The uptake of radio-labeled trastuzumab in tumors is higher than that of affibodies, whereas the latter provides better tumor-to-tissue contrast at an earlier time than trastuzumab due to its quicker clearance of radioactivity from blood and normal organs; such finding illustrates that affibody-based molecules might serve as better tracers than antibodies for the imaging of primary HER2 over-expressing tumors with or without distant metastases[19]. A probe of a ^{64}Cu-labeled affibody was synthesized in both monomeric and dimeric forms, and the result showed higher tumor accumulation and higher tumor-to-blood ratio in the monomer form than in the dimer form[20]. A ^{18}F-labeled affibody was utilized to display HER2 expression tumors and HER2 metastatic tumors to the lung via PET[21,22]. Researchers also confirmed the feasibility of using ^{18}F-labeled affibody molecules for the quantitative assessment and monitoring of HER2 down-regulation after treatment of trastuzumab or an inhibitor of heat-shock protein[23,24]. The size of affibodies can be further reduced by truncating the third alpha-helix that does not contribute to receptor recognition during synthesis. For this purpose, a ^{68}Ga-labeled 2-helix small protein (MUT-DS) with a chelator of DOTA was adopted for PET imaging. The result showed high HER2 binding affinity and high tumor accumulation with rapid clearance from normal tissues, indicating the feasibility of a 2-helix protein scaffold serving as a probe to monitor HER2 expression *in vivo*. The tumor uptake and tumor-to-organ ratio of ^{68}Ga-DOTA-MUT-DS were approximately 10^3 times lower than those of many 3-helix-affibody molecules. This result is probably due to the relatively weak HER2 binding affinity of the probe, but such characteristic is still acceptable, making ^{68}Ga-DOTA-MUT-DS an alternative candidate with high imaging contrast[25-27]. Recently, ^{68}Ga-labeled anti-HER2 single-chain variable fragment (scFv) was developed to achieve rapid blood clearance and good tumor-to-blood ratio at an early time point. HER2-positive xenografts were visualized successfully with ^{68}Ga-labeled scFv with high accumulation in the tumors, although the tumor-to-blood ratio was relatively low[28].

A good chelator should be able to connect the receptor-binding part and the radionuclide for HER2 imaging. Structures of chelators can affect the clearance rate from blood and tumor uptake, as well as the uptake of normal organs. A study conjugated the following macrocyclic chelators with ^{68}Ga to the N-terminus of the affibody: DOTA, 1,4,1,4,7-7-triazacyclononane-N,N,N-triaceticacid (NOTA), and 1-(1,3-carboxypropyl)-1,4,7-triazacyclononane-4, 7-diacetic acid (NODAGA); their biodistributions and targeting properties were then compared *in vivo*[29]. The results showed chelator-dependent differences. The tumor uptake for the ^{68}Ga-DOTA-affibody was significantly higher than that for both ^{68}Ga-NODAGA-affibody and ^{68}Ga-NOTA-affibody at 2 h after injection. This outcome was interpreted as different off-target interactions due to the local charge distribution and conformation of the N-terminus caused by different structures of chelators. The ^{68}Ga-NODAGA-affibody had the highest tumor-to-blood ratio and showed a two fold higher tumor-to-liver ratio than the ^{68}Ga-NOTA-affibody, which is important for detecting liver metastases. Another study compared ^{64}Cu-labeled DOTA and NODAGA antibodies using PET imaging and found that the latter showed favorable performance *in vivo* with tumor-to-tissue ratios of 2-fold and 1.5-fold higher than the former agent at 24 h and 48 h, respectively[30].

