



Molecular probes targeting HER2 PET/CT and their application in advanced breast cancer

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Abstract

Purpose Human epidermal growth factor receptor 2 (HER2)-positive breast cancer cases are among the most aggressive breast tumor subtypes. Accurately assessing HER2 expression status is vital to determining whether patients will benefit from targeted anti-HER2 treatment. HER2-targeted positron emission tomography (PET/CT) is noninvasive, enabling the real-time evaluation of breast cancer patient HER2 status with accuracy.

Methods We summarize the research progress of PET/CT targeting HER2 in breast cancer, focusing on PET/CT molecular probes targeting HER2 and their clinical application in the management of advanced breast cancer.

Results At present, a variety of different HER2 targeted molecular probes for PET/CT imaging have been developed, including nucleolin-labeled antibodies, antibody fragments, nanobodies, and peptides of various affinities, among others. HER2-targeted PET/CT can relatively accurately evaluate HER2 expression status in advanced breast cancer patients. It has good performance in the early detection of small HER2-positive lesions, evaluation of HER2 status in lesions that cannot be readily biopsied, evaluation of the heterogeneity of multiple metastases, identification of lesions with altered HER2 status, and evaluation of the efficacy of anti-HER2 drugs.

Conclusion HER2-targeted PET/CT offers a promising noninvasive approach for real-time assessment of HER2 status, which can be guide targeted treatment for HER2-positive breast cancer patients. Future prospective clinical studies will be invaluable for fully evaluating the importance of HER2-targeted molecular imaging in the management of breast cancer.

Keywords HER2 · PET/CT · Advanced breast cancer · Molecular probes · Clinical applications

Significance of HER2-targeted PET/CT in breast cancer

Breast cancer is among the most prevalent malignancies in the world and the fifth most common cause of cancer-associated mortality among women, with roughly 2.3 million

diagnoses and 685,000 deaths throughout the world in 2020 alone (Sung et al. 2021). Mortality rates associated with recurrent metastatic breast cancer are particularly high, and advanced breast cancer patients exhibit a 5-year survival rate of just 20% (Gonzalez-Angulo et al. 2007). The administration of appropriately targeted drugs selected based on the molecular characteristics of metastatic tumors can

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effectively prolong the survival of these patients, underscoring the need to establish approaches to reliably determine the characteristics of target tumors.

Optimal antitumor drugs are those exhibiting a high degree of anticancer activity while causing minimal normal tissue toxicity. One of the most effective approaches to achieving such efficacy is by targeting particular proteins on the surface of actively proliferating malignant cells. In clinical practice, the most common targets of drugs aimed at the treatment of breast cancer include estrogen/progesterone receptor (ER/PR) and human epidermal growth factor receptor 2. Primary HER2-targeted treatments include monoclonal antibodies (mAbs), such as trastuzumab and pertuzumab, small molecule tyrosine kinase inhibitors including lapatinib and pyrotinib, and antibody–drug conjugates including TDM-1 and DS-8201. Anti-HER2-targeted treatment has been repeatedly linked to improvements in disease-free and overall survival in advanced breast cancer patients with HER2-positive disease. These targeted therapies are, however, limited by their higher cost and potential for side effects that can include cardiotoxicity. It is thus vital that the HER2 status of tumors be reliably determined in order to identify patients who are likely to benefit from targeted interventions (Koleva-Kolarova et al. 2017).

At present, HER2 status is primarily evaluated through the evaluation of pathological sections via immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). HER2 positivity is diagnosed when IHC (+++) or (++) and FISH assays show HER2 gene amplification. The ability to obtain a sufficient pathological specimen is thus a prerequisite for an accurate diagnosis. In many cases, however, adequate pathological samples cannot be collected as the target mass is too deep, too small, or located in proximity to large vasculature and nerves. In these patients, targeted treatment recommendations can only be made based on the HER2 status of the primary tumor. This is a problem given the potential for inconsistencies in the receptor status of primary and metastatic tumors, as evidenced by the fact that roughly 20% of metastases in patients with HER2-negative primary tumors are HER2-positive (Priedigkeit et al. 2017). Approximately 16.6% of patients with HER2-positive primary tumors may exhibit HER2-negative metastases (Sari et al. 2011). Altered receptor expression can contribute to improper or off-target treatment. In patients with multiple metastatic lesions, substantial heterogeneity can exist among these metastases whereas treatment-related decisions are generally made based on biopsy results from a single lesion, contributing to the potential for off-target treatment decisions.

PET/CT imaging is an advanced, repeatable, noninvasive approach that can enable the simultaneous systemic detection of multiple target lesions. HER2-targeted PET/CT

scans provide an opportunity to specifically examine HER2 expression in multiple lesions in breast cancer patients in real time such that therapeutic planning can be performed in an individualized manner. This approach can serve as both a supplemental means of evaluating patients for whom traditional pathological analyses were conducted, as well as a primary approach to providing treatment recommendations for patients without any accessible metastatic specimens. There has been a growing interest in recent years focused on HER2-targeted PET/CT imaging in the context of the clinical management of breast cancer.

