Theranostic Approach in Breast Cancer

A Treasured Tailor for Future Oncology

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Abstract: Breast cancer is the most frequent invasive malignancy and the second major cause of cancer death in female subjects mostly due to the considerable diagnostic delay and failure of therapeutic strategies. Thus, early diagnosis and possibility to monitor response to the treatment are of utmost importance. Identification of valid biomarkers, in particular new molecular therapeutic targets, that would allow screening, early patient identification, prediction of disease aggressiveness, and monitoring response to the therapeutic regimen has been in the focus of breast cancer research during recent decades. One of the intensively developing fields is nuclear medicine combining molecular diagnostic imaging and subsequent (radio)therapy in the light of theranostics. This review aimed to survey the current status of preclinical and clinical research using theranostic approach in breast cancer patients with potential to translate into conventional treatment strategies alone or in combination with other common treatments, especially in aggressive and resistant types of breast cancer. In addition, we present 5 patients with breast cancer who were refractory or relapsed after conventional therapy while presumably responded to the molecular radiotherapy with ^{177}Lu-trastuzumab (Herceptin), ^{177}Lu-DOTATATE, and ^{177}Lu-FAPI-46.

Key Words: breast cancer, theranostics, ^{177}Lu-trastuzumab (Herceptin), DOTATATE, PSMA, FAPI, SRS

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Breast cancer is the most frequent invasive cancer and the second major cause of cancer death in female subjects, after lung cancer.[1] The incidence of breast cancer is predicted to reach 85 per 100,000 female subjects by 2021.[2] The heterogeneous origin of breast cancer presents challenges to patient management.[3,4] Various factors such as tumor type, histological grade, lymph node metastasis, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) affect the prognosis, aggressive phenotype, and treatment response of breast cancer. Breast cancer has been classified into molecular subtypes to assist clinicians to select an optimal therapeutic approach. According to the St Gallen Consensus 2011, systemic treatment recommendations are based on the following breast cancer molecular subtypes, classified according to the expressed genes: luminal A (ER-positive/PR-positive/HER2-negative/low Ki-67); luminal B including 2 subtypes HER2-negative (ER-positive/PR-positive/HER2-negative/high Ki-67) and HER2 overexpressed (ER-positive/PR-positive/any Ki-67/HER2-positive); HER2-positive or nonluminal (ER and PR absent/HER2 overexpressed); and basal-like or triple-negative breast cancers (TNBCs) (ER-negative/PR-negative/HER2-negative).[5,6] Other factors influencing the therapy selection are cancer grade and stage, general health condition, and menopausal status. The major challenge in patient management is the individual variability among the patients.

In the current decades, a deeper understanding of breast cancer biology underlying the cancer phenotype has led to the identification of specific targets, expressed on cancer cells, that can be used for diagnosis and therapy on molecular level of a specific individual and thus improve the therapeutic outcome.[7–9] The combination of the diagnostic and therapeutic agents targeted at the same biomarker specific for a certain disease is known as theranostics. It is aimed at the prediction of the efficacy of a treatment on an individual basis and monitoring response to the treatment.[10–13] The most important advantage of the theranostic approach is the identification of those individuals who would benefit from a specific treatment and thus prevention of potentially futile treatments resulting in improved cost-efficiency.

With respect to theranostics, an accelerated development has been occurring in the field of nuclear medicine wherein the molecular diagnostic imaging using PET or SPECT can be combined with subsequent targeted molecular radiotherapy or chemotherapy.[14,15] This strategy involves targeting cancer cells with radiopharmaceuticals specific to the biomarkers, for example, receptors that are overexpressed during the period of the disease.[16] The cornerstone of this approach is development and availability of radiopharmaceuticals for the diagnostic imaging and molecular radiotherapy preferably comprising the same vector molecule to bind to the molecular targets[11,15] (Fig. 1). PET and SPECT molecular imaging agents comprise radionuclides emitting, respectively, positrons (eg, ^{68}Ga [$t_{½}$ = 68 minutes]) and γ-ray photons (eg, ^{99m}Tc [$t_{½}$ = 6 hours]; ^{123}I [$t_{½}$ = 13.3 hours]).[17] Radiotherapeutical agents rely on α- and β^--emitting radionuclides. The most commonly used β^--emitting radionuclides include ^{131}I ($t_{1/2}$ = 8 days), ^{90}Y ($t_{1/2}$ = 64.1 hours), and ^{177}Lu ($t_{1/2}$ = 6.73 days). The additional γ emission of ^{131}I and ^{177}Lu allows simultaneous SPECT imaging, providing essential information on biodistribution and dosimetry of the radiopharmaceutical.[18] The current development of peptide receptor–targeted molecular radiotherapy is directed also toward the use of α-emitting

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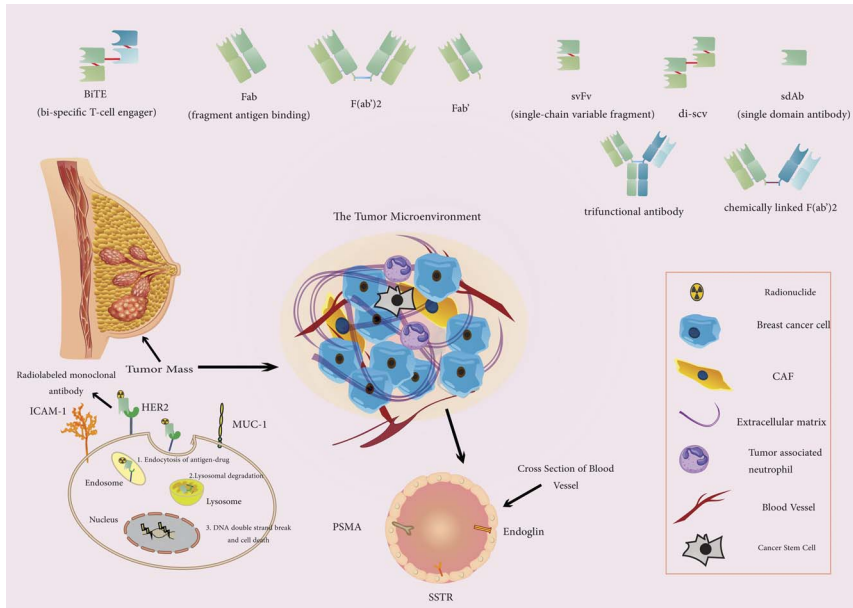

FIGURE 1. The schematic of different kinds of engineered mAbs and potential biological targets for a radiotheranostic approach in breast cancer treatment.

radionuclides such as ^{213}Bi ($t_{1/2}$ = 10.6 hours), ^{223}Ra ($t_{1/2}$ = 11.4 days), ^{225}Ac ($t_{1/2}$ = 10 days), and ^{227}Th ($t_{1/2}$ = 18.7 days).

It is commonly recognized that there is a medical need for individualized treatment of breast cancer and thus improvement of patient management and care. This review is focused on the contribution of nuclear medicine field to the development of the solution in the context of theranostics. The preclinical and clinical research using such biomarkers as HER2, somatostatin receptors (SSTRs), prostate-specific membrane antigen (PSMA), fibroblast activation protein (FAP), mucin 1 (MUC1), syndecan 1 (CD138), endoglin (CD105), and intercellular adhesion molecule 1 (ICAM-1) is comprehensively presented.

Human Epidermal Growth Factor Receptor 2

The HER2 is a transmembrane tyrosine kinase receptor overexpressed on breast cancer cells in 15% to 25% of cases.[19,20] The HER2 overexpression is associated with development and progression of the disease and is 1 of the most important biomarkers indicating poor survival. Disruption of HER2 signaling by, for example, monoclonal antibody (mAb) trastuzumab or tyrosine kinase inhibitor lapatinib improves survival of patients with metastatic breast cancer.[21] Successful decision making in HER2-targeted therapy depends on an accurate characterization of HER2 expression in breast cancer patients. Biopsy with subsequent tissue immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the major currently used diagnostic tools for the quantification of HER2 expression; however, HER2 testing may show false results in up to 20% of the cases most probably due to the heterogeneity of the receptor expression and inaccurate biopsy.[22,23] The FISH technique visualizes specific genes or portions of genes and demonstrates high accuracy in the measurement of HER2 expression that correlated with the rate of responsiveness to trastuzumab or lapatinib therapy.[24,25] According to the American Society of Clinical Oncology/College of American Pathologists guidelines, a scoring system (0–3+) is considered for IHC assay to evaluate HER2 expression, wherein an IHC score of 0 is negative; an IHC score of 1+, negative; an IHC score of 2+, equivocal; and an IHC score of 3+, positive. The HER2 test result is reported as positive (*a*) if the IHC score is 3+ based on circumferential membrane staining or (*b*) if the FISH assay is positive using either a single-probe or dual-probe FISH. If the IHC result is 2+, then confirmation by FISH is required for the selection of trastuzumab treatment.[26] This approach results in a decreased risk of not treating the patients who might benefit and treating the patients who are improbable to benefit from trastuzumab treatment.[27] However, the core needle biopsy presents potential adverse effects and increased dissemination of cancer cells from the primary lesion to distant metastasis. To overcome the drawbacks of conventional biopsy, a "digital biopsy" concept has currently been introduced. It is based on the premise that the data derived from, for example, molecular noninvasive imaging correlate with underlying biological processes.[28]

Molecular imaging can detect physiological changes providing specific information on heterogeneous cells related to breast cancer. It applies specific probes to detect and quantify HER2 overexpression noninvasively and to aid the selection of patients who could benefit from HER2-targeted therapy, as well as to monitor patients between treatment sessions and after therapy to evaluate response to the treatment. A number of anti-HER2 probes have been developed based on intact mAbs, antibody fragments (Fab), nanobodies, and affibodies for specific binding to HER2 and comprising radioactive atoms for tomographic detection.[8,29] Nevertheless, it has been demonstrated that their high affinity prevents homogeneous tumor penetration and intratumoral diffusion, and leads to a suboptimal therapeutic efficiency.[30–32] Also, some drawbacks such as almost high molecular weight, extravascular binding of antibodies, and higher interstitial pressure result in the heterogeneous distribution of the conventional antibodies in the tumor.[33,34] Moreover, the kidneys become unable to filter antibodies due to their relatively high molecular weight. This inappropriate filtration will lead to hematotoxicity and accumulation of antibodies in the liver. Monoclonal antibodies show a significant ratio of nonspecific uptake at the target regions, generally at the earlier time points of administration.[35,36] These limitations have developed smaller antibody fragments for better tissue penetration, favorable biodistribution, higher safety, and treatment efficacy[37] (Fig. 1).