SPECT

Radionuclides that are usually used for SPECT imaging are ^{86}Y, ^{99m}Tc, ^{111}In, and ^{125}I. ^{111}In-diethylenetriaminepentaacetic acid (DTPA)-trastuzumab was used for HER2-positive tumor imaging and found to be efficiently labeled with high yields, high stability, and good biodistribution in mice. The uptake of the ^{111}In-labeled trastuzumab in the liver, spleen, and kidney was expected, and no uptake was observed in the brain due to its size[18]. In elevating *in vivo* targeting capabilities, minimal monovalent binding fragments, such as Fab (~55 kDa) and scFv (~28 kDa) with retained binding specificity, are widely used in molecular imaging studies. Trastuzumab Fab labeled with different radionuclides of ^{111}In and ^{99m}Tc are similar in terms of visualizing HER2 xenografts, but ^{111}In-trastuzumab-Fab shows higher tumor-to-tissue ratios that favor the delayed imaging of tumors. Compared with that of intact trastuzumab, the HER2 binding affinity of trastuzumab Fab fragments shows a 2-fold decrease because of their smaller size, which is acceptable[31].

In a study, an affibody-based probe labeled with ^{99m}Tc was developed for SPECT imaging. The result revealed high uptake in HER2-expressing tumors and low uptake in the liver. The moderate- and high-level HER2-expressing xenografts can be clearly visualized 1 h after injection of the ^{99m}Tc-labeled affibody, thus proving that it can be a potential agent for SPECT imaging to detect HER2-positive tumors[32]. Compared with the ^{125}I-labeled affibody, the ^{99m}Tc-labeled agent had higher accumulation in the liver after injection[33].

MRI

In recent years, great progress has been made in MRI, especially for soft tissue imaging. Conventional breast MRI can identify tumors with their manifestations, including size, shape, margin, and signal changes. Currently, the contrast agents commonly used for MR imaging are gadolinium DTPA (decreases T1 relaxation time) and supraparamagnetic iron oxide (SPIO, decreases T2 relaxation time), which are non-specific but can increase the signal contrast between tumors and normal tissues. Molecular imaging can be performed upon MRI by using probes that bind to the target protein or molecule specifically. Nanoparticles (NPs) attract great interest in biomedical applications as imaging probes because of their extraordinary properties, including small size, well-modified structure, high binding affinity, long circulating half-life, and high permeability and retention effects that allow an effective accumulation at tumor sites[34-36]. In the literature, magnetic iron oxide nanoparticles (IONPs) conjugated with trastuzumab was designed to detect HER2 expression *in vitro* and *in vivo* using 3T MRI[15]. A 45% signal drop was found at the tumor site on T2WI after administration of the agent, proving that trastuzumab-NPs are able to distinguish cells and xenografts with different levels of HER2 expression while ensuring low cytotoxicity and good dispersity. On the basis of this work, the researchers developed HER2-targeted IONPs coated with block copolymer poly (PEO-b-PγMPS) and revealed a signal reduction in the tumor but with a low level of uptake by the reticuloendothelial system; PEO-b-PγMPS was found to be superior to most IONPs that require several weeks to be cleared out of the body from the liver and spleen[37]. Affibody-based SPIO NPs were generated for HER2-specific MR imaging. The agent showed good binding ability, and the tumors were clearly displayed on a gradient-echo sequence on MRI, thus confirming that the affibody-SPIO offers advantageous features that make it a suitable alternative for molecular imaging in comparison with antibody-based MR probes[38].

Optical imaging

Optical imaging is a non-invasive technique to detect and analyze signals originating from bioluminescent and fluorescent probes. It uses visible light and the special properties of photons to obtain detailed images of organs and tissues as well as cells and molecules. Optical imaging technologies rely on light-producing optical reporters, such as luciferase and fluorescent proteins, fluorescent dyes, and NPs. Quantum dots (QDs) are fluorescent nanocrystals (2–10 nm), and QD-based imaging can be used in cancer research to localize tumors and monitor possible changes in receptor expression during the treatment process. As an optional probe for determining HER2 expression levels, QD-based nanotechnology has attracted significant interest in the detection of HER2 breast cancer *in vivo* and *in vitro*. Studies have demonstrated that QD-based immunofluorescence technology can be used to quantitatively determine HER2 expression levels and serve as a potentially new method for HER2 detection in clinical practice[39,40]. A study synthesized QD-labeled anti-HER2 antibody and used it for both fixed and live cells. The result showed that the QD bioconjugates can rapidly localize HER2 positive cells, indicating its potential ability for targeted therapy and image-guided surgery[41]. A QD-based double-color imaging technique was reported to show the HER2 levels on breast cancer cells in the tumor matrix. This technique provided direct visible evidence that HER2 expression levels are directly related to cancer invasion[42]. QD-labeled anti-HER2 antibody was applied in the single molecular imaging of breast cancer cells with a high-resolution *in vivo* 3D microscopic system, and the result showed that cancer cells expressing HER2 can be visualized by the NPs *in vivo* at subcellular resolution[43]. In animal studies, HER2-xenografts can be visualized with the QD-linked anti-HER2 antibody[43,44]. A QD-conjugated antibody was also used to evaluate its delivery process to tumors, and valuable information on antibody-conjugated therapeutic NPs was obtained to help increase therapeutic efficacy[45].

