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Targeting HER2 in Nuclear Medicine for Imaging and Therapy

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Abstract

Since its discovery, the human epidermal growth factor 2 (HER2) has been extensively studied. Presently, there are 2 standard diagnostic techniques to assess HER2 status in biopsies: immunohistochemistry and fluorescence in situ hybridization. While these techniques have played an important role in the treatment of patients with HER2-positive cancer, they both require invasive biopsies for analysis. Moreover, the expression of HER2 is heterogeneous in breast cancer and can change over the course of the disease. Thus, the degree of HER2 expression in the small sample size of biopsied tumors at the time of analysis may not represent the overall status of HER2 expression in the whole tumor and in between tumor foci in the metastatic setting as the disease progresses. Unlike biopsy, molecular imaging using probes against HER2 allows for a noninvasive, whole-body assessment of HER2 status in real time. This technique could potentially select patients who may benefit from HER2-directed therapy and offer alternative treatments to those who may not benefit. Several antibodies and small molecules against HER2 have been labeled with different radioisotopes for nuclear imaging and/or therapy. This review presents the most recent advances in HER2 targeting in nuclear medicine focusing on preclinical and clinical studies.

Keywords

breast cancer, HER2-positive cancer, HER2 imaging, human epidermal growth factor 2, molecular imaging, PET/CT, SPECT/CT, receptor radionuclide therapy

Introduction

The human epidermal growth factor 2 (HER2) has been extensively studied since its discovery in 1987 by Dr Slamon and colleagues,[1] mainly as its overexpression in tumors has been associated with more aggressive tumor types. Human epidermal growth factor 2 is also known as erbB-2 and Neu; it is a member of the ErbBs or type I receptor tyrosine kinase family, which also includes the epidermal growth factor receptor 1 (EGFR or HER1), erbB-3 (HER3), and erbB-4 (HER4).[2,3] The HER family includes transmembrane proteins that activate intracellular signaling pathways in response to extracellular signals. Their structure consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. These proteins are expressed in a variety of tissues of epithelial, mesenchymal, and neuronal origin, where they are involved in cell development, proliferation, and differentiation.[2,4,5] Some cancer cells exhibit amplification of the HER2 gene, which often leads to the overexpression of the HER2 protein on the cell surface. This overexpression occurs in several cancers, including bladder, lung, gastric, ovarian, prostate, and breast cancer (BCa).[6] Tumor cells that overexpress HER2 frequently have a high rate of proliferation and are associated with more aggressive disease, poor prognosis, and shorter overall survival.[7,8] The development of HER2-targeted treatments using monoclonal antibodies (mAbs) has significantly improved patient survival, particularly in up to 20% of patients with BCa.[9,10] Patients with the triple negative subtype of BCa, whose tumors do not express estrogen receptor, progesterone receptor, and HER2,[11] cannot take advantage of HER2-directed therapies. Molecular imaging using anti-HER2 agents cannot diagnose the triple negative subtypes; however, it can exclude those who will not

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benefit from anti-HER2 therapy such that patients can have alternative treatments. As new targeted agents are currently being developed for the HER2-negative subtypes of BCa, molecular imaging approaches of emerging biomarkers have the potential to predict response to these investigational treatments. The lessons we learn from developing imaging agents for HER2 can be applied to future imaging agents for the HER2-negative subtypes of BCa.

Trastuzumab (Herceptin; Genentech, South San Francisco, CA) was the first humanized mAb against HER2 to be approved by the Food and Drug Administration (FDA) for HER2-positive BCa. Trastuzumab binds to domain IV of the extracellular domain of the HER2 epitope which ultimately suppresses cancer cell proliferation, growth, and survival through the following mechanism of actions: (1) downregulating total levels of HER2; (2) blocking cleavage of the extracellular domain of HER2; (3) inhibiting HER2 homodimerization, which inhibits the PI3K intracellular signaling pathway; (4) reducing angiogenesis; and finally (5) inducing antibody-dependent cellular cytotoxicity or lysing the antibody-bound cells via recruitment of immune cells.[12,13]

Despite the success of trastuzumab, HER2-targeted therapy remains a challenge. A significant number of patients are primarily resistant to this drug (intrinsic resistance), and prolonged treatment often ends with the majority of patients who initially had a clinical benefit becoming resistant (acquired resistance). The mechanisms of intrinsic resistance to trastuzumab are most often associated with an inactive target, whereas acquired resistance mostly occurs due to modifications in the target signaling level or the loss of HER2 expression over the course of the disease.[10] Another important limitation of trastuzumab is that some patients can have cardiotoxicity, as HER2 is expressed in the heart.[14] Studies in tumor-bearing mice cannot determine cardiotoxicity because trastuzumab is not cross-reactive with murine HER2.

In order to overcome this resistance in advanced disease, other drugs can be combined with trastuzumab. Pertuzumab, trastuzumab emtansine (T-DM1), and lapatinib are approved for inhibiting HER2 activity in the treatment of HER2-positive metastatic BCa. Pertuzumab (Perjeta; Genentech, South San Francisco, CA) is a humanized mAb that binds to domain II of the extracellular domain of the HER2 epitope and functions by inhibiting HER2 dimerization with other growth factor receptors, particularly the HER2-HER3 dimerization.[15] Preclinical experiments showed that the combination of trastuzumab and pertuzumab enhanced the antitumor effect compared to trastuzumab or pertuzumab alone due to complementary mechanisms of action that promote tumor regression more effectively.[15] This combination has also been tested in patients with metastatic BCa in a recent study with the CLEOPATRA trial.[16] The CLEOPATRA trial showed that patients who received trastuzumab, pertuzumab, and docetaxel (or paclitaxel) had a median overall survival of 56.5 months compared to 15 months for those who received trastuzumab alone. Since this trial, the combination of trastuzumab, pertuzumab, and docetaxel is used as first-line therapy for metastatic HER2-positive BCa.[16]