Besides, the treatment challenges, especially in end stages and metastatic cases of breast cancer, remain unresolved. The hallmarks of cancer cells are apoptosis evasion, which makes a barrier to immunotherapy success in breast cancer patients.[38] Because most of the anticancer therapies including chemotherapies and immunotherapies trigger apoptosis induction, a tumor sensitivity to anticancer treatments will remarkably depend on the level of expression of antiapoptotic proteins and on the existence of a prosurvival profile specification (increased ratio between antiapoptotic and proapoptotic protein).[39] The increase of the response rate for immunotherapy remains a big challenge for breast cancer patients, and further research in this era is needed.[40]

The enhancement of the therapeutic response can be achieved by incorporation of cytotoxic radioisotopes (α or β particle emitters) into the antibodies that specifically bind to the cancer cells, wherein the ionization radiation causes complex DNA double strands to break, thus eradicating the cancer cells. This strategy for systemic treatment known as radioimmunotherapy (RIT) was introduced in the early 1950s,[41] and currently, procedure guidelines for specific diseases are available.[42]

One of the major advantages of targeting RIT is minimizing the adverse effect on nontargeted cells due to the binding of the mAb to specific overexpressed antigens on the tumor cells.[43] Such radionuclides as ^{64}Cu, ^{90}Y, ^{111}In, ^{131}I, ^{177}Lu, ^{186}Re, and ^{211}At have been used for preclinical and clinical RIT studies of breast cancer. In addition to cytotoxic β^-, ^{64}Cu and ^{177}Lu also emit, respectively, positrons and γ's that can be used for the imaging, dosimetry calculations, as well as monitoring disease course and treatment response.[44–46]

Trastuzumab comprising ^{177}Lu demonstrated promising results preclinically.[47,48] The cytotoxic effect of the ^{177}Lu-DOTA-trastuzumab was found superior to that of the nonradioactive counterpart.[47] ^{177}Lu-trastuzumab was found safe to be administered intravenously to the patients with HER2-positive primary and metastatic breast lesions.[16] The lesion uptake of ^{177}Lu-trastuzumab was found relevant for palliative treatment in combination with other conventional treatments for HER2-positive metastatic breast cancer.[16]

The incidence of brain metastases related to the breast cancer is estimated to be 5.1%[49]; however, it varies depending on the subtype of breast cancer.[50] Expression of some markers, including HER2 and EGFR, on primary tumor might demonstrate increased risk of metastasis and support targeted therapeutic approaches.[51] The efficacy of α particle–emitting ^{211}At-labeled trastuzumab in a rat model of breast carcinomatous meningitis was demonstrated preclinically.[52] In addition, ^{68}Ga-HER2-nanobody PET/CT has been successfully tested as a safe procedure, with tolerability, favorable biodistribution, and high uptake in HER2-positive lesions of breast carcinoma compared with normal peripheral tissues. Phase 2 clinical trial of the ^{68}Ga-DOTA-anti-HER2 VHH1 for PET/CT imaging of breast cancer brain metastases is ongoing. If successful, it might warrant the development of therapeutic analogs of this radiotracer for targeted radionuclide therapy as theranostic strategy.[53,54] Phase 1 trial (NCT04467515) demonstrated safety and imaging potential of ^{131}I-SGMIB-anti-HER2 VHH1 in breast cancer patients.[55] The biodistribution was favorable with distinct uptake in lesions of breast cancer and metastatic regions, and no adverse effects were observed after low-dose IV administration of ^{131}I-GMIB-anti-HER2 VHH1.[55] These promising results encouraged phase 1/2 study for dose escalation and investigation of the therapeutic potential of this compound.[55] Single-domain antibody fragments (CAM-H2) labeled with radioiodine (^{131}I) were investigated in a phase 1 study targeting HER2-expressing malignancies.[56] The SPECT imaging demonstrated focal uptake of ^{131}I-CAM-H2 in metastatic lesions, relatively fast healthy tissue clearance, and favorable biodistribution. The dosimetry calculation estimated strong potential for the administration of therapeutic doses with a low risk of toxicity to the healthy organs.[56]

Two patients aged 46 and 49 years who were presented with HER2-positive breast cancer and brain metastases were referred to our department. In both cases, the IHC staining was negative for ER and PR. One of the patients had a history of several cycles of chemotherapy sessions and radiotherapy using γ knife. After relapsing of the brain lesions and confirmed HER2-positive lesions based on IHC result (IHC 3+), she underwent 2 cycles of ^{177}Lu-trastuzumab. Posttherapy scintigraphy and brain MRI revealed a remarkable decrease in the lesion size (Fig. 2). The other woman had history of several cycles of chemotherapy and radiotherapy. Immunohistochemistry analysis reported positive result permitting trastuzumab treatment strategy (IHC 3+). She underwent 1 treatment cycle of ^{177}Lu-trastuzumab. Posttherapy scintigraphy after the first cycle showed intensive radiotracer uptake in the lesion (Fig. 3).

Some patients demonstrate recurrent tumor cells and metastasis. One of the proposed hypotheses for the resistance is the overexpression of other EGFR family members (eg, EGFR and HER3).[57] This co-overexpression was used for the development of bispecific radioimmunoconjugates for imaging and therapeutic application. Razumienko et al[58] designed ^{177}Lu-DOTA-Fab-PEG24-EGF and ^{111}In-DOTA-Fab-PEG24-EGF to target overexpression of both HER2 and EGFR receptors, as well as compared therapeutic efficiency of these bispecific radiopharmaceuticals in a preclinical study.

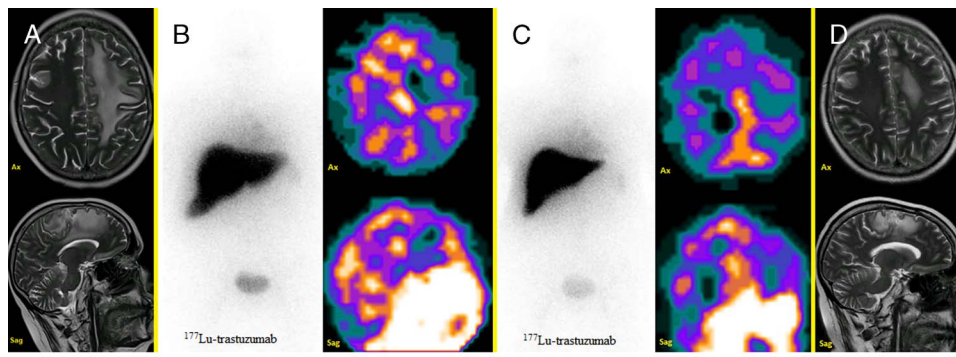

FIGURE 2. A 46-year-old woman with HER2-positive breast cancer and brain metastasis observed on MRI (**A**) refractory to conventional therapy methods underwent 2 cycles of ^{177}Lu-trastuzumab (Herceptin) (3.7 GBq). Posttherapy scintigraphy after the first cycle of ^{177}Lu-trastuzumab (Herceptin) (**B**) showed intensive radiotracer uptake in the lesion and in its periphery, which decreased in posttherapy scintigraphy of the second cycle (**C**). Also, brain MRI done 2 weeks after the second cycle of therapy revealed a remarkable decrease in the peripheral lesion (**D**).