PET/CT molecular probes targeting HER2

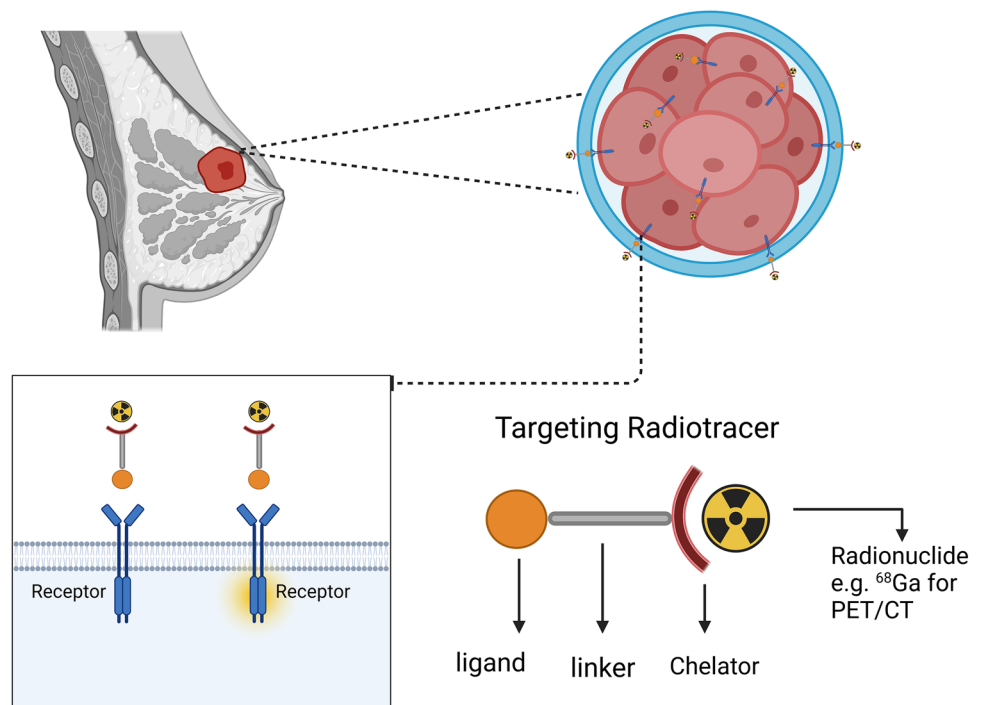
Several different HER2-targeting molecular probes for PET/CT imaging have been developed to date, including nucleolin-labeled antibodies, antibody fragments, nanobodies, and peptides of various affinities, among others (see Fig. 1).

Monoclonal antibody-based molecular probes

To date, both trastuzumab and pertuzumab are monoclonal antibodies that have been subjected to radionuclide labeling. Trastuzumab recognizes a HER2 epitope located on extracellular region IV of this protein and can suppress tumor cell growth through several mechanisms including the overall downregulation of HER2 expression, the disruption of the cleavage of this HER2 extracellular region. In addition, this therapeutic mAb can inhibit the heterodimerization of HER2 and HER3, thereby disrupting intracellular PI3K signaling. Trastuzumab can also suppress angiogenesis, and trastuzumab-coated HER2-positive tumor cells can undergo antibody-dependent cellular cytotoxicity-mediated lysis upon immune cell recognition (Nahta 2012). Pertuzumab binds to a HER2 epitope located in extracellular region II of this protein and functions by inhibiting the ability of HER2 to dimerize with other growth factor receptors, exhibiting a particularly potent suppressive effect on HER2–HER3 heterodimerization (Scheuer et al. 2009).

Whole antibodies, which exhibit a molecular weight of approximately 150 kDa, often only achieve a sufficient tumor-to-blood ratio after multiple days such that they exhibit relatively limited tumor penetration and gradual clearance. As a result, short-lived radionuclides are poorly suited to antibody-mediated PET imaging. However, the relatively long half-lives of ^{89}Zr and ^{64}Cu make them better suited for antibody labeling and use in PET/CT imaging.