Another approach to overcoming resistance is the use of antibody drug conjugates, whereby antigen binding on the cell surface mediates the internalization of the antibody and subsequent delivery of the toxic payload to increase the selectivity and potency of this drug. The FDA has approved T-DM1 as second-line therapy for patients with HER2-positive metastatic BCa who previously received trastuzumab. DM1 (a derivative of maytansine) is a potent inhibitor of microtubule polymerization and is linked to trastuzumab to form the T-DM1 antibody–drug conjugate. T-DM1 has been well tolerated by patients. The only grade $\geq$3 adverse events are reversible thrombocytopenia (decrease in platelet count) and elevations in hepatic transaminase, which are present in $\geq$5% of patients.[17] Currently, there are new drugs under evaluation in clinical trials which might give new options to the treatment of advanced HER2-positive BCa such as HER2 vaccines, new antibodies (ertumaxomab and margetuximab), and defucosylated trastuzumab.[3]

The availability of several drugs targeting HER2 on the extracellular and/or intracellular domain provides several options for treatment, which places nuclear medicine in a unique position to help guide decisions for treatment. Targeting HER2 for imaging and/or therapy in nuclear medicine has generated a wide interest, resulting in many recent studies with a focus on developing new radiopharmaceuticals. This review highlights recent advances in HER2-targeted imaging and therapy from both preclinical and clinical oncological studies published since 2015.

Radiopharmaceuticals Targeting HER2 for Diagnostic Use

It is evident that an accurate characterization of HER2 expression is the key for the success of HER2-targeted therapy. At present, there are 2 types of tests to determine the HER2 status in BCa: (1) immunohistochemistry (IHC), which detects the HER2 protein and (2) fluorescence in situ hybridization (FISH), which detects the copy number of the HER2 gene.[18] However, it has been reported that up to 20% of results using these methods may be inaccurate.[18] Both methods are performed on biopsied tissues. Mathenge et al demonstrated that the use of core needle biopsy before surgical excision of BCa tumors can significantly increase lung metastasis in mice.[19] Therefore, biopsy may unintentionally promote metastasis by dissemination of cancer cells from the primary lesion to distant organs.[19] Many protocols encourage the use of repeated biopsies during the course of treatment, since HER2 expression can change over the course of the disease.

In addition, intratumoral heterogeneity and small sample size of biopsied tumors may not represent the status of HER2 expression in the whole tumor or between tumor foci in the metastatic setting.[6,20] Thus, a more accurate method for assessment of the HER2 status is needed.

In this scenario, molecular imaging using specific radiopharmaceuticals to target HER2 exhibits an immense advantage. This new technique may provide a complementary and

noninvasive option to identify patients who may be responsive and those who may not respond to HER2-targeted therapy. Since molecular imaging is a noninvasive procedure, it may have an advantage over biopsy-based approaches and has the potential to help guide physicians to tailor the treatment for each patient. Furthermore, it may be possible to monitor the response to the HER2-targeted therapy and identify those patients who become resistant.

Anti-HER2 probes have the ability to bind to the HER2 protein regardless of its gene amplification. Imaging HER2 using these agents is an advantage over FISH, as gene amplification does not always lead to the overexpression of the HER2 protein.[21,22] Also, some somatic mutations can lead to a negative result in gene amplification, while the tumor cells continue to express the HER2 protein.[21]

Several studies have been published using radiolabeled intact mAbs, antibody fragments (for instance, Fab), nanobodies, and affibodies for the development of new imaging agents for HER2. Low-molecular-weight constructs in these studies have been developed to reduce the blood residency time to allow for imaging at earlier time points (hours) than that achieved by intact mAb (days). Tables 1 and 2 summarize the results of the main studies performed in preclinical and clinical stages.

Intact Antibodies

Due to the long circulation time of antibodies in the blood, the choice of suitable radionuclides based on their physical half-lives ($t_{1/2}$) is crucial. Zirconium-89 (^{89}Zr) provides good positron emission tomography (PET) spatial resolution, as it decays via positron emission with a low average energy ($E\beta^+$, average = 396 keV) and has a half-life of 3.27 days that matches the long biological half-life of antibodies.[46,47]

Frequently, the radiolabeling of intact antibodies with ^{89}Zr is performed through the modification of a native lysine side chain with desferrioxamine-B (DFO).[47,48] Nevertheless, it has been suggested that this chelator is not considered ideal for satisfying the coordination sphere of the Zr^{4+} cation, which can lead to the release of the radiometal from its chelator in rodent studies, leading to an accumulation in the bone.[49] In rodents, it has been shown that ^{89}Zr-oxalate and ^{89}Zr-chloride show uptake in the bone (15%-20% injected dose per gram [ID/g]).[49] However, most clinical studies showed that the dose absorbed by the bone/red bone marrow is almost negligible for ^{89}Zr-mAb. For instance, Laforest et al[24] showed that patients with BCa who were administered with 65 $\pm$ 18 MBq of ^{89}Zr-trastuzumab had only 0.69 mGy/MBq in the red bone marrow. With the goal of creating more stable chelators for ^{89}Zr, some groups have dedicated efforts to create new chelators as alternatives to DFO; however, none of these new chelators have yet to show significant improvement in their in vivo stability over DFO when conjugated with an antibody.[50,51]