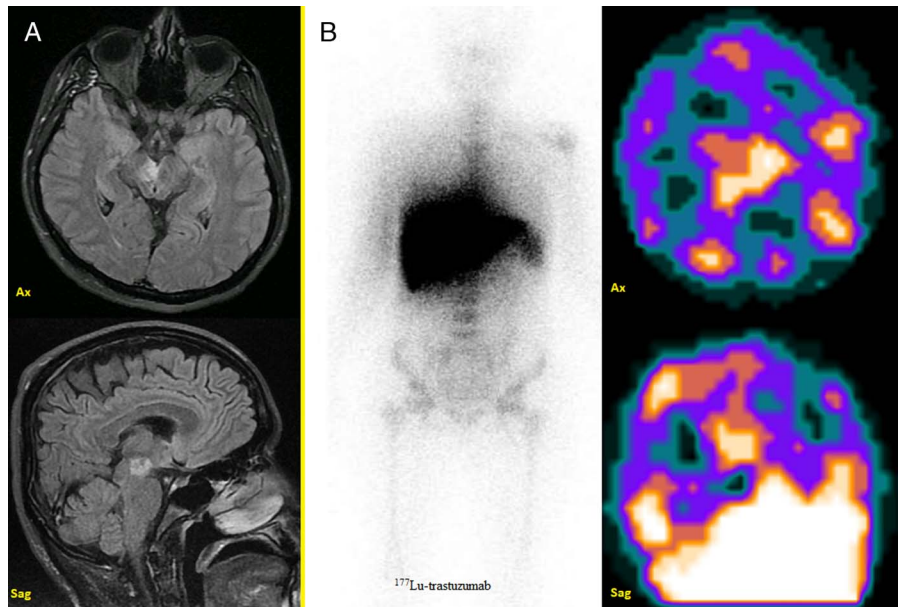

FIGURE 3. A 49-year-old woman with HER2-positive breast cancer and brain metastasis in the brain stem observed on MRI (**A**). She underwent 1 cycle of ^{177}Lu-trastuzumab (Herceptin) (1.85 GBq) that showed intensive radiotracer uptake in the lesion according to the posttherapy scintigraphy (**B**).

In addition, these preclinical results were compared with monospecific radiotracers ^{177}Lu- or ^{111}In-labeled trastuzumab Fab or EGF.[58] Gamma emission of ^{177}Lu and ^{111}In was used for micro-SPECT imaging to assess dosimetry data and estimation of radiation absorbed dose. The study showed promising results of bispecific radiopharmaceuticals for the eradication of tumor cells in vitro, as well as in vivo breast cancer xenografts. Radiation-absorbed dose in tumor cells using ^{177}Lu-DOTA-Fab-PEG24-EGF was 9-fold higher than that of ^{111}In-DTPA-Fab-PEG24-EGF. This study demonstrated the feasibility and potential of dual receptor–targeted RIT of breast cancer patients, especially those of trastuzumab-resistant types. In another similar study by Kwon et al,[59] bispecific radiopharmaceuticals comprising ^{64}Cu radioisotope were compared with monospecific ^{64}Cu-NOTA-trastuzumab-Fab and ^{64}Cu-NOTA-EGF targeting HER2 and EGFR and showed superior characteristics in terms of uptake in tumor xenografts expressing either one or both receptors.[59]

Somatostatin Receptors

The expression of SSTRs on various hormone-producing carcinoids of breast cancer, paragangliomas, pancreatic islet cell tumors, meningiomas, neuroendocrine tumors, small cell lung cancer, renal cell carcinoma, malignant lymphoma, and prostate cancer has been demonstrated in vitro and in vivo.[60–62]

The Analysis of the Cancer Genome Atlas data demonstrated the expression of SSTR2 by high proportion of samples from breast invasive carcinoma and variability of the SSTR2 expression level dependent on genomic and clinical characteristics of breast invasive carcinoma.[63] Overexpression of SSTR2 indicated poor prognosis in TNBC subjects.[63] The overexpression of SSTR2 mRNA was detected in 169 infiltrating breast cancers; particularly, it was significantly higher in ER-positive samples, suggesting that the SSTR status of tumors must be considered during the treatment planning because the therapeutic effect of somatostatin analogs might be influenced by the ER antagonist therapy.[64] The correlation between mRNA and enhanced expression of all subtypes of SSTR (1–5) was detected in primary ductal not otherwise specified breast tumors.[65] A weak to moderate expression of SSTR2A and SSTR5 was observed immunohistochemically in 50% to 70% of the patients with neuroendocrine breast cancer (presented with incidence of 0.1%–18%).[66,67] However, the relevance of SSTR-targeted therapy can be justified for neuroendocrine breast cancer with strong SSTR2A expression.[66,67] The SSTR-mediated therapeutic effect on breast cancer was attributed to inhibition of cell proliferation and induction of apoptosis (so-called direct mechanism).[68,69]

Somatostatin receptor autoradiography using ^{125}I-[tyr3]-SMS201-995 revealed receptor-specific uptake in 342 breast tumor samples including both primary tumors and distant metastases with positive SSTR.[70] In in vivo PET visualization using ^{68}Ga-labeled agonist (^{68}Ga-DOTATOC and ^{68}Ga-DOTATATE) and antagonist (^{68}Ga-NODAGA-JR11), somatostatin analogs were studied in mice bearing breast cancer xenografts expressing SSTR type 2 (ZR-75-1 cell culture).[68,69] The antagonist counterpart demonstrated lower uptake in contrast to previous studies wherein ^{177}Lu-DOTA-JR11 antagonist showed higher uptake.[71,72] The discrepancy of these studies illustrates the complexity of the biological system and consequently sensitivity of the method to the experimental conditions, biological models, and variability in radiopharmaceutical construct. A head-to-head comparative study providing the same experimental conditions would be needed to confirm the advantages of using antagonist ligands. Another study demonstrated the uptake of ^{68}Ga-DOTATOC in breast cancer xenograft mouse twice as high as that of ^{18}F-FDG.[73]

^{111}In-Pentetreotide scintigraphy of the breast cancer detected overexpressed SSTR2 and SSTR5 in vivo in patients[60,74–76]; it particularly detected primary breast cancer in 75% of randomly selected patients.[60,74] The method was found more sensitive than conventional serum cancer markers and was suggested for the selection of patients who might benefit from peptide receptor radionuclide therapy (PRRT). The lesion uptake was well correlated with SSTR2A and SSTR5 but not with SSTR1 and SSRT3 expression determined by IHC.[75] A case report presented an asymptomatic patient with breast cancer wherein the early diagnosis of breast cancer resulted in the improvement of patient care strategy. The coauthors considered a potential benefit from the treatment with somatostatin

analogs.[76] These clinical studies present the potential relevance of SSTR imaging for breast cancer management; however, a more extensive and standardized clinical investigation is required to prove the concept. Moreover, the PET technique offering higher sensitivity, resolution, and accurate quantification as compared with ^{111}In-scintigraphy would be more relevant to use for such study.

A case report, wherein a patient with liver metastases from breast cancer distinctly responded to PRRT, demonstrated the potential benefit of this radiotherapeutic approach in metastatic breast cancer treatment.[66,67] One of the recent preliminary studies showed ^{68}Ga-DOTATOC uptake and partial tumor remission after subsequent ^{177}Lu-DOTATOC PRRT in a patient with breast invasive ductal carcinoma and primary large-cell neuroendocrine breast carcinoma.[77]

A 42-year-old woman with invasive ductal carcinoma, positive for ER and PR and negative for HER2, referred to our department. Whole-body ^{99m}Tc-octreotide scintigraphy showed multiple bone lesions. Because she was refractory to conventional chemoradiotherapy, she underwent PRRT with ^{177}Lu-DOTATATE. She received 3 cycles of PRRT (22 GBq) that were well tolerated, and the patient declared a significant reduction in bone pain (Fig. 4).

Another case was a 45-year-old woman with history of surgery for breast angiosarcoma. The patient was referred to our department for whole-body bone scan because of bone pain in the right shoulder. Pathology result showed concurrent endometrial and ovarian involvement by angiosarcoma. The bone scan revealed multiple skeletal metastases. Whole-body ^{99m}Tc-octreotide scintigraphy showed positive lesions in the right humerus and left iliac bone regions, qualifying her for ^{177}Lu-DOTATATE therapy (Fig. 5). ^{177}Lu-DOTATATE therapy was performed every 2 months for 3 cycles (22.2 GBq).

Prostate-Specific Membrane Antigen

The PSMA is a transmembrane glycoprotein overexpressed not only by normal prostate and prostate cancer cells, but also by hepatocellular, glioma, thyroid, bronchial, ovarian, and breast cancer cells.[78,79] Importantly, it is presented in tumor entities lacking other specific cell surface markers, for example, in the case of TNBC without HER2, estrogen, and progesterone receptors, which stands for almost 10% to 15% of all breast cancers.[80] The overall survival of TNBC patients is lower than that of patients with cancer positive for hormone receptors and negative for HER2 receptors.[81] Different adjuvant and neoadjuvant modalities such as chemotherapy and novel-targeted agents such as poly (ADP-ribose) polymerase inhibitors, antiandrogen agents, and immunotherapy were used; however, overall survival could not be increased.

The first detailed assessment of PSMA expression in the tumor-associated vasculature of invasive breast carcinoma with brain metastasis was performed by Wernicke and coworkers.[82] In this study, patients with negative ER and PR had more overexpression of PSMA compared with patients with positive ER and PR tumor cells. Immunohistochemical analysis of PSMA expression and angiogenetic activity in normal breast tissue and breast cancer tissues from primary tumors and distant metastases demonstrated higher expression in metastases than in primary tumors.[83] The strong immunohistochemically determined expression of PSMA in bone metastases was visualized by ^{68}Ga-PSMA-HBED-CC PET in 1 patient. ^{68}Ga-PSMA-HBED-CC PET examination of a patient with TNBC was conducted with the aim to investigate the possibility of PSMA-targeted therapy.[84] The uptake was observed in tumor neovasculature and metastatic lesions, and PSMA-based therapy was considered after failed standard-of-care treatments.