Fig. 1 Schematic overview of targeted receptor imaging. Ligands capable of binding to specific targets that are overexpressed by breast cancer cells can be coupled via a linker domain to a chelator labeled with ^{68}Ga or other radionuclides, providing an effective probe for PET/CT imaging



^{89}Zr -Trastuzumab

Owing to its prolonged 78.4 h half-life (Dijkers et al. 2010), relatively low positron energy (average: 0.396 MeV), and short positron-free travel distance, ^{89}Zr can provide a high degree of spatial resolution when used for PET/CT imaging (Laforest et al. 2016). In an effort to thoroughly evaluate the most optimal dosing and timing of ^{89}Zr -trastuzumab administration, Dijkers et al. (2010) evaluated 14 HER2-positive patients with metastatic breast cancer via ^{89}Zr -trastuzumab PET/CT imaging. In patients that had not undergone prior trastuzumab treatment, they found that the pre-injection of a 50 mg trastuzumab dose was necessary to achieve effective imaging, whereas only a 10 mg dose was necessary for patients with a history of prior trastuzumab treatment. Ulaner et al. (2016, 2017) experimentally determined that ^{89}Zr -trastuzumab administration was associated with false-positive metastatic foci, however, highlighting an important limitation of this molecular probe.

^{89}Zr -Pertuzumab

Ulaner et al. (2018) employed ^{89}Zr -pertuzumab to detect HER2-positive metastatic breast cancer. In addition to evaluating the dosing, pharmacokinetics, and biodistribution of this labeled antibody, the authors demonstrated that this drug was not associated with any apparent toxicity. The highest dose of radiation was delivered to the liver, kidneys, and heart wall. They also found that the probe was able to facilitate tumor imaging at an optimal imaging timepoint

of 5–8 days post-administration, allowing for the detection of pathologically confirmed brain metastases not detectable through other approaches, thus highlighting the value of ^{89}Zr -pertuzumab.

Studies employing both trastuzumab and pertuzumab can achieve superior antitumor activity owing to the enhanced affinity of these antibodies and their complementary effects, respectively inhibiting HER2 dimerization and p95HER2 formation (Scheuer et al. 2009). In mice bearing breast tumor xenografts, ^{89}Zr -pertuzumab uptake was enhanced in the presence of trastuzumab (Marquez et al. 2014). Efforts to detect HER2 status using ^{89}Zr -pertuzumab may be more effective in patients undergoing trastuzumab or T-DM1 (trastuzumab emtansine) treatment.

^{64}Cu -Trastuzumab

^{64}Cu offers a short 12.8 h half-life such that it exposes patients to a lower dose of radiation as compared to ^{89}Zr -trastuzumab (Mortimer et al. 2014). Mortimer et al. (2014) demonstrated the ability of ^{64}Cu -DOTA-trastuzumab to detect HER2-positive lesions. Trastuzumab pretreatment (45 mg) was sufficient to reduce hepatic uptake by ~75% without any impact on tumoral uptake. In vivo, this probe yielded a radiation uptake dose similar to that of ^{18}F -FDG. While these results are promising, much as with ^{89}Zr -trastuzumab, ^{64}Cu -DOTA-trastuzumab can be taken up by HER2-negative tumors, and owing to the 46 h half-life of ^{64}Cu -DOTA-trastuzumab in the blood such that imaging must be performed within 48 h post-administration,

contributing to suboptimal imaging contrast (Carrasquillo et al. 2019).

Fab and F(ab)₂ antibody fragment-based probes

As mAbs exhibit a high molecular weight, they are cleared relatively slowly from the blood such that visualization is best performed 4–8 days following administration (Laforest et al. 2016). These antibodies can also be nonspecifically taken up by tumors as a result of the enhanced permeability and retention effect (EPR), limiting their clinical utility (Mendler et al. 2015). These mAbs also expose health organs to high levels of radiation and can reportedly yield false-positive findings in certain patients (Ulaner et al. 2017). To overcome this issue, proteases can be used to digest whole mAbs to isolate the Fab domains. The papain-mediated digestion of an IgG molecule yields a single Fc domain fragment and two Fab domain fragments, whereas pepsin-mediated IgG digestion yields two Fab domains linked by a hinge domain [F(ab)₂] and one Fc domain fragment. Owing to their lower molecular weight, Fab and F(ab)₂ fragments can be rapidly cleared at earlier time points such that superior tumor contrast can be achieved, while also eliminating the Fc domain such that these imaging probes have no impact on anti-Fc-mediated antibody detection.

⁶⁴Cu-BFC-Fab-Trastuzumab

Moreau et al. (2017) employed ⁶⁴Cu-BFC-Fab-trastuzumab for molecular imaging and biodistribution analyses of mice bearing breast tumor xenografts, achieving a tumor uptake at 24 h after injection ranging from 8.9 to 12.8% ID/g. Using this probe, the most effective visualization was achieved at 4 and 24 h after injection.