The most widely used agents for imaging of HER2 are based on trastuzumab. In a preclinical study, Dijkers et al[51] compared ^{89}Zr-trastuzumab with ^{111}In-trastuzumab, a radiopharmaceutical developed for antibody single-photon emission computed tomography (SPECT), and showed similar immunoreactivity and tumor uptake in xenograft models of BCa. Dijkers et al then conducted the first-in-human ^{89}Zr-trastuzumab PET imaging clinical trial in patients with metastatic BCa.[40] In this trial, ^{89}Zr-trastuzumab showed excellent tumor uptake and visualization of HER2-positive metastatic lesions. These lesions were generally in agreement with available data from computed tomography (CT) and magnetic resonance imaging scans. It is well known that mAbs such as trastuzumab cannot cross an intact blood–brain barrier due to their large size, presenting a challenge in drug delivery for brain metastasis. However, Dijkers et al observed uptake in brain metastasis, likely due to the disruption of the blood–brain barrier at the site of the brain metastasis. During this study, no infusion-related reactions or adverse events were observed and the total radiation dose estimated was comparable to 2 abdominal CT scans (18 mSv).[40]

Similarly, a phase 0 study involving 12 patients with HER2-positive BCa confirmed that ^{89}Zr-trastuzumab is safe and does not induce adverse effects. However, as ^{89}Zr emits high-energy (909 keV) gamma rays associated with a high branching ratio (99.0%) coupled with the 511 keV gamma ray (22% branching ratio) for PET, dosimetry is an important concern.[46] Laforest et al showed that ^{89}Zr-trastuzumab had high residence times in several organs, mainly in the liver, kidney, and heart muscle. The liver was determined to be the critical organ, with a dose of 1.63 mSv/MBq (6.02 rad/mCi) due to the highest uptake of ^{89}Zr-trastuzumab in this organ, as opposed to dose received from neighboring organs.[24] An example of an image from this study is shown in Figure 1.

^{89}Zr-trastuzumab was also used to identify patients with HER2-positive metastatic BCa who were originally diagnosed with HER2-negative primary BCa.[38] Ulaner et al evaluated 9 patients who received 185 MBq of ^{89}Zr-trastuzumab. In this study, PET-CT visualized tumor foci, which were then biopsied and analyzed with IHC to confirm HER2 positivity. Five (56%) patients presented uptake of ^{89}Zr-trastuzumab in metastatic foci, but only 2 were considered HER2-positive after the biopsy.[38] Although this study involved a small sample population, the authors concluded that ^{89}Zr-trastuzumab may help identify patients who will benefit from targeted therapy. In addition, they suggested that a larger sample population would be beneficial for future studies.[38]

Further, ^{89}Zr-trastuzumab was studied in patients with advanced HER2-positive BCa to predict which patients are likely or unlikely to benefit from the use of T-DM1.[43] The ZEPHIR study was a well-designed multicenter trial that enrolled patients from Belgium and the Netherlands who were eligible to receive T-DM1 for HER2-positive advanced disease.[43] Gebhart et al evaluated 56 patients who received T-DM1 (3.6 mg/kg, every 3 weeks) using ^{8}F-Fluodeoxyglucose(^{18}F-FDG) and ^{89}Zr-trastuzumab PET imaging. This study reported that 16 patients, who were previously diagnosed with HER2-positive metastasis by biopsy, did not present ^{89}Zr-trastuzumab uptake in their lesions. The HER2-positive lesions were identified in 39 patients, and among them, 28 showed responses to the T-DM1 treatment.[43]

Table 1. Preclinical Studies Performed With Probes Against HER2 Receptor.

Probe, Dose, and Modality	Main Findings	Study
^{64}Cu-NOTA-pertuzumab, F(ab′)2; 1-3 MBq; PET	High accumulation in the kidneys; predicted total body dose in humans was 0.015 mSv/MBq	Lam et al[23]
^{89}Zr-HOPO-trastuzumab; 0.5 MBq; PET	Good tumor uptake despite lower purity and stability	Tinianow et al (2016)[24]
^{64}Cu-NOTA-Fab-PEG$_{24}$-EGF; 15-25 MBq; PET	High accumulation in the liver and kidneys 48 hours PI; clear visualization of tumor xenografts expressing one or both receptors (PI HER2 and EGF)	Kwon et al[25]
^{177}Lu-trastuzumab-AuNP; 3 MBq, intratumorally injection; therapy	In xenograft BCa tumors, the DNA damage caused by the gold nanoparticles modified with trastuzumab was at least 2.8-fold higher than the nanoparticle without trastuzumab	Cai et al[26]
^{111}In-trastuzumab-AuNP; 10 MBq, intratumorally injection; therapy	^{111}In-trastuzumab-AuNP inhibited tumor growth in mice with SC HER2-positive BC xenografts; no toxicity was found in normal tissues	Cai et al[27]
^{177}Lu-DOTA-Fab-PEG$_{24}$-EGF and ^{111}In-DOTA-Fab-PEG$_{24}$-EGF; 11.1 MBq, intraperitoneal injection; therapy	^{177}Lu-DOTA-Fab-PEG$_{24}$-EGF demonstrated stronger tumor growth inhibition than ^{111}In-DOTA-Fab-PEG$_{24}$-EGF even in tumors that are trastuzumab resistant	Razumienko et al[28]
^{99m}Tc-HYNIC-H6F; 37 MBq; SPECT	Peptide demonstrated excellent HER2 binding specificity both in vitro and in vivo. Uptake in tumor was not blocked by coinjection of excess of trastuzumab	Li et al[29]
^{99m}Tc-CGGG-LTVSPWY and ^{99m}Tc-CSSS-LTVSPWY; 7.4 MBq; SPECT	Both peptides showed specific binding; the CSSS ligand showed more favorable uptake in the tumor	Sabahnoo et al[30]
^{99m}Tc-trastuzumab-PCSN; 5.9 MBq; SPECT	Good uptake in tumor; poor radiochemical yield	Yamaguchi et al[20]
^{64}Cu-NOTA-pertuzumab; 5-10 MBq; PET	Clear tumor visualization including tumors orthotropic in the peritoneal cavity	Jiang et al[31]
^{177}Lu-pertuzumab; 5-7 MBq; therapy	Specific and high tumor uptake; elevated absorbed dose in tumors contributing to the inhibition of tumor progression	Persson et al[32]
^{89}Zr-pertuzumab; 3.7 MBq; PET	Optimal image timing: 7 days PI; tumor uptake was increased in presence of unlabeled trastuzumab	Marquez et al[33]
^{90}Y-CHX-A″-DTPA-trastuzumab and ^{90}Y-octapa-trastuzumab; 3.7 MBq; therapy	Both chelators provided high radiochemical yields; high tumor uptake after 72 hours PI; significant decrease in tumor growth compared to controls after 36 days of therapy	Price et al[34]
^{131}I-trastuzumab; 0.7-0.55 MBq; biodistribution evaluation	Good affinity for HER2-positive cells; immunoreactivity not compromised; significant tumor uptake after 24 hours PI; high uptake in the liver, lungs, and spleen	Kameswaran et al[35]
^{111}In-trastuzumab-NLS-S and -L; 0.37 MBq; therapy; cytotoxicity	^{111}In-trastuzumab-NLS showed higher cytotoxicity compared to ^{111}In-trastuzumab and cytotoxicity was enhanced in the presence of bortezomid	Li et al[36]
^{188}Re-HYNIC-trastuzumab; 0.037-0.74 MBq; cell therapy	^{188}Re-HYNIC-trastuzumab enhanced the cytotoxicity to nearly 100-fold than trastuzumab alone; ^{188}Re-HYNIC-trastuzumab prolongs the effects of apoptosis	Luo et al[37]