Multimodal imaging

The development of probes has enhanced the possibility of multimodality imaging of HER2. Sampath et al.[17] first designed a dual-labeled HER2 imaging agent that conjugated trastuzumab with ^{111}In and a fluorescent dye to detect HER2 expression using SPECT and near-infrared (NIR) fluorescence imaging *in vivo* and *in vitro*. The outcome indicated the molecular specificity of targeting and the correlation

between nuclear and optical imaging results, thus confirming the stability of the dual-labeled agent. A few years later, on the basis of their previous work, the authors developed the agent of ^{64}Cu-DOTA-trastuzumab-IRDye800, which was dual-labeled for both PET and NIR fluorescence imaging, to detect metastases of HER2-positive breast cancer[14]. The detection capabilities of the dual-labeled agent and ^{18}F-FDG-PET were similar for primary tumors, but only the former was able to identify lung metastases . The *in vitro* NIR fluorescence imaging enabled the visualization of channels between the primary tumor and the axillary lymph nodes, thus providing the ability to track the lymphatic route for cancer cells. Qiao et al.[46] developed a novel class of multiple modality contrast agents for MRI and NIR imaging. They designed a Gd^{3+} binding site into a stable scaffold protein to increase the relaxivity value of both T1 and T2 so that the sensitivity of disease detection was increased, resulting in 100-fold lower dose usage than the clinically used non-targeting agent DTPA. Gao et al.[47] designed affibody-based nanoprobes using NIR QDs and IONPs to image HER2-expressing cells and tumors and found that nanoparticle-affibody conjugates may be excellent candidates as targeting probes for HER2 overexpressing tumor diagnosis. Similarly, antibody conjugated multilayered nanoprobes and NIR QDs were successfully developed and used for both NIR and MR imaging of HER2 cells and tumors[48]. Probes with different HER2-targeting and/or signaling components for HER2 imaging are listed in Table 1.