⁸⁹Zr-Df-Fab-PAS200 and ¹²⁴I-Fab-PAS200

Mendler et al. recently demonstrated the ability of a 200-amino-acid chain composed of Pro, Ala, and Ser residues (PAS200) to effectively bind to Fab antibody fragments while modulating their pharmacokinetic properties so as to enhance that imaging utility by overcoming limitations associated with rapid Fab clearance (Mendler et al. 2015). Using the ⁸⁹Zr•Df-Fab-PAS200 and ¹²⁴I-Fab-PAS200 probes, these authors were able to detect the tumor-specific uptake of these molecules at 24 h post-injection. Of these two probes, ¹²⁴I was associated with greater thyroid uptake while greater renal ⁸⁹Zr uptake was observed, with ⁸⁹Zr•Df-Fab-PAS200 exhibiting superior stability and tumor uptake (Mendler et al. 2015).

⁶⁸Ga-NOTA-F(ab')₂-Trastuzumab and ⁶⁴Cu-NOTA-Pertuzumab F(ab')₂

Rathore et al. (2022) performed PET/CT imaging with a ⁶⁸Ga-labeled probe prepared from the Fab domains of trastuzumab that exhibited a molecular weight of just 46.3 kDa, roughly one-third that of the full antibody. Immunohistochemistry was used to successfully confirm the identification of breast and lymphatic lesions detected with this ⁶⁸Ga-NOTA-F(ab')₂-trastuzumab probe. Suman et al. (2023) also demonstrated that ⁶⁸Ga-NOTA-F(ab')₂-trastuzumab was capable of identifying tumor cells overexpressing HER2. Lam et al. (2017) similarly evaluated changes in the expression of HER2 over the course of trastuzumab treatment using a ⁶⁴Cu-NOTA-pertuzumab F(ab')₂ probe, thereby delivering a lower dose of whole-body radiation.

Imaging probes using engineered antibody fragments and their derivatives

Single-chain Fv (scFv)

Noncovalent single-chain Fv (scFv) dimers can be generated by introducing a short (5 amino acid) linker between the variable light (V_L) and variable heavy (V_H) chain domains of an antibody while preventing the homodimeric pairing of the V_H and V_L chains. The resultant scFv molecules are capable of simultaneously binding to two target antigen molecules (Robinson et al. 2008). Ueda et al. (2015) demonstrated a high degree of ⁶⁸Ga-Df-anti-HER2 scFv accumulation in mice bearing HER2-positive tumor xenografts such that changes in HER2 status over the course of anti-HER2 treatment could be monitored in a noninvasive fashion.

Single-domain antibody fragments (sdAbs) / 2Rs15d / nanobody

HER2-targeted nanobodies have been designed for use as molecular imaging probes. Nanosomes are comprised of antigen-binding antibody heavy chain fragments, and are the smallest antigen-binding antibody fragments (12–15 kDa) that exhibit optimal PET/CT imaging properties (Chakravarty et al. 2014).

Small molecule (12–15 kDa) single-domain antibody fragments can serve as ideal tumor contrast agents and are particularly well suited to use with ¹⁸F. As they are relatively small, however, they are more susceptible than whole mAbs to undesirable changes upon chemical modification that may result in normal tissue retention or altered binding to target proteins (Zhou et al. 2021). In an effort to overcome

this limitation, Zhou et al. (2021) employed a thiol-maleimide reaction-based approach to the site-specific ligation of 5F7GGC to a tetrazine-bearing agent. Their resultant ^{18}F -5F7GGC single-domain antibody fragment construct was still able to bind HER2 with high affinity and strong immunoreactivity while also undergoing more rapid renal clearance.

In a phase I study focused on ^{68}Ga -NOTA-2Rs15d that was conducted by Keyaerts et al. (2016), researchers demonstrated that the radiation dose associated with this probe was comparable to that for other PET/CT tracers in routine use. A high degree of imaging contrast was achieved in both primary and metastatic breast cancer. However, additional phase II trials will be necessary to further evaluate the preferential accumulation of this novel tracer in HER2-positive metastases relative to proximal normal tissues. While it exhibited reduced in vitro and in vivo tumor cell retention, the ability of [^{18}F]RL-I-2Rs15d to being a HER2 epitope distinct from that recognized by the HER2-targeted therapeutic antibodies trastuzumab and pertuzumab makes it ideally suited for the PET/CT-based assessment of the HER2 status of patients who have undergone treatment with either of these mAbs (Zhou et al. 2017).

Mice bearing HER2-positive xenograft tumors have been shown to exhibit strong uptake of the [^{18}F]FB-anti-HER2 nanobody, allowing for the high-contrast imaging of tumors overexpressing HER2. This tracer can also be combined with trastuzumab such that it is ideally positioned for use in patients undergoing treatment with this mAb (Xavier et al. 2016).