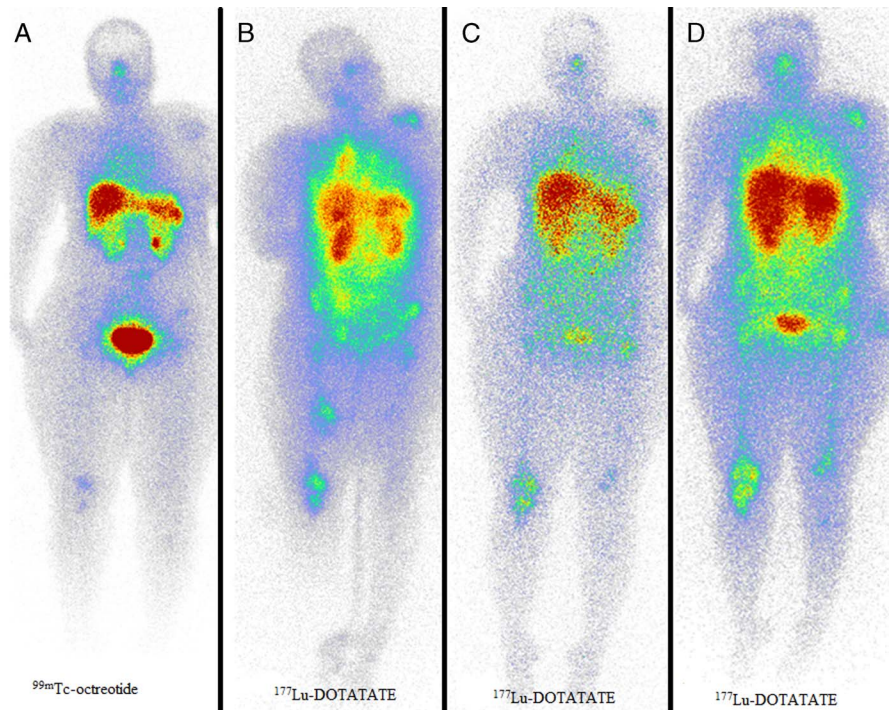

FIGURE 4. A 42-year-old woman with invasive ductal carcinoma underwent ^{99m}Tc-octreotide scintigraphy showed multiple bone metastases on the left shoulder, ribs, spinal cord, pelvic, right knee (**A**). Therefore, the patient was candidate for PRRT with ^{177}Lu-DOTATATE. She received 3 cycles of PRRT that resulted partial response according to the post-PRRT cycles images (**B**–**D**) and a significant reduction in bone pain.

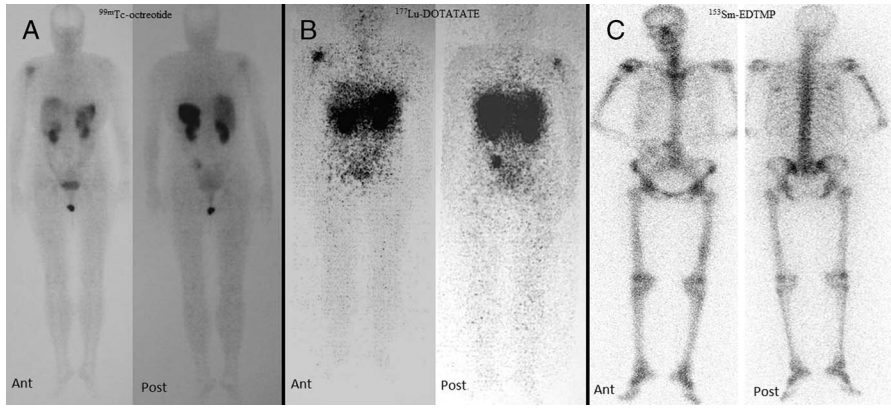

FIGURE 5. A 45-year-old woman with bone pain utmost right shoulder and previous surgery for breast angiosarcoma. Whole-body scintigraphy using ^{99m}Tc-octreotide scan revealed lesions in the right humerus and left iliac bone regions (**A**); therefore, she underwent 3 cycles of ^{177}Lu-DOTATATE treatment with 2-month interval (22.2 GBq) (**B**). She showed a pathologic fracture in the right humerus, so she performed surgery for this fracture and received ^{153}Sm-EDTMP (3.7 GBq) for bone pain palliation (**C**).

Preclinical studies using mice with TNBC cell xenografts demonstrated imaging and radiotherapeutic potential of targeting PSMA of TNBC.[85] The xenograft localization by ^{68}Ga-PSMA-11 PET correlated with ex vivo immunofluorescence analysis of the respective tissue, and the treatment using ^{177}Lu-PSMA-617 significantly reduced the vitality and angiogenic potential of the cancer cells.

These initial preclinical and clinical studies indicate the potential of PSMA-targeted imaging and therapy for TNBC treatment.

Fibroblast Activation Protein

The FAP is a transmembrane serine protease expressed by activated fibroblasts. It is involved in the remodeling of the extracellular matrix (eg, keloid formation, rheumatoid arthritis, hepatic and pulmonary fibrosis, and osteoarthritis) and is highly upregulated in the microenvironment of a wide variety of cancers. Fibroblast activation protein is overexpressed on activated carcinoma-associated fibroblasts in more than 90% of common human epithelial malignancies, for example, colorectal, ovarian, oropharynx, and pancreatic carcinoma. It promotes tumor growth, progression, tumorigenesis, and cell migration. Fibroblast activation protein inhibition decreases tumor growth, making it useful for therapeutic applications. The combination of chemotherapy with cyclophosphamide and FAP immunotherapy was considered as a promising treatment for breast malignancy.[86–89]

A number of radioactive fibroblast activation protein inhibitors (FAPIs) based on *N*-(4-quinolinoyl)glycyl-(2-cyanopyrrolidine) and quinoline scaffolds were developed for targeted imaging and molecular radiotherapy of various malignancies.[87,90–95] Importantly, FAP is typically low to undetectable on normal fibroblast cells, thus providing high image contrast in, for example, PET and favorable dosimetry for molecular radiotherapy. High lesion uptake of ^{68}Ga-FAPI-04 in patients with metastatic breast cancer followed by a reduction in pain symptoms after the treatment with ^{90}Y-FAPI-04 suggested the strong potential of FAP-targeted radiotheranostic approach for the treatment of tumors containing a high level of activated fibroblasts such as breast cancer.[94]

A first-in-human study demonstrated favorable biodistribution with high tumor uptake and low uptake in normal tissues of theranostic pair, ^{68}Ga/^{177}Lu-FAP-2286 in 11 advanced adenocarcinoma patients including 5 pancreases, 4 breasts, 1 rectum, and 1 ovarian tumor.[96] ^{177}Lu-FAP-2286 was highly accumulated with long retention in the lesions. The radiopharmaceuticals were well tolerated. This new theranostic platform was recommended as a highly promising treatment option in a wide spectrum of malignancies.[96]

Fibroblast activation protein inhibitors radiolabeled with ^{68}Ga and ^{177}Lu demonstrated potential for theranostic application in a case study with end-stage breast cancer.[97] Multiple malignant lesions were localized by ^{68}Ga-DOTA.SA.FAPi PET in breast, lung, liver, and bones. The diagnostic imaging was followed by a single cycle of ^{177}Lu-DOTA.SA.FAPi (3.2 GBq [86 mCi]) wherein the in vivo distribution of ^{177}Lu-DOTA.SA.FAPi, monitored by STECT/CT, correlated with that of ^{68}Ga-DOTA.SA.FAPi PET/CT. ^{68}Ga-DOTA.SA.FAPi PET/CT-guided ^{177}Lu-DOTA.SA.FAPi therapy was found specially beneficial for the patients refractory to common treatment approaches.[97]

Recently, a 47-year-old woman with ductal breast carcinoma metastases who had failed conventional treatment was referred to our department to perform the bone scan. The immunohistopathological result demonstrated ER-positive, PR-positive, and HER2-negative for this case. Bone scan revealed multiple skeletal metastases. The patient received a diagnostic dose of ^{177}Lu-FAPI-46 to evaluate radiotracer distribution, which showed intensive uptake on bone lesions. Subsequently, she was selected for the ^{177}Lu-FAPI-46 treatment approach and received 2 cycles of ^{177}Lu-FAPI-46 3.7 GBq (Fig. 6).

Mucin 1

Mucin 1 is 1 of the 12 mucin gene products that is hypoglycosylated and upregulated in more than 90% of breast malignancies such as TNBCs.[98] It is characterized by a peptide core comprising a high number of tandem repeat amino acid sequences.[99] The 58-aa MUC1-C extracellular domain of it can be considered as a potential target for antibody-mediated therapy.[98,100] Multiple antibodies have been established to target MUC-1 epitopes related to epithelial tumors.[99]

A humanized murine antibody, hTAB004 targeting MUC1 was labeled with ^{111}In and ^{225}Ac, and preclinically investigated for the feasibility of TNBC theranostics.[101] The organ distribution of ^{111}In-DOTA-hTAB004 was favorable with as high tumor uptake as 65% ± 15% ID/g. The subsequent single dose of ^{225}Ac-DOTA-hTAB004 resulted in a significant tumor volume reduction encouraging the further development and clinical translation.