Abbreviations: EGF, epidermal growth factor; HER2, human epidermal growth factor 2; PET, positron emission tomography; PI, postinjection; SC, subcutaneous; SPECT, single-photon emission computed tomography.

As mentioned previously, pertuzumab is currently being used in combination with trastuzumab.[16] In nuclear medicine, this humanized mAb has been labeled with a variety of radiometals (Indium-111 [^{111}In], Lutetium-177 [^{177}Lu], and Copper-64 [^{64}Cu])[31,32,52-54] and has been proposed to be used as the imaging agent during monotherapy with trastuzumab[33] because it binds to a different epitope (domain II of HER2) from that of trastuzumab (domain IV). Molecular biology studies have shown that the association of trastuzumab and pertuzumab promotes enhanced antitumor effect and increases binding affinity of both antibodies.[15,33] A recent PET study has also complemented these studies showing that the tumor uptake of ^{89}Zr-pertuzumab was increased in the presence of trastuzumab in BCa xenografts.[33] Therefore, using ^{89}Zr-pertuzumab as the imaging agent while treating with trastuzumab or T-DM1 may allow a more sensitive detection of HER2 without competing for binding.

In addition to BCa, HER2 is also overexpressed in other types of cancer, and thus, the advantages of molecular imaging can be extended to assess the HER2 status in other tumor types. Ovarian cancer (OVCa) has a lower incidence than BCa, with an estimated 22 440 new cases in 2017 in the United States. However, it is estimated that 62.74% of these new cases will result in death, making the mortality rate for this cancer higher than any other cancer of the female reproductive system.[55,56] This mortality is attributed to late diagnosis when the disease has already advanced to late stages with metastasis in the peritoneal cavity.[31,56]

In animal models of OVCa, ^{64}Cu-pertuzumab was used as an agent for imaging of HER2 and it was able to detect small

Table 2. Clinical Studies Performed With Probes Against HER2 Receptor.

Probe, Dose, and Modality	Patient Population	Main Findings	Study
^{89}Zr-trastuzumab; 185 MBq; PET	Metastatic HER2-negative primary BCa (n = 9)	Five patients presented uptake in metastasis focus, indicating that HER2-negative primary BCa can generate HER2-positive metastases	Ulaner et al[38]
^{68}Ga-HER2-nanobody; 53-174 MBq; PET	Early and metastatic breast carcinoma (n = 20)	Highest organ dose, respectively: urinary bladder wall, kidneys, liver, intestines; optimal image timing: 90 minutes PI; uptake in tumor lesions in 19 patients; clear tracer accumulation in metastatic lesions	Keyaerts et al[39]
^{89}Zr-trastuzumab; 43.3-88.8 MBq; PET	Patients with BCa with at least 1 lesion determined by another imaging method (n = 12)	Optimal image timing: 5 days PI; liver was the dose-limiting organ; no adverse or clinically detectable pharmacological effects; uptake in at least 1 known lesion in 10 patients	Laforest et al[40]
^{68}Ga-ABY-025; 215 MBq; PET	Metastatic BCa (n = 8)	Highest absorbed organ doses in the kidneys and liver, respectively; high dose of peptide gives low effective dose (5.6 mSv) than low dose (6.0 mSv); however, dose is much higher compared to ^{68}Ga-DOTATATE and ^{68}Ga-DOTATOC	Sandstrom et al[41]
^{68}Ga-ABY-025; 212 MBq; PET	Metastatic BCa (n = 16)	Optimal image timing: 4 hours PI; PET imaging was accurate in identifying HER2-positive metastases, and PET SUV correlated with biopsy HER2-scores; noncompetitive binding with trastuzumab and pertuzumab	Sörensen et al[42]
^{89}Zr-trastuzumab (37 MBq) + ^{18}F-FDG; PET	Advanced BCa (n = 56)	The association of molecular imaging and metabolic imaging helped to identify lesions that do not respond to T-DM1 therapy and demonstrated that advanced HER2 BCa is highly heterogeneous disease	Gebhart et al[43]
^{111}In-ABY-025; 142.6 MBq; SPECT	Recurrent metastatic breast cancer (n = 7)	High uptake in the kidneys, liver, and spleen; effective dose of 0.15 mSv/MBq; no drug-related adverse events; high-contrast HER2 images within 4 to 24 hours; visualization of metastases in the liver and brain	Sörensen et al[44]
^{177}Lu-trastuzumab; 140.6-925 MBq; SPECT/ Therapy	Early and advanced BCa (n = 10)	Optimal image timing: 5 or 7 days PI; uptake in primary and metastatic BCa lesion; accumulation in heart, liver, spleen, and nasopharynx; no leukopenia or liver toxicity was observed	Abbas et al[45]