Aptamer-based imaging probes

As short 20–90 base pair single-stranded oligonucleotides, aptamers exhibit strong and selective binding activity such that they can bind to targets of interest via the systematic evolution of ligands by exponential enrichment (Stoltenburg et al. 2007). When using an ^{18}F -labeled HER2-targeting aptamer to conduct PET/CT imaging, Kim et al. (2019) demonstrated the ability of this molecule to facilitate cell surface HER2 recognition and to enable the preferential binding of HER2-positive breast cancer cells in vitro and in xenograft-bearing mice. In a similar vein, Gijs et al. (2016) were able to utilize ^{68}Ga -labeled NOTA oligonucleotides to conduct molecular imaging, leveraging their advantages over antibodies or traditional proteins including their reduced immunogenicity, smaller size, and less substantial toxicity. These oligonucleotides can be prepared in an inexpensive manner via chemical synthesis and can be readily subjected to chemical modification to enhance their bioavailability, stability, and pharmacokinetic properties (Syed and Pervaiz 2010).

Engineered scaffold protein-based imaging probes

While antibodies naturally exhibit a high degree of affinity for a wide range of molecular targets, novel affinity ligands can also be developed by leveraging a wider repertoire of natural binding proteins. These novel binding proteins are characterized by a robust scaffold skeleton, ensuring that variable amino acids of interest are stably positioned while minimizing the entropy penalty. Large combinatorial libraries generated via the randomization of unstable amino acids provide an opportunity to select for proteins with specific high-affinity binding activity for targets of interest. These engineered scaffold proteins have been used to develop a range of imaging probes including Affibody molecules, Albumin-binding domain (ABD)-Derived Affinity ProTeins (ADAPT), and Designed ankyrin repeat proteins (Tolmachev and Orlova 2020).

Affibody molecules

Affibody molecules are engineered scaffold proteins that are just 58 amino acids in length and that exhibit a high level of affinity for various target proteins. Radiotracer-labeled Affibodies have demonstrated excellent utility in preclinical research when used to sensitively and precisely image target tumors on the day of injection (Tolmachev and Orlova 2020).

$Z_{\text{HER2}:342}$ and derivatives thereof are second-generation HER2-targeting antibodies with a wide range of uses. The chimeric Affibody-peptide nucleic acid chimera $Z_{\text{HER2}:342}\text{-SR-HP1}$ was developed by Honarvar et al. (2016) in an effort to overcome the high levels of renal absorption and consequent reductions in tumor contrast evident for unmodified Affibodies, successfully enhancing radiotracer accumulation within tumors. Building on these promising results, the $Z_{\text{HER2}:342}$ derivative $\text{DOTA}^0\text{-}Z_{\text{HER2}:342}\text{-pep2}$ (ABY-002) was designed containing a DOTA chelator conjugated to an N-terminal valine residue to facilitate site-specific radiometal labeling while binding to HER2 with a K_D of 65 pmol/L (Orlova et al. 2007). Baum et al. (2010) evaluated three patients that underwent PET/CT imaging using ^{111}In - or ^{68}Ga -labeled ABY-002 with upfront ^{18}F -FDG-PET/CT, revealing that ^{111}In - or ^{68}Ga -labeled ABY-002 was sufficient to enable the localization of otherwise undetectable metastases and the assessment of their HER2 status.

The recombinant Affibody, ABY-025, recognizes a HER2 epitope located in the extracellular domain III region distinct from that recognized by other HER2-targeting therapeutic agents. ABY-025 also exhibits an excellent tumor/background ratio, enabling the detection of tumor metastases (Sorensen et al. 2014).

Trousil et al. (2014) designed [^{18}F]GE-226 ($Z_{\text{HER2}:2891}$) as a next-generation Affibody-based radiotracer, amenable to

large-scale synthesis while exhibiting superior pharmacokinetic characteristics. [¹⁸F]GE-226 can function as an imaging probe to differentiate among lesions with varying levels of HER2 expression in metastatic breast cancer patients. However, the efficacy of this probe remains uncertain as imaging results have yet to be published.

ADAPT molecules

ADAPT molecules employ a novel scaffolding probe based on the stabilization provided by the Streptococcal Protein G ABD domain. These ADAPT probes can enable the high-contrast imaging of HER2-positive lesions in breast patients (Garousi et al. 2015). In mice bearing human tumor xenografts exhibiting varying HER2 expression levels, DOTA-C⁵⁹-DEAVDANS-ADAPT6-GSSC and DOTA-C⁶¹-(HE)₃DANS-ADAPT6-GSSC were both reportedly able to differentiate between tumors expressing low and high levels of HER2 (Lindbo et al. 2018). ⁶⁸Ga-(HE)₃DANS-ADAPT6-GSSC-NODAGA was also able to serve as an imaging probe with excellent contrast when used for the PER/CT-based evaluation of HER2 status (von Witting et al. 2019).