A murine IgG1 mAb, BrE-3 antibody that can react with an epitope on the tandem repeat of the peptide core of MUC-1[102] was humanized (h) to reduce the immunogenicity.[103] Immunohistochemical analysis revealed the activity of BrE-3 in more than 95% of biopsy samples from patients with ductal carcinoma of the breast.[104] Imaging and pharmacokinetic results of ^{111}In-labeled

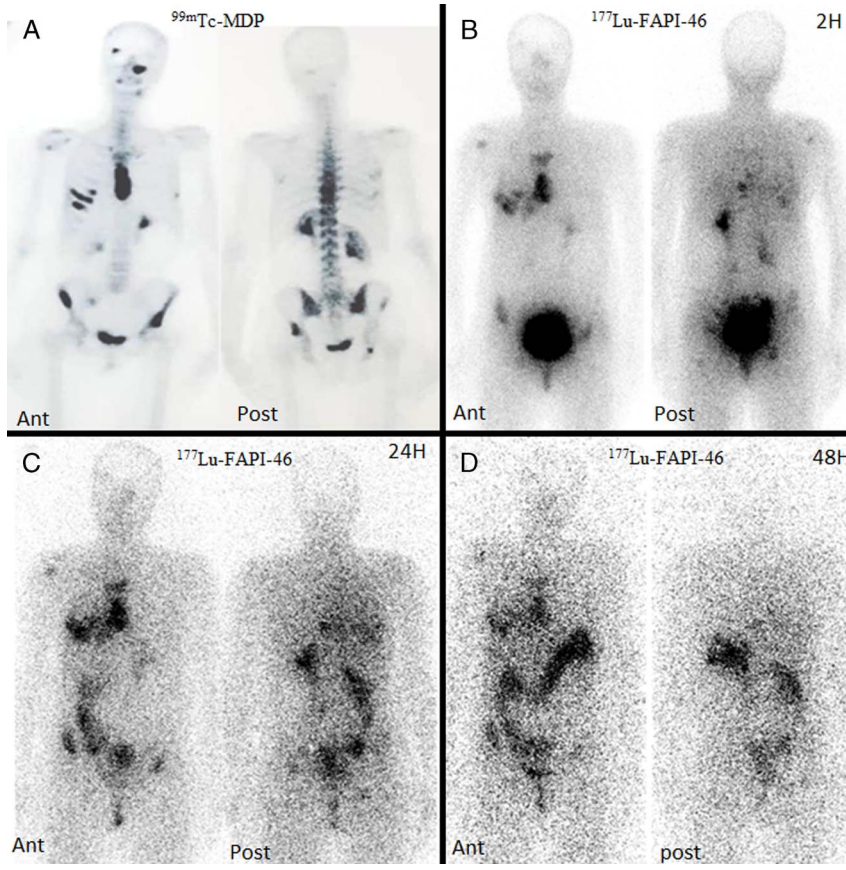

FIGURE 6. A 47-year-old woman with breast cancer and refractory to conventional chemotherapy underwent bone scan revealed multiple skeletal metastases (**A**). For metastatic workup and radiotracer distribution, a diagnostic dose of ^{177}Lu-FAPI-46 was injected and whole-body imaging showed metastases regions in the skull, right shoulder, clavicles, sternum, ribs, spine, sacroiliac joints, and pelvis. Therefore, the patient was candidate for ^{177}Lu-FAPI-46 and underwent 2 therapy cycles of ^{177}Lu-FAPI-46 (3.7 GBq) (**B**–**D**).

BrE-3 suggested therapeutic radiation dose for radiotherapy with ^{90}Y-MX-DTPA-BrE-3 targeting the peptide core of MUC-1 in patients with advanced breast cancer.[105,106] The data advocated that multiple treatment sessions of ^{90}Y-MX-DTPA-BrE-3 could result in frequent and constant response. Phase 1 clinical trial using ^{111}In-labeled BrE-3 for the pretherapeutic lesion localization and dosimetry evaluation for the subsequent high-dose ^{90}Y-BrE-3 radiotherapy revealed negligible toxicity and 50% response rate to the radiotherapy.[103]

Syndecan 1

Syndecan 1 or CD138 is an integral membrane proteoglycan present on epithelial cells holding together the cytoskeleton and the components of extracellular matrix.[107–109] CD138 plays an important role as coreceptors for growth factors, matrix proteins, cytokines, and chemokines as well as in interceding different biological functions, such as proliferation, adhesion, differentiation, migration, angiogenesis, and malignant progression.[109,110] It is also present on pre-B cells and plasma cells that regulate multiple myeloma.[108,111] The expression of CD138 is associated with poor prognosis for TNBC patients. A preliminary theranostic study using the antihuman syndecan 1 B-B4 mAb radiolabeled with either ^{124}I or ^{131}I in tumor-bearing mice (^{124}I-B-B4, ^{131}I-B-B4) confirmed the visualizing of CD138-expressing cells along with great uptake of B-B4 and its retention within the TNBC cells. This promising result in TNBCs expressing syndecan 1 demonstrated the suitability of this biomarker as a target in quantitative imaging and therapy of patients with TNBC; it even might be considered as a treatment approach to decrease brain metastasis of breast cancers.[108,110]

Endoglin

Angiogenesis is considered a key process in the progression of a cancer disease. Lymphovascular invasion and neovascularization indicate poor prognosis in patients with breast cancer.[112,113] Endoglin, also named CD105, is expressed on the endothelial cells of around- and within-tumor blood vessels as well as overexpressed in tumor stromal components. The anti-CD105 antibodies have shown a high affinity for activated endothelial cells in tissues associating angiogenesis and are considered as a therapeutic option for patients with breast cancer, especially of triple-negative type.[114,115] The preliminary study of CD105 staining revealed that microvascular density has a positive and negative relationship, respectively, with HER2 and hormone receptor expression. Consequently, targeting CD105 was suggested as a potential strategy in the anticancer treatment approach.[116] Radiolabeled anti-endoglin/CD105 mAb was used for tumor imaging and demonstrated a rapid and high uptake in mammary canine adenocarcinomas.[117] The data suggested that targeting of CD105 on tumor vasculature may demonstrate a new policy for in vivo imaging of solid malignancies with any histological origin.[118] Radioimmunotherapy can also be used

in targeting the vascular endothelial growth factor pathways. Recently, TRC105 labeled with ^{86}Y ($t_{1/2}$ = 14.7 hours) and ^{90}Y has been introduced as theranostic pair to target overexpressed CD105 on angiogenic vessels in a murine breast cancer model.[119] High uptake of ^{86}Y-DTPA-TRC105 in tumor cells and remarkably strong tumor growth inhibition upon the therapeutic cycle with ^{90}Y-DTPA-TRC105 were observed. This strategy may be synergistic with additional treatment options such as immunotherapy to complete eradication of tumor cells.

Intercellular Adhesion Molecule 1

Intercellular adhesion molecule 1 as a cell surface glycoprotein is expressed on various types of cells including vascular endothelial cells, leukocytes, and cancer cells. The expression of ICAM-1 antigen surface molecule correlates with tumor aggressiveness and metastatic potential in a wide spectrum of diseases such as breast cancer, prostate cancer, and myeloma.[120,121] As previously mentioned, TNBCs have currently no definitive curative option and present a high death rate. Intercellular adhesion molecule 1 has been suggested as a biomarker and target for diagnostics and treatment of such malignancies with poor prognosis.[122] Radioiodinated anti–ICAM-1 antibody localized TNBC MDA-MB-231 xenografts in nude mice using ^{125}I/SPECT and inhibited the tumor growth using ^{131}I isotope instead of ^{125}I.[123]

C-X-C Chemokine Receptor 4

C-X-C chemokine receptor 4 (CXCR4) is expressed in more than 23 various types of tumors including breast cancer cells.[124] It promotes cancer metastases, and its inhibition has been investigated for therapeutic purposes. Many studies evaluated CXCR4 expression and its role in the diagnosis and prognosis of breast cancer.[125–131] It was found that the female subjects with a high CXCR4 expression had more lymph node lesions and shorter disease-free and overall survival compared with those with the low level of CXCR4. However, the feasibility study of ^{68}Ga-pentixafor PET imaging targeted at CXCR4 as a diagnostic tool in BC patient treatment demonstrated a considerably lower lesion detection rate as compared with ^{18}F-FDG PET.[132] Another ligand of CXCR-4, ubiquitin radiolabeled with ^{64}Cu demonstrated promising results in a 4T1-xenograft mouse model of breast cancer.[133]

Various CXCR4 ligands of peptidic and nonpeptidic nature have been identified, opening the possibility for the development of radiolabeled analogs for diagnostic imaging and molecular radiotherapy.[134]

SUMMARY

The major purpose of preclinical and clinical studies presented herein is the development of diagnostic and therapeutic strategies on molecular level and of a high therapeutic index and safety along with minimal toxicity. Nuclear medicine is projected toward a personalized approach, an effective and safe therapy using the concept of theranostics. Molecular imaging of breast cancer is a useful technique to identify disease heterogeneity or differentiate between indolent and aggressive diseases that might help to select the best therapeutic management.[135] The strong potential of the theranostic strategy to treat metastatic breast cancer has already been demonstrated.[15,16,55,58,77,85,103,108,119,136] The discovery of specific biomarkers and the development of the respective radiopharmaceuticals is the cornerstone of the ever-advancing theranostics in nuclear medicine.[15] Some other critical aspects are the regulation and standardization of radiopharmaceutical production, clinical examination procedure, and evaluation.