Abbreviations: BCa, breast cancer; EGF, epidermal growth factor; HER2, human epidermal growth factor 2; PET, positron emission tomography; PI, postinjection; SC, subcutaneous; SPECT, single-photon emission computed tomography.

peritoneal tumors in an orthotopic tumor model.[31] Additionally, ^{89}Zr-trastuzumab provided specific and high uptake in an HER2-positive model of OVCa in mice.[50] These agents warrant potential investigation in patients with OVCa.

As shown, mAbs are being widely used in molecular imaging because they are specific for their targets and the established chemistry permits a convenient and fast production of these radiopharmaceuticals in high yields for preclinical and clinical studies. Nonetheless, their high molecular weight and consequently slow clearance make the best time for imaging at 4 to 7 days after injection due to the optimal tumor to background ratios achieved at these later time points. Thus, several strategies have been developed to design low-molecular-weight probes to accelerate rates of clearance and allow for imaging at earlier time points.

Antibodies Fragments and Other Small Molecules

With the goal to improve upon antibody pharmacokinetics without compromising affinity and specificity, several molecules have been bioengineered, including Fab and F(ab')2 fragments (2 antigen-binding Fab portions linked together via disulfide bonds), affibodies, nanobodies, and minibodies.[57] These relatively smaller scaffolds typically have shorter residence times in the bloodstream. Consequently, the imaging acquisition can be performed much sooner when compared to intact antibodies (hours versus days). Certainly, this approach represents greater convenience to the patient, as imaging can be performed within hours after being injected with the radiotracer. For example, nanobodies are considered the smallest naturally derived antigen-binding fragment (12-15 kDa), which consist of the variable domain of the heavy-chain portion of the immunoglobulin G.[57] Recently, Keyaerts et al[39] reported the safety, biodistribution, dosimetry, and tumor-targeting potential of a ^{68}Ga-anti-HER2-nanobody in patients with breast carcinoma. Twenty patients were divided into 3 groups, and each group received a different dose of ^{68}Ga-anti-HER2-nanobody (0.01-1 mg; 53-174 MBq). The biological half-life was estimated to be 1 hour, with the renal system being the main route for the elimination of the

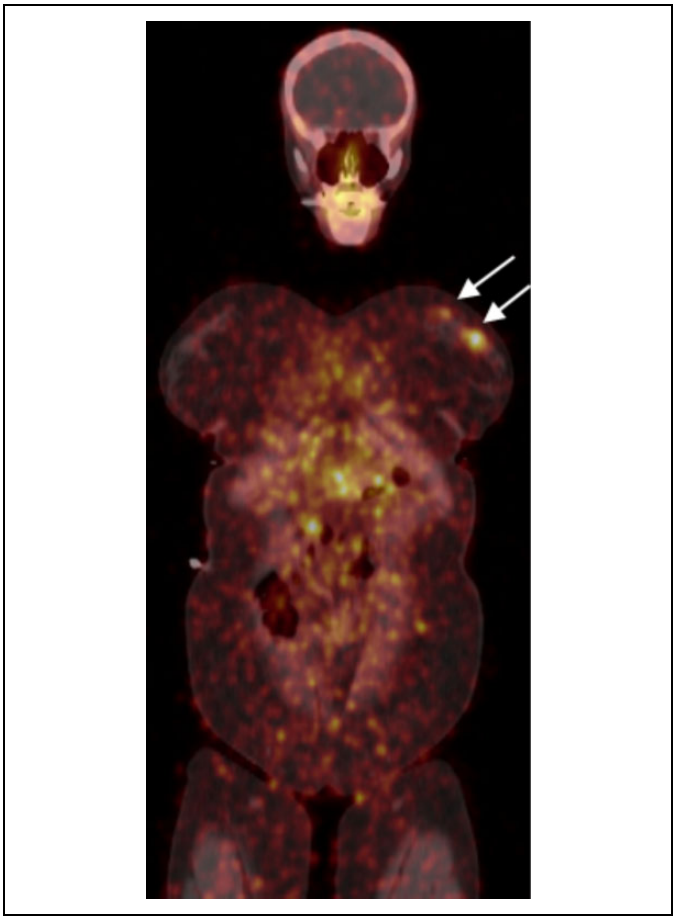

Figure 1. ^{89}Zr-trastuzumab imaging at 5 days postinjection in a patient with ER+/PR−/human epidermal growth factor 2 (HER2)+ multicentric primary breast cancer in the neoadjuvant setting.

radiopharmaceutical. The organs that received the highest radiation doses were the urinary bladder wall (0.406 mGy/MBq), kidney (0.216 mGy/MBq), liver (0.0778 mGy/MBq), lower large intestine wall (0.0759 mGy/MBq), and upper large intestine wall (0.0619 mGy/MBq), respectively. The authors concluded that the use of ^{68}Ga-anti-HER2-Nanobody is safe, up to 174 MBq, with no adverse effects.[39]