Designed ankyrin repeat proteins (DARPin)

DARPin represent a novel binding molecule class capable of recognizing HER2 or other target proteins of interest with a high degree of selectivity (Zahnd et al. 2007). These proteins contain tightly packed 33-amino acid residues repeats that comprise structural units composed of a β-turn and two antiparallel α-helices, with up to 29 consecutive repeats per DARPin (Walker et al. 2000). These ankyrin repeat domains most often contain 4–6 repeating units, yielding proteins with a right-handed solenoid structure, a continuous hydrophobic core, and a large surface domain accessible by solvents. Researchers have established a DARPin library of molecules with fixed domains important for the structural framework of these proteins and six variable positions per repeat corresponding to nonconserved, surface-exposed residues with the potential to interact with targets (Pluckthun 2015).

DARPin exhibit several attractive properties, as in the case of ⁸⁹ZrDFO-G3-DARPin, which is taken up and retained by tumors while rapidly being cleared from normal tissues, thus yielding PET/CT images with high tumor-to-background contrast (Fay et al. 2022).

Small molecule peptides

Peptides offer multiple attractive properties that make them well-suited to use as molecular probes for PET/CT imaging. For one, unlabeled small-molecule precursors exhibit well-defined chemical structures, a lack of immunogenicity,

regulated pharmacokinetic properties, amenability to a range of modifications, and ease of synthesis that most often occurs via solid-phase peptide synthesis. Following radionuclide labeling, peptides exhibit greater tissue permeability and more rapid blood circulation (Fani et al. 2012; Fosgerau and Hoffmann 2015). Radionuclide-labeled peptides can thus reduce the imaging time necessary to evaluate HER2-positive tumors while accurately informing HER2 expression-based treatment efforts.

⁶⁸Ga-DOTA-(Ser)₃-LTVSPWY

⁶⁸Ga-DOTA-(Ser)₃-LTVSPWY can specifically accumulate within HER2-positive tumors, enabling the PET/CT-based identification of tumors overexpressing this receptor (Bianani et al. 2021).

DOTA-PEG2-GSGKCCYSL (P5) and DOTA-PEG2-DTFPYLGWWNPNEYRY (P6)

Researchers evaluated the HER2 binding activity of ⁶⁸Ga-labeled peptides in vitro and in vivo in xenograft model mice. At 2 h post-injection, they found that [⁶⁸Ga]P5 exhibited a significant increase in binding to HER2-positive tumors as measured in percentage injected dose per gram (%ID/g) relative to tumors negative for HER2 expression (0.24 ± 0.04 vs. 0.12 ± 0.06; *P* < 0.05), while [⁶⁸Ga]P6 exhibited similarly enhanced binding at 1 h post-injection (0.98 ± 0.22 vs. 0.51 ± 0.08; *P* < 0.05). These peptides can thus be leveraged to enable the visual detection of HER2-positive breast tumors (Ducharme et al. 2022).

It has been shown that the ligand portion (including trastuzumab, pertuzumab, affinity, and peptides) of the HER2-targeting radioactive molecular probes is responsible for the specific detection of HER2-positive tumor cells by targeting and binding to the extracellular domains IV or II of HER2 (Carter et al. 1992). Theoretically, alterations in either of the extracellular structural domains IV or II of HER2 could result in negative results on PET/CT molecular imaging targeting HER2, despite the presence of the HER2 receptor. Clinically, this is particularly common in patients with advanced metastatic breast cancer following treatment with frontline trastuzumab and pertuzumab therapy. There are several possible explanations for this. 1. The p95HER2 receptor is a truncated form of the full-length p185HER2 receptor, which lacks the trastuzumab binding site and is therefore unable to detect lesions on HER2-targeted molecular imaging. The p95HER2 receptor is present in about 30% of HER2-positive breast cancers and has been found to be a marker of poor prognosis (Molina et al. 2002). 2. As mucin-4 (MUC4) is a surface glycoprotein, it can physically block the binding sites of mAbs, leading to negative visualization (Oshima et al. 2014; Rowson-Hodel et al. 2018). 3.

HER2/neu mutations could theoretically alter the structure of the binding site, inducing off-target effects, although this has not been reported. In summary, structural alterations in the binding site or steric hindrance from surface proteins could affect the binding of the tracer to the extracellular domain of HER2, resulting in false-negative results.

Clinical applications for HER2-targeted PET/CT scanning in advanced breast cancer

Early detection of small HER2-positive lesions undetectable via traditional PET/CT

Owing to their ability to specifically bind to HER2, HER2-targeted molecular imaging probes can yield superior specificity and sensitivity when detecting tumors overexpressing these receptor molecules, enabling the clinical detection of small HER2-positive lesions not visible via traditional PET/CT imaging. Dijkers et al. (2010) first reported human PET/CT imaging performed in 14 HER2-positive metastatic breast cancer patients using ^{89}Zr -Trastuzumab, revealing that this approach was capable of detecting small liver, lung, bone, and brain metastases that were undetectable via FDG-PET scanning. Laforest et al. (2016) also demonstrated the efficacy of high-dose ^{89}Zr -Trastuzumab for PET/CT imaging, while Alhuseinalkudhur et al. (2020) demonstrated that ^{68}Ga -ABY-025 was able to function as an effective imaging probe for dynamic scanning and parametric imaging, mitigating the limited ability of conventional PET/CT scans to detect small liver lesions as a consequence of high levels of background probe uptake such that these metastatic lesions were detectable with greater sensitivity.