REFERENCES

1. Lacey JV Jr, Devesa SS, Brinton LA. Recent trends in breast cancer incidence and mortality. *Environ Mol Mutagen*. 2002;39:82–88.
2. Han SJ, Guo QQ, Wang T, et al. Prognostic significance of interactions between ER alpha and ER beta and lymph node status in breast cancer cases. *Asian Pac J Cancer Prev*. 2013;14:6081–6084.
3. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406:747–752.
4. Li SG, Li L. Targeted therapy in HER2-positive breast cancer. *Biomed Rep*. 2013;1:499–505.
5. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol*. 2011;22:1736–1747.
6. Al-thoubaity FK. Molecular classification of breast cancer: a retrospective cohort study. *Ann Med Surg (Lond)*. 2019;49:44–48.
7. Tang X, Ding CK, Wu J, et al. Cystine addiction of triple-negative breast cancer associated with EMT augmented death signaling. *Oncogene*. 2017;36:4235–4242.
8. Mezni E, Vicier C, Guerin M, et al. New therapeutics in HER2-positive advanced breast cancer: towards a change in clinical practices? *Cancers (Basel)*. 2020;12:1573.
9. Acar A, Simões BM, Clarke RB, et al. A role for notch signalling in breast cancer and endocrine resistance. *Stem Cells Int*. 2016;2016:2498764.
10. Langbein T, Weber WA, Eiber M. Future of theranostics: an outlook on precision oncology in nuclear medicine. *J Nucl Med*. 2019;60:13S–19S.
11. Jokar N, Assadi M, Yordanova A, et al. Bench-to-bedside theranostics in nuclear medicine. *Curr Pharm Des*. 2020;26:3804–3811.
12. Oberg K. Molecular imaging radiotherapy: theranostics for personalized patient management of neuroendocrine tumors (NETs). *Theranostics*. 2012;2:448–458.
13. Velikyan I. Molecular imaging and radiotherapy: theranostics for personalized patient management. *Theranostics*. 2012;2:424–426.
14. Velikyan I. Radionuclides for imaging and therapy in oncology. In: Chen X, Wong S, eds. *Cancer Theranostics*. London, United Kingdom: Elsevier; 2014:285–325.
15. Velikyan I. (Radio) theranostic patient management in oncology exemplified by neuroendocrine neoplasms, prostate cancer, and breast cancer. *Pharmaceuticals*. 2020;13:39.
16. Bhusari P, Vatsa R, Singh G, et al. Development of Lu-177-trastuzumab for radioimmunotherapy of HER2 expressing breast cancer and its feasibility assessment in breast cancer patients. *Int J Cancer*. 2017;140:938–947.
17. Sgouros G, Bodei L, McDevitt MR, et al. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nat Rev Drug Discov*. 2020;19:589–608.
18. Cremonesi M, Ferrari M, Bodei L, et al. Dosimetry in peptide radionuclide receptor therapy: a review. *J Nucl Med*. 2006;47:1467–1475.
19. Cesca MG, Vian L, Cristóvão-Ferreira S, et al. HER2-positive advanced breast cancer treatment in 2020. *Cancer Treat Rev*. 2020;88:102033.
20. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol Biol Int*. 2014;2014:852748.
21. Awada G, Gombos A, Aftimos P, et al. Emerging drugs targeting human epidermal growth factor receptor 2 (HER2) in the treatment of breast cancer. *Expert Opin Emerg Drugs*. 2016;21:91–101.
22. Sauter G, Lee J, Bartlett JM, et al. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. *J Clin Oncol*. 2009;27:1323–1333.
23. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*. 2007;131:18–43.
24. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177–182.
25. Colomer R, Montero S, Lluch A, et al. Circulating HER2 extracellular domain and resistance to chemotherapy in advanced breast cancer. *Clin Cancer Res*. 2000;6:2356–2362.
26. Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med*. 2014;138:241–256.

27. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. *N Engl J Med*. 2007;357:39–51.
28. Massicano AVF, Marquez-Nostra BV, Lapi SE. Targeting HER2 in nuclear medicine for imaging and therapy. *Mol Imaging*. 2018; 17:1536012117745386.
29. Doddamane I, Butler R, Jhaveri A, et al. Where does radioimmunotherapy fit in the management of breast cancer? *Immunotherapy*. 2013;5:895–904.
30. Weinstein JN, van Osdol W. Early intervention in cancer using monoclonal antibodies and other biological ligands: micropharmacology and the "binding site barrier". *Cancer Res*. 1992;52:2747s–2751s.
31. Luque-Cabal M, García-Teijido P, Fernández-Pérez Y, et al. Mechanisms behind the resistance to trastuzumab in HER2-amplified breast cancer and strategies to overcome it. *Clin Med Insights Oncol*. 2016;10:21–30.
32. Beckman RA, Weiner LM, Davis HM. Antibody constructs in cancer therapy: protein engineering strategies to improve exposure in solid tumors. *Cancer*. 2007;109:170–179.
33. Jain RK, Baxter LT. Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure. *Cancer Res*. 1988;48:7022–7032.
34. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci*. 2004;93:2645–2668.
35. Jovčevska I, Muyldermans S. The therapeutic potential of nanobodies. *BioDrugs*. 2020;34:11–26.
36. Vaneycken I, Devoogdt N, Van Gassen N, et al. Preclinical screening of anti-HER2 nanobodies for molecular imaging of breast cancer. *FASEB J*. 2011;25:2433–2446.
37. Altunay B, Morgenroth A, Beheshti M, et al. HER2-directed antibodies, affibodies and nanobodies as drug-delivery vehicles in breast cancer with a specific focus on radioimmunotherapy and radioimmunoimaging. *Eur J Nucl Med Mol Imaging*. 2020.
38. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674.
39. García-Aranda M, Pérez-Ruiz E, Redondo M. Bcl-2 inhibition to overcome resistance to chemo- and immunotherapy. *Int J Mol Sci*. 2018;19:3950.
40. García-Aranda M, Redondo M. Immunotherapy: a challenge of breast cancer treatment. *Cancer*. 2019;11:1822.
41. Pressman D, Korngold L. The in vivo localization of anti-Wagner-osteogenic-sarcoma antibodies. *Cancer*. 1953;6:619–623.
42. Ogawa K, Ono M, Tian M, et al. Development of radiolabeled compounds for molecular imaging and imaging-based therapy. *ScientificWorldJournal*. 2015;2015:365418.
43. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256:495–497.
44. Djokić D, Nikolić NS, Janković DL, et al. Development, preparation and quality assurance of radiopharmaceuticals based on ^{188}Re and ^{90}Y for radionuclide therapy: in house production of radioisotopes at Vinča Instute of Nuclear Sciences Chapter 10. *International Atomic Energy Agency (IAEA)*. 2015;46:169–205.
45. Boswell CA, Brechbiel MW. Development of radioimmunotherapeutic and diagnostic antibodies: an inside-out view. *Nucl Med Biol*. 2007;34:757–778.
46. Aghevlian S, Boyle AJ, Reilly RM. Radioimmunotherapy of cancer with high linear energy transfer (LET) radiation delivered by radionuclides emitting α-particles or Auger electrons. *Adv Drug Deliv Rev*. 2017;109: 102–118.
47. Ramli M, Hidayat B, Aguswarini S, et al. Preclinical study of ^{177}Lu-DOTA-trastuzumab: a potential radiopharmaceutical for therapy of breast cancer positive HER-2. *Jurnal Ilmu Kefarmasian Indonesia*. 2013;11:116–122.
48. Ray GL, Baidoo KE, Keller LM, et al. Pre-clinical assessment of Lu-labeled trastuzumab targeting HER2 for treatment and management of cancer patients with disseminated intraperitoneal disease. *Pharmaceuticals (Basel)*. 2011;5:1–15.
49. Lombardi G, Di Stefano AL, Farina P, et al. Systemic treatments for brain metastases from breast cancer, non-small cell lung cancer, melanoma and renal cell carcinoma: an overview of the literature. *Cancer Treat Rev*. 2014;40:951–959.
50. Kodack DP, Askoxylakis V, Ferraro GB, et al. Emerging strategies for treating brain metastases from breast cancer. *Cancer Cell*. 2015;27:163–175.
51. Zhang L, Ridgway LD, Wetzel MD, et al. The identification and characterization of breast cancer CTCs competent for brain metastasis. *Sci Transl Med*. 2013;5:180ra48.
52. Boskovitz A, McLendon RE, Okamura T, et al. Treatment of HER2-positive breast carcinomatous meningitis with intrathecal administration of alpha-particle-emitting (211)At-labeled trastuzumab. *Nucl Med Biol*. 2009;36:659–669.
53. Keyaerts M, Xavier C, Everaert H, et al. Phase II trial of HER2-PET/CT using ^{68}Ga-anti-HER2 VHH1 for characterization of HER2 presence in brain metastases of breast cancer patients. *Ann Oncol*. 2019;30:iii25–iii26.
54. Keyaerts M, Xavier C, Heemskerk J, et al. Phase I study of ^{68}Ga-HER2-nanobody for PET/CT assessment of HER2 expression in breast carcinoma. *J Nucl Med*. 2016;57:27–33.
55. D'Huyvetter M, De Vos J, Caveliers V, et al. Phase I trial of ^{131}I-GMIB-Anti-HER2-VHH1, a new promising candidate for HER2-targeted radionuclide therapy in breast cancer patients. *J Nucl Med*. 2020;jnumed-120.
56. Keyaerts M, De Vos J, Duhoux FP, et al. Phase I results of CAM-H2: safety profile and tumor targeting in patients. *Am J Clin Oncol*. 2018;36:–e13017.
57. Gallardo A, Lerma E, Escuin D, et al. Increased signalling of EGFR and IGF1R, and deregulation of PTEN/PI3K/Akt pathway are related with trastuzumab resistance in HER2 breast carcinomas. *Br J Cancer*. 2012; 106:1367–1373.
58. Razumienko EJ, Chen JC, Cai Z, et al. Dual-receptor–targeted radioimmunotherapy of human breast cancer xenografts in athymic mice coexpressing HER2 and EGFR using ^{177}Lu-or ^{111}In-labeled bispecific radioimmunoconjugates. *J Nucl Med*. 2016;57:444–452.
59. Kwon LY, Scollard DA, Reilly RM. ^{64}Cu-labeled trastuzumab fab-PEG24-EGF radioimmunoconjugates bispecific for HER2 and EGFR: pharmacokinetics, biodistribution, and tumor imaging by PET in comparison to monospecific agents. *Mol Pharm*. 2017;14:492–501.
60. Van Eijck CH, Krenning EP, Bootsma A, et al. Somatostatin-receptor scintigraphy in primary breast cancer. *Lancet*. 1994;343:640–643.
61. Reubi J, Lang W, Maurer R, et al. Distribution and biochemical characterization of somatostatin receptors in tumors of the human central nervous system. *Cancer Res*. 1987;47:5758–5764.
62. Vikić-Topić S, Raisch KP, Kvols LK, et al. Expression of somatostatin receptor subtypes in breast carcinoma, carcinoid tumor, and renal cell carcinoma. *J Clin Endocrinol Metab*. 1995;80:2974–2979.
63. Lee H, Suh M, Choi H, et al. A pan-cancer analysis of the clinical and genetic portraits of somatostatin receptor expressing tumor as a potential target of peptide receptor imaging and therapy. *EJNMMI Res*. 2020;10:42.
64. Orlando C, Raggi CC, Bianchi S, et al. Measurement of somatostatin receptor subtype 2 mRNA in breast cancer and corresponding normal tissue. *Endocr Relat Cancer*. 2004;11:323–332.
65. Kumar U, Grigorakis SI, Watt HL, et al. Somatostatin receptors in primary human breast cancer: quantitative analysis of mRNA for subtypes 1–5 and correlation with receptor protein expression and tumor pathology. *Breast Cancer Res Treat*. 2005;92:175–186.
66. Terlević R, Perić Balja M, Tomas D, et al. Somatostatin receptor SSTR2A and SSTR5 expression in neuroendocrine breast cancer. *Ann Diagn Pathol*. 2019;38:62–66.
67. Savelli G, Zaniboni A, Bertagna F, et al. Peptide receptor radionuclide therapy (PRRT) in a patient affected by metastatic breast cancer with neuroendocrine differentiation. *Breast Care*. 2012;7:408–410.
68. Pollak M. The potential role of somatostatin analogues in breast cancer treatment. *Yale J Biol Med*. 1997;70:535–539.
69. Dude I, Zhang Z, Rousseau J, et al. Evaluation of agonist and antagonist radioligands for somatostatin receptor imaging of breast cancer using positron emission tomography. *EJNMMI Radiopharm Chem*. 2017;2:4.
70. Reubi JC, Waser B, Foekens JA, et al. Somatostatin receptor incidence and distribution in breast cancer using receptor autoradiography: relationship to EGF receptors. *Int J Cancer*. 1990;46:416–420.
71. Dalm SU, de Jong M. Comparing the use of radiolabeled SSTR agonists and an SSTR antagonist in breast cancer: does the model choice influence the outcome? *EJNMMI Radiopharm Chem*. 2017;2:11.
72. Dalm SU, Nonnekens J, Doeswijk GN, et al. Comparison of the therapeutic response to treatment with a ^{177}Lu-labeled somatostatin receptor agonist and antagonist in preclinical models. *J Nucl Med*. 2016;57:260–265.
73. Chereau E, Durand L, Frati A, et al. Correlation of immunohistopathological expression of somatostatin receptor-2 in breast cancer and tumor detection with ^{68}Ga-DOTATOC and ^{18}F-FDG PET imaging in an animal model. *Anticancer Res*. 2013;33:3015–3019.
74. Bajc M, Ingvar C, Palmer J. Dynamic indium-111-pentetreotide scintigraphy in breast cancer. *J Nucl Med*. 1996;37:622–626.
75. Schulz S, Helmholz T, Schmitt J, et al. True positive somatostatin receptor scintigraphy in primary breast cancer correlates with expression of sst2A and sst5. *Breast Cancer Res Treat*. 2002;72:221–226.