Table 1 Targeting probes for HER2-positive breast cancer imaging

Imaging modality	Year	Researchers	Probes	Source of signal	Agent category
PET	2006	Smith-Jones PM, et al.[49]	^{68}Ga-DOTA-HER2-F(ab')$_2$	^{68}Ga	Antibody fragments
	2009	Orlova A, et al. [19]	^{124}I/^{125}I-PIB-$Z_{HER2:342}$ ^{124}I/^{125}I-PIB-trastuzumab	^{124}I/^{125}I	Affibody Antibody
	2009	Ren G, et al.[25]	^{68}Ga-DOTA-MUT-DS	^{68}Ga	Affibody (2-helix)
	2011	Kramer-Marek G, et al.[50]	^{68}Ga-DOTA-$Z_{HER2:2891}$	^{68}Ga	Affibody
	2011	Miao Z, et al.[51]	^{18}F-FBO-MUT-DS	^{18}F	Affibody (2-helix)
	2012	Kramer-Marek G, et al. [23]	^{18}F-FBEM-$HER_{2:342}$	^{18}F	Affibody
	2012	Wållberg H, et al. [12]	^{68}Ga-$Z_{HER2:342}$-DOTA ^{11}C-$Z_{HER2:342}$-DOTA	^{68}Ga	Affibody
	2016	Honarvar H, et al. [8]	^{44}Sc-DOTA-$Z_{HER2:2891}$ ^{68}Ga-DOTA-$Z_{HER2:2891}$	^{44}Sc ^{68}Ga	Affibody
PET/CT	2012	Kramer-Marek G, et al. [22]	^{18}F- $Z_{HER2:342}$	^{18}F	Affibody
	2013	Xavier C, et al. [52]	^{68}Ga-NOTA-2Rs15	^{68}Ga	Nanobody
	2013	Strand J, et al.[53]	^{68}Ga-DOTA-$Z_{HER2:S1}$ ^{68}Ga-NODAGA-$Z_{HER2:S1}$ ^{68}Ga-NOTA-$Z_{HER2:S1}$	^{68}Ga	Affibody
SPECT	2004	Lub-de Hooge MN, et al. [13]	^{111}In-DTPA-trastuzumab	^{111}In	Antibody
	2005	Tang Y, et al. [31]	^{99m}Tc-trastuzumab-Fab ^{111}In-trastuzumab-Fab	^{99m}Tc ^{111}In	Antibody fragments
	2006	Orlova A, et al.[54]	^{125}I-$Z_{HER2:4}$/$Z_{(HER2:4)2}$/$Z_{HER2:342}$	^{125}I	Affibody
	2006	Orlova A, et al. [33]	^{99m}Tc-His6-$(Z_{HER2:4})_2$ ^{125}I-His6-$(Z_{HER2:4})_2$	^{99m}Tc ^{125}I	Affibody
	2006	Steffen AC, et al. [55]	^{125}I-$(Z_{HER2:4})_2$	^{125}I	Affibody
	2009	Chen KT, et al. [16]	^{188}Re (I)-trastuzumab	^{188}Re	Antibody

Continued

Continued

Imaging modality	Year	Researchers	Probes	Source of signal	Agent category
	2009	McLarty K, et al. [18]	^{111}In-DTPA-trastuzumab	^{111}In	Antibody
	2009	Ahlgren S, et al. [32]	^{99m}Tc-$Z_{HER2:2395}$	^{99m}Tc	Affibody
	2011	Wållberg H, et al. [56]	^{99m}Tc-$Z_{HER2:342}$	^{99m}Tc	Affibody
	2012	Rosik D, et al.[27]	^{111}In-PEP09239 ^{111}In-ABY-002	^{111}In	Affibody (2-helix) Affibody (3-helix)
MRI	2009	Chen T, et al.[15]	Herceptin-SPIO	SPIO	Antibody
	2010	Kinoshita M, et al. [38]	HER2-affibody-SPIO	SPIO	Affibody
SPECT/NIR	2007	Sampath L, et al.[17]	^{111}In-DTPA-trastuzumab-IRDye800*	^{111}In/IR-Dye800	Antibody
PET/NIR	2010	Sampath L, et al.[14]	^{64}Cu-DOTA-trastuzumab-IRDye800*	^{64}Cu/IR-Dye800	Antibody
MRI/NIR	2011	Gao J, et al. [47]	IO-affibody-QD800*	IO/QD800	Affibody
MRI/NIR	2011	Qiao J, et al. [46]	Gd^{3+}-affibody-Cy5.5*	Gd^{3+}/IR-Dye Cy5.5	Affibody
MRI/NIR	2012	Ma Q, et al. [48]	IO-antibody-QD780*	IO/QD780	Antibody

Note: * Dual-labeled probes; ABY-002: $Z_{HER2:342\text{-}pep2}$; DOTA: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA: diethylenetriaminepentaacetic acid; FBEM: (2-(4-^{18}F-fluorobenzamido) ethyl) maleimide; FDG: fluorodeoxyglucose; HER2: human epidermal growth factor receptor 2; IO: iron oxide (Fe_3O_4); MRI: magnetic resonance imaging; NIR: near-infrared; NODAGA:1-(1,3-carboxypropyl)-1,4,7- triazacyclononane-4,7-diacetic acid; NOTA: 1,4,7-triazacyclononane-N,N,N-triacetic acid; PET: positron emission tomography; PET/CT: positron emission tomography/ computed tomography; SPECT: single photon emission computed tomography; SPIO: supraparamagnetic iron oxide.