Evaluation of HER2 status in lesions that cannot readily be biopsied

Selecting treatment approaches for tumors that cannot be biopsied, such as deep metastases, brain metastases, and lesions, closely associated with major nerves or large blood vessels is challenging. The application of molecular imaging probes in these cases can provide a more reliable means of determining whether patients are likely to benefit from targeted treatment efforts.

Bensch et al. (2018) employed ^{89}Zr -Trastuzumab PET/CT imaging as a means of evaluating the HER2 expression status of 7 breast tumor metastases not accessible for biopsy, enabling more reliable patient dosing. Ulaner et al. (2018) similarly conducted the PET/CT imaging of metastatic breast cancer patients with ^{89}Zr -Pertuzumab as a molecular probe, enabling the identification of brain metastases that were not detectable via ^{18}F -FDG imaging. Relative to normal brain tissue, this probe exhibited

18-fold higher uptake levels in tumor tissues, potentially owing to the disruption of the blood–brain barrier at metastatic sites (Dijkers et al. 2010). Lee et al. (2017) further demonstrated the utility of ^{64}Cu -MM-302 nanoparticles as a PET/CT imaging probe and successfully detected the HER2 status of lesions not readily accessible for biopsy.

Controversy remains regarding the use of ^{64}Cu -DOTA-Trastuzumab as a probe for the detection of brain metastases. Tamura et al. (2013) and Kurihara et al. (2015) both successfully applied this probe to detect the HER2 status of metastatic lesions in the brain, consistent with its ability to cross the blood–brain barrier. In contrast, Mortimer et al. (2018) failed to effectively detect brain metastases when utilizing ^{64}Cu -DOTA-Trastuzumab for imaging, suggesting that the restricted ability of this antibody-based probe to cross physiological barriers warrants consideration when conducting HER2-targeted PET/CT imaging.

Evaluating the heterogeneity of multiple metastases

In cancer patients with metastatic disease, metastases are often observed in the form of multiple heterogeneous foci. Treatment planning is generally based on the biological characteristics of one or a small number of these metastases, potentially resulting in the application of therapies that cannot effectively target certain lesions. HER2-targeted PET/CT scanning can simultaneously establish the HER2 status of each of these lesions, thus providing more detailed insight into tumor characteristics and supporting the informed adjustment of patient treatment plans.

Inki (Lee et al. 2022) described the case of a breast cancer patient with HER2-positive disease that experienced local recurrence and pulmonary metastasis following postoperative chemotherapy and dual-target therapy. When they applied ^{64}Cu -DOTA-Trastuzumab for whole-body PET/CT imaging, this probe was readily taken up by local tumor tissue in the left breast and left axillary lymph nodes but not by the pulmonary metastases. Consistent with this observation, anti-HER2-targeted treatment resulted in the significant reduction of the left breast and left axillary lesions whereas the lung metastases progressed, providing strong support for the heterogeneous nature of recurrent metastatic lesions. Mortimer et al. (2014) similarly demonstrated the ability of ^{64}Cu -DOTA-Trastuzumab to highlight significant variability in the uptake of this probe among lesions in a given patient, with foci lacking any apparent uptake further highlighting the potential for intratumoral HER2 heterogeneity.

Identifying lesions with altered HER2 status

Much as heterogeneity can exist among lesions in a given patient, so too can HER2 status vary between primary and

metastatic lesions. HER2-targeted PET/CT imaging thus provides a basis for potential targeted treatment planning in patients with HER2-negative primary disease.

In their analysis of 20 metastatic breast cancer patients with HER2-negative primary disease, Ulaner et al. (2016, 2017) conducted ^{89}Zr -Trastuzumab PET/CT imaging and identified 9 putatively HER2-positive lesions of which 3 were confirmed to be HER2-positive on biopsy while the remaining 5 were false-positives. Similarly, Bensch et al. (2018) used the results of ^{89}Zr -Trastuzumab PET/CT imaging to modulate treatment planning in 8 enrolled patients (40% of the overall cohort), including 5 that began anti-HER2 treatment and 3 that did not based on these ^{89}Zr -Trastuzumab scans. Ulaner et al. (2020) were also able to apply ^{89}Zr -Pertuzumab PET/CT to facilitate the successful detection of HER2-positive metastatic lesions in patients with biopsy-confirmed HER2-negative primary breast tumors. They found that 6 of 24 analyzed patients exhibited suspected HER2-positive lesions upon PET/CT imaging, with 3 of these lesions ultimately being confirmed to be HER2-positive, while 2 were HER2-negative and 1 yielded unspecified results. These results highlight the value of assessing the HER2 status of metastatic lesions such that patients with HER2-negative primary tumors have the potential to benefit from targeted therapy if HER2-positive metastatic lesions are detected.