76. Gómez-de la Fuente FJ, Jiménez-Bonilla J, Estevez J, et al. Somatostatin receptors expression in breast cancer. *Cir Esp*. 2017;95:545–547.
77. Liu Q, Zhang J, Kulkarni HR, et al. ^{177}Lu-DOTATOC peptide receptor radionuclide therapy in a patient with neuroendocrine breast carcinoma and breast invasive ductal carcinoma. *Clin Nucl Med*. 2020;45:e232–e235.
78. Wernicke AG, Kim S, Liu H, et al. Prostate-specific membrane antigen (PSMA) expression in the neovasculature of gynecologic malignancies: implications for PSMA-targeted therapy. *Appl Immunohistochem Mol Morphol*. 2017;25:271–276.
79. Haffner MC, Kronberger IE, Ross JS, et al. Prostate-specific membrane antigen expression in the neovasculature of gastric and colorectal cancers. *Hum Pathol*. 2009;40:1754–1761.
80. Lebert JM, Lester R, Powell E, et al. Advances in the systemic treatment of triple-negative breast cancer. *Curr Oncol*. 2018;25:S142–S150.
81. Lin NU, Vanderplas A, Hughes ME, et al. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the National Comprehensive Cancer Network. *Cancer*. 2012;118: 5463–5472.
82. Wernicke AG, Varma S, Greenwood EA, et al. Prostate-specific membrane antigen expression in tumor-associated vasculature of breast cancers. *APMIS*. 2014;122:482–489.
83. Kasoha M, Unger C, Solomayer E-F, et al. Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases. *Clin Exp Metastasis*. 2017;34:479–490.
84. Passah A, Arora S, Damle NA, et al. ^{68}Ga-prostate-specific membrane antigen PET/CT in triple-negative breast cancer. *Clin Nucl Med*. 2018; 43:460–461.
85. Morgenroth A, Tinkir E, Vogg ATJ, et al. Targeting of prostate-specific membrane antigen for radio-ligand therapy of triple-negative breast cancer. *Breast Cancer Res*. 2019;21:116.
86. Fang J, Xiao L, Joo KI, et al. A potent immunotoxin targeting fibroblast activation protein for treatment of breast cancer in mice. *Int J Cancer*. 2016; 138:1013–1023.
87. Loktev A, Lindner T, Mier W, et al. A tumor-imaging method targeting cancer-associated fibroblasts. *J Nucl Med*. 2018;59:1423–1429.
88. Xia Q, Geng F, Zhang F-F, et al. Cyclophosphamide enhances anti-tumor effects of a fibroblast activation protein α-based DNA vaccine in tumor-bearing mice with murine breast carcinoma. *Immunopharmacol Immunotoxicol*. 2017;39:37–44.
89. Lindner T, Loktev A, Giesel F, et al. Targeting of activated fibroblasts for imaging and therapy. *EJNMMI Radiopharm Chem*. 2019;4:16.
90. Jansen K, Heirbaut L, Verkerk R, et al. Extended structure–activity relationship and pharmacokinetic investigation of (4-quinolinoyl) glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP). *J Med Chem*. 2014;57:3053–3074.
91. Brennen WN, Isaacs JT, Denmeade SR. Rationale behind targeting fibroblast activation protein–expressing carcinoma-associated fibroblasts as a novel chemotherapeutic strategy. *Mol Cancer Ther*. 2012;11:257–266.
92. Jansen K, Heirbaut L, Cheng JD, et al. Selective inhibitors of fibroblast activation protein (FAP) with a (4-quinolinoyl)-glycyl-2-cyanopyrrolidine scaffold. *ACS Med Chem Lett*. 2013;4:491–496.
93. Giesel FL, Adeberg S, Syed M, et al. FAPI-74 PET/CT using either ^{18}F-AlF or cold-kit ^{68}Ga labeling: biodistribution, radiation dosimetry, and tumor delineation in lung cancer patients. *J Nucl Med*. 2021;62:201–207.
94. Lindner T, Loktev A, Altmann A, et al. Development of quinoline-based theranostic ligands for the targeting of fibroblast activation protein. *J Nucl Med*. 2018;59:1415–1422.
95. Loktev A, Lindner T, Burger EM, et al. Development of fibroblast activation protein-targeted radiotracers with improved tumor retention. *J Nucl Med*. 2019;60:1421–1429.
96. Baum R, Chantadisai M, Smerling C, et al. Peptide-targeted radionuclide therapy (PTRT) using Lu-177 FAP-2286 in diverse adenocarcinomas: feasibility, biodistribution and preliminary dosimetry in a first-in-human study. *J Nucl Med*. 2020;61(suppl 1):633.
97. Ballal S, Yadav MP, Kramer V, et al. A theranostic approach of [^{68}Ga]Ga-DOTA.SA.FAPi PET/CT-guided [^{177}Lu]Lu-DOTA.SA.FAPi radionuclide therapy in an end-stage breast cancer patient: new frontier in targeted radionuclide therapy. *Eur J Nucl Med Mol Imaging*. 2020.
98. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol*. 2005;18: 1295–1304.
99. Price MR, Rye PD, Petrakou E, et al. Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. San Diego, Calif., November 17–23, 1996. *Tumour Biol*. 1998;19:1–20.
100. Kufe DW. MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. *Oncogene*. 2013;32: 1073–1081.
101. Kelly VJ, Wu ST, Gottumukkala V, et al. Preclinical evaluation of an ^{111}In/^{225}Ac theranostic targeting transformed MUC1 for triple negative breast cancer. *Theranostics*. 2020;10:6946–6958.
102. Couto JR, Blank EW, Peterson JA, et al. Engineering of antibodies for breast cancer therapy: construction of chimeric and humanized versions of the murine monoclonal antibody BrE-3. In: Ceriani RL, eds. *Antigen and Antibody Molecular Engineering in Breast Cancer Diagnosis and Treatment. Advances in Experimental Medicine and Biology*. Boston, MA: Springer; 1994;353:55–59.
103. Schrier DM, Stemmer SM, Johnson T, et al. High-dose ^{90}Y Mx-diethylenetriaminepentaacetic acid (DTPA)-BrE-3 and autologous hematopoietic stem cell support (AHSCS) for the treatment of advanced breast cancer: a phase I trial. *Cancer Res*. 1995;55:5921s–5924s.
104. Howell LP, DeNardo SJ, Levy NB, et al. Immunohistochemical staining of metastatic ductal carcinomas of the breast by monoclonal antibodies used in imaging and therapy: a comparative study. *Int J Biol Markers*. 1995;10:129–135.
105. Richman CM, DeNardo SJ. Systemic radiotherapy in metastatic breast cancer using ^{90}Y-linked monoclonal MUC-1 antibodies. *Crit Rev Oncol Hematol*. 2001;38:25–35.
106. Kramer EL, DeNardo SJ, Liebes L, et al. Radioimmunolocalization of metastatic breast carcinoma using indium-111-methyl benzyl DTPA BrE-3 monoclonal antibody: phase I study. *J Nucl Med*. 1993;34:1067–1074.
107. Solursh M, Reiter RS, Jensen KL, et al. Transient expression of a cell surface heparan sulfate proteoglycan (syndecan) during limb development. *Dev Biol*. 1990;140:83–92.
108. Rousseau C, Ruellan AL, Bernardeau K, et al. Syndecan-1 antigen, a promising new target for triple-negative breast cancer immuno-PET and radioimmunotherapy. A preclinical study on MDA-MB-468 xenograft tumors. *EJNMMI Res*. 2011;1:20.
109. Baba F, Swartz K, van Buren R, et al. Syndecan-1 and syndecan-4 are overexpressed in an estrogen receptor-negative, highly proliferative breast carcinoma subtype. *Breast Cancer Res Treat*. 2006;98:91–98.
110. Sayyad MR, Puchalapalli M, Vergara NG, et al. Syndecan-1 facilitates breast cancer metastasis to the brain. *Breast Cancer Res Treat*. 2019; 178:35–49.
111. Rane SS, Sultana Javad JM, Rees RC. Tumor antigen and epitope identification for preclinical and clinical evaluation. In: Rezaei N, ed. *Cancer Immunology: Bench to Bedside Immunotherapy of Cancers*. Berlin, Germany: Springer; 2015:55–71.
112. Gasparini G. Clinical significance of the determination of angiogenesis in human breast cancer: update of the biological background and overview of the Vicenza studies. *Eur J Cancer*. 1996;32:2485–2493.
113. Folkman J, Watson K, Ingber D, et al. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature*. 1989;339:58–61.
114. Rau KM, Su YL, Li SH, et al. High expression of endoglin in primary breast cancer may predict response to neoadjuvant chemotherapy. *Mol Med Rep*. 2017;16:7185–7190.
115. Tanaka F, Otake Y, Yanagihara K, et al. Evaluation of angiogenesis in non-small cell lung cancer: comparison between anti-CD34 antibody and anti-CD105 antibody. *Clin Cancer Res*. 2001;7:3410–3415.
116. Rau K-M, Huang C-C, Chiu T-J, et al. Neovascularization evaluated by CD105 correlates well with prognostic factors in breast cancers. *Exp Ther Med*. 2012;4:231–236.
117. Bredow S, Lewin M, Hofmann B, et al. Imaging of tumour neovasculature by targeting the TGF-β binding receptor endoglin. *Eur J Cancer*. 2000; 36:675–681.
118. Fonsatti E, Jekunen AP, Kairemo KJ, et al. Endoglin is a suitable target for efficient imaging of solid tumors: in vivo evidence in a canine mammary carcinoma model. *Clin Cancer Res*. 2000;6:2037–2043.
119. Ehlerding EB, Ferreira CA, Aluicio-Sarduy E, et al. 86/90Y-based theranostics targeting angiogenesis in a murine breast cancer model. *Mol Pharm*. 2018;15:2606–2613.
120. Hubbard AK, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. *Free Radic Biol Med*. 2000;28: 1379–1386.
121. Roland CL, Harken AH, Sarr MG, et al. ICAM-1 expression determines malignant potential of cancer. *Surgery*. 2007;141:705–707.

122. Guo P, Huang J, Wang L, et al. ICAM-1 as a molecular target for triple negative breast cancer. *Proc Natl Acad Sci U S A*. 2014;111:14710–14715.
123. You L, Wang X, Guo Z, et al. MicroSPECT imaging of triple negative breast cancer cell tumor xenografted in athymic mice with radioiodinated anti-ICAM-1 monoclonal antibody. *Appl Radiat Isot*. 2018;139:20–25.
124. Balkwill FR. The chemokine system and cancer. *J Pathol*. 2012;226:148–157.
125. Xu C, Zhao H, Chen H, et al. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. *Drug Des Devel Ther*. 2015;9:4953–4964.
126. Boudot A, Kerdivel G, Habauzit D, et al. Differential estrogen-regulation of CXCL12 chemokine receptors, CXCR4 and CXCR7, contributes to the growth effect of estrogens in breast cancer cells. *PloS One*. 2011;6:e20898.
127. Kerdivel G, Boudot A, Pakdel F. Estrogen represses CXCR7 gene expression by inhibiting the recruitment of NFκB transcription factor at the CXCR7 promoter in breast cancer cells. *Biochem Biophys Res Commun*. 2013;431:729–733.
128. Boudot A, Kerdivel G, Lecomte S, et al. COUP-TFI modifies CXCL12 and CXCR4 expression by activating EGF signaling and stimulates breast cancer cell migration. *BMC Cancer*. 2014;14:407.
129. Lefort S, Thuleau A, Kieffer Y, et al. CXCR4 inhibitors could benefit to HER2 but not to triple-negative breast cancer patients. *Oncogene*. 2017;36:1211–1222.
130. Chen IX, Chauhan VP, Posada J, et al. Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer. *Proc Natl Acad Sci U S A*. 2019;116:4558–4566.
131. Marques CS, Santos AR, Gameiro A, et al. CXCR4 and its ligand CXCL12 display opposite expression profiles in feline mammary metastatic disease, with the exception of HER2-overexpressing tumors. *BMC Cancer*. 2018;18:741.
132. Vag T, Steiger K, Rossmann A, et al. PET imaging of chemokine receptor CXCR4 in patients with primary and recurrent breast carcinoma. *EJNMMI Res*. 2018;8:90.
133. Li H, Zhang X, Wu HY, et al. ^{64}Cu-labeled ubiquitin for PET imaging of CXCR4 expression in mouse breast tumor. *ACS Omega*. 2019;4:12432–12437.
134. Walenkamp AME, Lapa C, Herrmann K, et al. CXCR4 ligands: the next big hit? *J Nucl Med*. 2017;58:77S–82S.
135. Boers J, de Vries EFJ, Glaudemans AWJM, et al. Application of PET tracers in molecular imaging for breast cancer. *Curr Oncol Rep*. 2020;22:85.
136. Hermanto S, Haryuni RD, Ramli M, et al. Synthesis and stability test of radioimmunoconjugate ^{177}Lu-DOTA-F (ab′) 2-trastuzumab for theranostic agent of HER2 positive breast cancer. *J Radiat Res Appl Sci*. 2016;9:441–448.