Clinical application of HER2-specific imaging

Thus far, specific HER2 imaging is not widely performed on patients. Most probes are antibody (trastuzumab)-based agents that are labeled with different radionuclides for PET or SPECT imaging. Perik and his colleagues[57] developed ^{111}In-DTPA-trastuzuma for preclinical study in 2004 and used it to predict the cardiotoxicity of trastuzumab and identify tumors in a clinical observation in 2006. Fifteen HER2-positive breast cancer patients with metastases were enrolled, and they underwent SPECT from 15 min to 7 days after administration of 150 MBq radio-labeled trastuzumab. ^{111}In-DTPA-trastuzumab was able to identify HER2-positive tumors, but it cannot predict trastuzumab-associated cardiotoxicity. Dijkers et al.[58] employed ^{89}Zr-trastuzumab as a tracer for PET imaging of patients with HER2-positive metastatic breast cancer and concluded that the best time for tumor visualization is 4–5 days after injection because of the long half-life of ^{89}Zr. A similar clinical study applied ^{89}Zr-trastuzumab to detect HER2-positive metastases and found no correlation between ^{89}Zr-trastuzumab uptake and HER2 expression level; however, almost half of the positive ^{89}Zr-trastuzumab foci on PET/CT were false positive[59]. Both ^{89}Zr- and ^{111}In- labeled trastuzumab were used to assess the clinical response of HER2 positive metastatic breast cancer to the therapy of a heat shock protein 90 inhibitor or trastuzumab, respectively[60-62]. HER2 imaging can also predict the response to trastuzumab if combined with the traditional ^{18}F-FDG PET and if responders can be differentiated from non-responders[62]. Probes radio labeled with ^{64}Cu have also been applied in clinical practice in some studies. PET or PET/CT with the probe of ^{64}Cu-DOTA-trastuzumab has been performed on patients with primary and/or metastatic HER2-positive breast cancer[63,64]. Both primary breast tumors and metastases in the brain, liver, bone, and lymph nodes were identified with generally high image quality and tumor-to-tissue contrast. Furthermore, CT-positive lesions were detected by ^{64}Cu-DOTA-trastuzumab but not by ^{18}F-FDG in some lesions, and false positivity of ^{64}Cu-DOTA-trastuzumab occurred in only one instance. According to the results, tumor uptake and the radiation exposure of ^{64}Cu-DOTA-trastuzumab were both comparable to those of ^{18}F-FDG, thus demonstrating the feasibility of ^{64}Cu-DOTA-trastuzumab as a potentially safe probe to detect the primary and metastatic lesions of HER2-positive breast cancer for clinical use[63]. Another study, which involved eight patients of HER2-positive metastatic breast cancer, preinjected 45 mg trastuzumab before the introduction of the ^{64}Cu-trastuzumab, and an approximately 75% reduction in liver

uptake of ^{64}Cu was found but without significant changes in tumor uptake. This result provided a new strategy for a favorable biodistribution of ^{64}Cu and visualization of liver metastases[64].