As mentioned previously, the mechanisms of resistance to trastuzumab are not well understood; however, Gallardo et al observed that trastuzumab resistance mechanisms are related to overexpression of EGFR and Insulin-like growth factor 1 receptor (IGF1R) in patients with HER2-positive carcinomas.[58] Bispecific radioimmunoconjugates (bsRICs) that are capable of binding to HER2 and EGFR were developed for both therapy and diagnostic applications. These bsRICs are composed of the trastuzumab Fab (ligand for HER2) linked to EGF (ligand for EGFR) via a long spacer (polyethylene glycol [PEG_{24}]).[28,25] This long spacer was chosen to increase the blood residence time of the molecule (^{64}Cu-NOTA-Fab-PEG_{24}-EGF; NOTA: 1,4,7-triazacyclononane-triacetic acid), since small proteins such as Fabs can have rapid clearance that can lead to poor tumor uptake. ^{64}Cu-NOTA-Fab-PEG_{24}-EGF exhibited preserved specific binding to both EGFR and HER2 *in vitro* and high tumor uptake (28.9% ±7.37% ID/g at 48 hours postinjection [PI]) in mice bearing subcutaneous (SC) SKOV-3 ($EGFR^{low}$/$HER2^{high}$) tumors.[28,25]

The pertuzumab Fab segment was also labeled with ^{64}Cu in order to create a radiopharmaceutical that is able to detect changes in HER2 expression associated with response to trastuzumab treatment in BT-474 xenografted mice.[23] Lam et al reported that ^{64}Cu-NOTA-pertuzumab F(ab')2 was able to detect a decrease in HER2 expression at 1 week after trastuzumab therapy. In addition, PET/CT images showed low uptake in normal organs except for the kidneys which presented the greatest uptake (52.4-65.6 %ID/g). The absorbed dose estimated for a human female adult was 1 mSv/MBq. The estimated whole-body equivalent dose was 0.015 mSv/MBq, which is 3.3 times less than the same probe labeled with ^{111}In.[23]

Affibodies are medium-sized peptides (6.5 kDa) derived from a nonimmunoglobulin α-helix-based scaffold with similar or higher affinity than some mAb.[59,42] ABY-025 is a second-generation affibody molecule with reduced nonspecific liver uptake that binds to domain III of the extracellular portion of HER2. Since trastuzumab and pertuzumab bind to domains IV and II, respectively, ABY-025 would promote a noncompetitive interaction, which may provide an important advantage for using this imaging agent in the presence of therapeutic concentrations of trastuzumab and/or pertuzumab.[41,42,60] ABY-025 has been successfully labeled with ^{68}Ga for PET[41,42] and ^{111}In for SPECT.[44] Both modalities were able to discriminate HER2-positive from HER2-negative tumors in patients with metastatic BCa. The effective dose for a typical 200 MBq administration of ^{68}Ga-ABY-025 was found to be between 5.6 and 6.0 mSv. The authors reported that this dose was lower than the effective dose for 200 MBq of ^{111}In-ABY-025 (21 mSv) and an ^{18}F-FDG PET scan, which usually delivers an effective dose of about 7 mSv.[44]

Another peptide that does not compete with trastuzumab for binding to HER2 is ^{99m}Tc-HYNIC-H6F.[29,30] Li et al developed this novel SPECT imaging agent for similar reasons as the affibody probe—for noncompetitive binding with trastuzumab. In a preclinical model, ^{99m}Tc-HYNIC-H6F yielded rapid accumulation and relatively high uptake (2.47 ± 0.12 %ID/g at 30 minutes PI) in SC HER2-positive tumors (MDA-MB-453 cells) and low uptake (0.99 ± 0.19 %ID/g at 30 minutes PI) in HER2-negative tumors (MDA-MB-231 cells). As H6F binds to a different domain of HER2 (domain II), excess of trastuzumab did not block the binding of ^{99m}Tc-HYNIC-H6F nor did it inhibit its tumor accumulation.[29]

Another recent work evaluated a heptapeptide (LTVSPWY) conjugated with 2 different chelators (CGGG and CSSS) and labeled with ^{99m}Tc. In this study, Sabahnoo et al found that both peptides have high affinity (dissociation constant [KD] of 4.3 ± 0.8 nmol/L and 33.9 ± 9.7 nmol/L for ^{99m}Tc-CGGG-LTVSPWY and ^{99m}Tc-CSSS-LTVSPWY, respectively) to the extracellular domain II of HER2. The reduction in ^{99m}Tc-heptapeptide binding to SKOV-3 cells in the presence of trastuzumab showed that both peptides compete with trastuzumab. The higher uptake of ^{99m}Tc-CGGG-LTVSPWY in blood

suggested that ^{99m}Tc was transchelated to plasma proteins. Both peptides showed similar tumor uptake (2.44 ± 1.12 %ID/g and 2.26 ± 1.28 %ID/g at 4 hours PI for ^{99m}Tc-CGGG-LTVSPWY and ^{99m}Tc-CSSS-LTVSPWY, respectively); however, the faster clearance of ^{99m}Tc-CSSS-LTVSPWY provided a higher tumor to normal organ ratio and better visualization of the tumor at 4 hours PI.[30]

The main advantage presented in using smaller scaffolds is the potential fast clearance with suitable affinity and specificity. Additionally, these molecules can pair well with shorter lived radioisotopes such as F-18 ($t_{1/2}$ = 1.8 hours), thus reducing the dose to patients. In general, this fast clearance is also responsible for lower tumor uptake of the radiopharmaceuticals and an increased dose to the renal system.