Sorensen et al. (2016) evaluated 16 metastatic breast cancer patients via ^{68}Ga -ABY-025 PET/CT imaging. Based on their results, treatment plans were altered for three patients including one individual with HER2-positive primary tumors but bone metastases exhibiting low levels of HER2 expression and two patients with HER2-negative primary tumors that were found to exhibit high HER2 expression following PET/CT scanning. All of these imaging results were ultimately confirmed via pathological biopsy.

Evaluating the efficacy of anti-HER-2 drugs

Gebhart et al. (2016) published the results of the multicenter ZEPHIR study of 60 HER2-positive metastatic breast cancer patients in which the association between ^{89}Zr -trastuzumab uptake and prognosis was prospectively evaluated following T-DM1 treatment. Patients with HER2-positive lesions, as designated based on the results of ^{89}Zr -trastuzumab PET/CT imaging, exhibited greater reductions in tumor size and improved progression-free survival following three T-DM1 treatment cycles. These data highlight the importance of molecular imaging as a noninvasive tool that can enable the dynamic evaluation of tumor responses to particular therapeutic agents, thereby enabling the design and application of antibody–drug conjugates on an individualized basis.

Poor preclinical-to-clinical translation of PET/CT targeting HER2

While there are currently many kinds of PET/CT molecular probes targeting HER2, difficulties remain in their application in large-scale clinical promotion due to the complexity of the labeling method and the high cost involved. Both PET/CT and SPET/CT can provide dynamic, visualized imaging of both local lesions or whole body imaging using a single drug administration. Compared with PET/CT, SPET/CT has the advantages of having a simple drug-labeling method, high yield, and low price, all of which are advantageous for its clinical promotion. To date, several HER2-targeted SPET/CT tracers have reached the stage of clinical application and have the potential for large-scale clinical promotion. Zhao et al. (2021) developed a novel $^{99\text{mTc}}$ -labeled anti-HER2 single-domain antibody ($^{99\text{mTc}}$ -NM-02) and investigated its safety, radiation dosimetry, biodistribution, and tumor-targeting potential in 10 patients with HER2-positive breast cancer. The results showed a mean effective dose of 6.56×10^{-3} mSv/MBq, together with good safety and imaging characteristics, with tracer uptake visible in both primary tumors and metastases. Altunay et al. (2023) performed SPET/CT imaging with a $^{99\text{mTc}}$ -labeled single-domain antibody (RAD201) in six patients with HER2-positive breast cancer and found that the tracer was able to discriminate HER2 status in advanced breast cancer, regardless of ongoing HER2-targeted antibody treatment. Cai et al. (2020) in an open-label phase I clinical trial (NCT03546478) involving patients with HER2-positive breast cancer demonstrated specific binding (overall specificity, 60%) of the $^{99\text{mTc}}$ -labeled HER2-targeted affinity ABH2 affibody to target molecules without noticeable adverse effects for the patient.

Conclusions and outlook

HER2 remains an essential biomarker when assessing patients with breast cancer that guides treatment-related decision-making and prognostic evaluation. HER2-targeted therapies can contribute to significant improvements in survival outcomes for patients with HER2-positive disease, and the ability to accurately evaluate patient HER2 status is thus vital to successful treatment planning. As obtaining pathological specimens can be challenging for certain metastases, HER2 expression levels in primary tumors and metastases may be inconsistent with one another, and different metastases can exhibit heterogeneous phenotypes, HER2 status has the potential to be inaccurately judged through conventional means. HER2-targeted PET/CT imaging, in contrast, offers a noninvasive and comprehensive approach that can enable

systemic screening for HER2-positive lesions in real time such that the HER2 status of metastases can be carefully assessed.

At present, mAbs molecular probes are most commonly deployed in clinical studies. These probes exhibit a range of limitations including high levels of nonspecific uptake, undesirably high levels of radiation exposure to health organs, and a risk of false-positive results. As such, protein-based probes, including single-domain antibodies and engineered scaffold proteins, may offer greater utility as molecular imaging agents. These probes are not subject to EPR effects such that the risk of false-positive results can be minimized and HER2 heterogeneity can be effectively assessed and monitored over the course of disease progression. While these findings are promising, future prospective clinical studies will be invaluable as a means of fully evaluating the importance of HER2-targeted molecular imaging in the management of breast cancer.

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Declarations

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