^{111}In- or ^{68}Ga-labeled affibody (ABY-002) molecules were first used in a clinical study involving only three patients with recurrent metastatic breast cancer. The blood kinetics analysis showed rapid blood clearance of the radio-labeled agents in humans with the first half-life of 4–11 min for the ^{111}In-labeled affibody and 10–14 min for the ^{68}Ga-labeled affibody. The images of SPECT or PET/CT were compared with those of ^{18}F-FDG PET/CT, and most of the lesions identified by ^{18}F-FDG PET/CT were also visualized with the ^{111}In- or ^{68}Ga-labeled affibody, except for one lesion of liver metastasis and another lesion close to the kidney. Both ^{18}F-FDG and ^{68}Ga-labeled affibody can improve the diagnosis of metastasis in comparison with CT. They concluded that the affibody is more adaptable for clinical practice with high detection rate compared with antibodies or fragments, although further clinical studies are still needed[65]. Considering the high liver uptake of radio-labeled affibodies, Sörensen et al.[66] developed a second-generation affibody with improved biochemical and biophysical characteristics and used it in seven patients of recurrent metastatic HER2-positive or HER2-negative breast cancer. The ^{111}In-labeled affibody can discriminate the metastases with high or low HER2 expression. The uptake of HER2-positive metastases increased between 4 and 24 h after injection, whereas it decreased in the negative metastases in the same time duration. Detailed comparisons of sensitivity and/or specificity of radio-labeled trastuzumab *vs.* ^{111}In-labeled affibody were not permitted due to limited clinical studies. Most recently, ^{68}Ga-HER2-nanobody was used for the visualization of both primary and metastatic HER2 positive lesions, and it showed favorable biodistribution and zero toxicity[67].

These clinical findings related to HER2 imaging confirm that HER2-targeted imaging can be applied to disease detection, staging, and therapy response monitoring through the determination of the HER2 expression of breast tumors. Probes and trials for clinical HER2-imaging are listed in **Table 2**.

Table 2 Clinical applications of probes for HER2-positive breast cancer imaging

Agent category	Year	Researchers	Imaging probes	Imaging approaches	Source of signal	Subjects
Antibody-based	2006	Perik, et al. [57]	^{111}In-DTPA-trastuzumab	SPECT	^{111}In	Patients with HER2-positive metastatic breast cancer
	2009	Dijkers, et al.[58]	^{89}Zr-trastuzumab	PET	^{89}Zr	Patients with HER2-positive metastatic breast cancer
	2013	Tamura, et al. [63]	^{64}Cu-DOTA-trastuzumab	PET/CT	^{64}Cu	Patients with primary or metastatic HER2-positive breast cancer
	2014	Mortimer, et al. [64]	^{64}Cu-DOTA-trastuzumab	PET/CT	^{64}Cu	Patients with HER2-positive metastatic breast cancer
	2014	Gaykema, et al. [60]	^{89}Zr-trastuzumab	PET/CT	^{89}Zr	Patients with HER2-positive metastatic breast cancer
	2014	Gaykema, et al.[61]	^{111}In-trastuzumab	SPECT	^{111}In	Patients with HER2-positive metastatic breast cancer
	2016	Ulaner, et al. [59]	^{89}Zr-trastuzumab	PET/CT	^{89}Zr	Patients with HER2-negative primary breast cancer
Affibody-based	2010	Baum, et al. [65]	^{111}In-ABY-002 ^{68}Ga-ABY-002	SPECT PET/CT	^{111}In ^{68}Ga	Patients with recurrent metastatic HER2-positive breast cancer
	2014	Sörensen, et al. [66]	^{111}In-ABY-025	SPECT/CT	^{111}In	Patients with metastatic HER2-positive and -negative breast cancer
Nanobody-based	2016	Keyaerter, et al. [67]	^{68}Ga-HER2-nanobody	PET/CT	^{68}Ga	Patients with HER2-positive breast cancer

Note: ABY-002: $Z_{HER2:342-pep2}$; DOTA: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA: diethylenetriaminepentaacetic acid; HER2: human epidermal growth factor receptor 2; PET: positron emission tomography; PET/CT: positron emission tomography/computed tomography; SPECT: single photon emission computed tomography.

Conclusions

HER2 plays an important role in cancer progression and prognosis of breast cancer. Increasing attention has been paid to the imaging of HER2 -positive breast cancer. This technology can help to identify patients who would benefit from trastuzumab therapy and monitor the response and efficacy of treatment. In addition, it can detect the discordance of HER2 status between primary breast cancer and distant metastases. Most HER2-targeting probes are still in experimental and preclinical status. Among these techniques, nuclear medicine imaging seems to be the most popular applied imaging modality for HER2 -positive tumors, although technical complexity in probe synthesis and the stability, safety, and toxicity of the probes themselves may limit the clinical applications of HER2 specific probes.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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