In addition to smaller scaffolds, nanoparticles have been used as targeting vectors for HER2. Yamaguchi et al[20] developed silica nanoparticles for multimodal imaging. They grafted hyperbranched polyamidoamine onto the surface of synthetic amorphous silica nanoparticles functionalized with indocyanine green (a fluorescence agent) and radiolabeled with ^{99m}Tc. Subsequently, anti-HER2 antibodies were conjugated to this nanoparticle to enable targeting of HER2-expressing cells. Although further studies will be necessary to increase *in vivo* stability, the authors conclude that these silica nanoparticles are promising for diagnostic and therapy applications. They also have the potential to be used as a drug delivery carrier for antitumor agents or β-emitting radioisotopes.[20]

Targeting of HER2 for Radionuclide Therapy

The term theranostic is defined as the integration of a diagnostic test with individualized therapy in the management of disease. Thus, a theranostic agent (or pair of imaging/therapeutic agents) can be used to identify those individuals who would benefit from a specific treatment, treat them, and monitor their responses to the treatment.[61,62] Human epidermal growth factor 2 is an important target for theranostic use because the same or similar radiopharmaceutical can be used for either therapy or diagnosis, depending on the radionuclide used to label the molecule.

Therapy using radiopharmaceuticals is known as receptor radionuclide therapy or targeted radionuclide therapy, and the possibilities of developing new agents are promising. Antibodies used for targeted radionuclide therapy can be labeled with alpha, beta, and auger electron-emitting radionuclides. Similar to the diagnostic field of research, trastuzumab has been the focus for the development of HER2-targeted radiotherapeutics agents in the last decade. This antibody has been labeled with ^{177}Lu, ^{64}Cu, ^{111}In,Thorium-227 (^{227}Th), Rhenium-188 (^{188}Re), Yttrium-90 (^{90}Y), and Iodine-131 (^{131}I).[34-37,45,63-66]

Iodine-131 is a β-emitter that is widely used in therapy owing to its cost-effective accessibility, long half-life ($t_{1/2}$ = 8.1 days), and ease of radiolabeling and imaging capabilities due to its gamma emission.[35] Radio-iodination can be damaging to mAbs; however, Kameswaran et al showed that ^{131}I-trastuzumab preserved its immunoreactivity, affinity, and specific binding to HER2-positive cells (BT-474 and MDA-MB-453).[35]

Indium-111 is a radioisotope that can be used for imaging (SPECT) and therapy because of its auger electron emission. An auger electron is a low-energy electron with a very short path length and its cytotoxic effect only occurs when its nucleus decays in close proximity to the cell's DNA. The high linear energy transfer of auger electrons enables In-111 to inflict lethal DNA damage.[36,27]

The use of a nuclear localizing signal (NLS) peptide has been proposed to achieve the efficient delivery of auger emitters into the tumor cell nucleus. Trastuzumab modified with NLS was labeled with ^{111}In and its cytotoxicity was evaluated in SKBR3, MCF7 and HMEpC (human mammary epithelial cell) cells. Li et al found that ^{111}In-trastuzumab-NLS was more cytotoxic than ^{111}In-trastuzumab and its cytotoxicity was higher in the HER2-overexpressing SKBR3 cells than in the low-level HER2-expressing MCF7 and HMEpC cells. After a transcriptome analysis, Li et al determined that the DNA damage by auger electrons activated the nuclear factor κB pathway.[36]

Lutetium-177 is a radiolanthanide that has been demonstrated to be applicable for theranostic applications. It is a low-energy β-emitter (0.497 MeV_{max}) with tissue penetration up to 1.6 mm.[65] Lutetium-177 is considered to be a viable alternative to ^{131}I due to its lower energy γ-emission. Lutetium-177 has gamma energies of 208 keV (11% abundant) and 113 keV (6.4% abundant), while ^{131}I has gamma energy of 364 keV (81% abundant). Thus, ^{177}Lu has a considerable advantage in lower patient dosimetry and postpatient care.[67]

The same bsRICs labeled with ^{64}Cu by Kwon et al were labeled with ^{177}Lu and ^{111}In, and the tumor growth inhibitory properties of ^{177}Lu-DOTA-Fab-PEG_{24}-EGF (DOTA: tetraazacyclododecane-1,4,7,10-tetraacetic acid) and ^{111}In-DTPA-Fab-PEG_{24}-EGF (DTPA: diethylenetriaminepentaacetic acid) were compared in mice implanted SC with trastuzumab-sensitive (HER2-positive MDA-MB-231/H2N) and acquired trastuzumab-resistant cells (HER2-positive TrR1—subclone of MDA-MB-231/H2N cells).[25] The radiation-absorbed dose in the tumor for ^{177}Lu-DOTA-Fab-PEG_{24}-EGF was 9.3-fold higher, and thus, it was 1.3-fold more effective in inhibiting tumor growth than the ^{111}In-labeled construct. The resistant tumor also responded to treatment with the ^{177}Lu-labeled construct. The normal tissue toxicity studies for ^{177}Lu-DOTA-Fab-PEG_{24}-EGF showed that the ID of 3.7 and 11.1 MBq had no toxicity. However, when mice received a dose of 18.5 MBq, leukocyte and erythrocyte counts, hemoglobin, and hematocrit were significantly reduced compared to mice that received saline. Kwon et al determined that 11.1 MBq of ^{177}Lu-DOTA-Fab-PEG_{24}-EGF was the dose of the no observable adverse effect level.[25]

Abbas et al performed a preliminary clinical study with ^{177}Lu-trastuzumab involving 10 patients in order to study the localization of the ^{177}Lu-trastuzumab in primary and/or metastatic lesions and in the nontarget organs.[45] ^{177}Lu-trastuzumab

accumulated in the HER2-positive lesions, but no uptake was observed in the HER2-negative sites (as determined by IHC). Uptake in normal organs was observed in the heart, liver, spleen, and nasopharynx. In this study, the authors did not evaluate the radiation dose and toxicity to the white and red blood cells.[45]

Pertuzumab has also been labeled with ^{177}Lu using the chelate isothiocyanate-benzyl-CHX-A″-DTPA. In a preclinical study using SKOV-3 OVCa xenografted Balb/c (nu/nu) mice, Persson et al found that ^{177}Lu-DTPA-pertuzumab delivered a high dose (50.86 $\pm$ 5.57 Gy—activity injected of 7.3 MBq) to the tumor. The low bone uptake (0.07 $\pm$ 0.01 %ID/g at 14 days PI) suggested that this radiopharmaceutical was stable in vivo. The study group treated with ^{177}Lu-pertuzumab showed a clear delay in tumor growth.[52]

Yttrium-90 is a pure β-emitting radioisotope with a path length of 5 mm and a high-energy β particle (2.296 MeV).[68] This high energy also allows for the delivery of dose to adjacent tumor tissue that could be inaccessible because of tumor bulk, poor vascular supply, and/or tumor heterogeneity.[68,69] Due to significant radiation dose that free ^{90}Y can deliver to the bone, the development of chelators that improve upon the *in vivo* stability has been investigated. Price et al studied 2 acyclic chelators (H_4octapa and CHX-A″-DTPA). This research demonstrated that both chelators were radiolabeled with fast kinetics without considerable heat (15 minutes at room temperature produced radiochemical yield >95%). Price et al determined that ^{90}Y-CHX-A-DTPA-trastuzumab and ^{90}Y-octapa-trastuzumab exhibited excellent *in vitro* and *in vivo* stability with high immunoreactivity. Also, both radiopharmaceuticals significantly inhibited tumor growth (tumor with 300 mm^3 of volume after 36 days PI) compared to the control group (tumor with 1000 mm^3 of volume after 36 days PI).[34]

Rhenium-188 is an attractive radionuclide for use in targeted radiotherapy due to its high-energy β particles (maximum energy = 2.11 MeV) and 155 keV gamma photons. Rhenium-188 has another advantage: It is conveniently produced from a transportable, alumina-based ^{188}W/^{188}Re generator, similar to a ^{99}Mo/^{99m}Tc generator.[37,70] Despite its attractive decay properties for targeted radiotherapy, ^{188}Re has rarely been used for the development of targeted therapeutics for HER2-expressing tumors. In a recent study, Luo et al showed that ^{188}Re-HYNIC-trastuzumab enhanced the cytotoxicity to tumor tissues to a level nearly 100-fold higher than that of the treatment with trastuzumab alone.[37] Additionally, Altai et al conducted studies using an affibody ($Z_{HER2:\ V2}$). Rhenium-188-$Z_{HER2:\ V2}$ showed high affinity (6.4 $\pm$ 0.4 pico molar [pM].) and uptake in SKOV-3 xenografted tumors (14% $\pm$ 2 %ID/g at 1 hour PI) and rapid blood clearance. The dosimetry extrapolated to humans suggests that ^{188}Re-$Z_{HER2:\ V2}$ may provide an absorbed dose to tumors of more than 70 Gy.[71] Future investigations in the clinical setting are needed to expand on this work.

An interesting approach combining targeted radiotherapy with brachytherapy is to inject radioisotopes, particularly β-emitters, directly in the tumor. For this purpose, gold nanoparticles (AuNP) were modified with trastuzumab and labeled with ^{177}Lu[26] and ^{111}In.[27] In previous work, these trastuzumab-AuNP were injected intravenously, resulting in moderate tumor uptake and high spleen uptake.[72] Cai et al modified their strategy to inject these agents intratumorally. Both molecules showed significantly higher binding to the HER2-positive cells than the nontargeted nanoparticles (AuNP-^{177}Lu and AuNP-^{111}In). In addition, both were able to inhibit tumor growth in mice without apparent toxicity to the other tissues.[27,26]

In nuclear medicine, about 90% of the procedures performed worldwide are for diagnosis. Research in the development of new radiopharmaceuticals is also abundant for this application. Consequently, the vast majority of clinical trials being conducted are for diagnosis. However, efforts to develop new probes for therapy employing radiopharmaceuticals are currently expanding. These new investigations for therapy are producing promising results in preclinical studies and warrant investigation in clinical trials in the future.

Conclusions and Perspectives

Human epidermal growth factor 2 is an important target in oncology. The methods currently available to determine HER2 status are invasive and can show discrepancies due to tumor heterogeneity among other reasons. These inconsistent results may lead to suboptimal selection of patients for HER2-targeted therapy. In addition, not all lesions are readily accessible to be biopsied. In this scenario, molecular imaging offers an advantage because it is potentially a less invasive solution for the diagnosis of HER2-positive tumors. Therefore, radiopharmaceuticals targeting HER2 can help to identify patients who may benefit from HER2-targeted therapy and to monitor the change in HER2 status during therapy. Equally as important is the identification of patients who may not respond to HER2-targeted therapy so that alternative treatment options can be initiated early.

The high overexpression of HER2 in cancer cells relative to normal cells also makes HER2 a candidate for targeted radionuclide therapy, especially in cases where tumors are HER2 positive but resistant to some HER2-directed therapies. Improvements in radioisotope production have made radionuclides more available to more investigators, which contribute to the increase in biomedical research in this area. Human epidermal growth factor 2 is a relevant target for both diagnostic and therapeutic (theranostic) applications, and the development of molecules designed for HER2-directed therapy has been heavily explored in the last few years. With regard to the selection of probes for the clinical setting, Gebhart et al[6] made an excellent point—the choice of the molecule will be guided by the desired application. While whole antibodies might be preferred for therapy approaches due to their longer residence time and consequently higher lethal radiation dose delivered to the tumor, smaller constructs such as nanobodies, peptides, and affibodies may be used as a diagnostic alternative to IHC or FISH. However, these probes may not necessarily reflect the

delivery of antibody-based therapeutics; in this case, the antibody may be the best choice to be used as a diagnostic agent as well.

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