Advances in the Application of Radionuclide-Labeled HER2 Affibody for the Diagnosis and Treatment of Ovarian Cancer

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Human epidermal growth factor receptor 2 (HER2) is a highly expressed tumor marker in epithelial ovarian cancer, and its overexpression is considered to be a potential factor of poor prognosis. Therefore, monitoring the expression of HER2 receptor in tumor tissue provides favorable conditions for accurate localization, diagnosis, targeted therapy, and prognosis evaluation of cancer foci. Affibody has the advantages of high affinity, small molecular weight, and stable biochemical properties. The molecular probes of radionuclide-labeled HER2 affibody have recently shown broad application prospects in the diagnosis and treatment of ovarian cancer; the aim is to introduce radionuclides into the cancer foci, display systemic lesions, and kill tumor cells through the radioactivity of the radionuclides. This process seamlessly integrates the diagnosis and treatment of ovarian cancer. Current research and development of new molecular probes of radionuclide-labeled HER2 affibody should focus on overcoming the deficiencies of non-specific uptake in the kidney, bone marrow, liver, and gastrointestinal tract, and on reducing the background of the image to improve image quality. By modifying the amino acid sequence; changing the hydrophilicity, surface charge, and lipid solubility of the affibody molecule; and using different radionuclides, chelating agents, and labeling conditions to optimize the labeling method of molecular probes, the specific uptake of molecular probes at tumor sites will be improved, while reducing radioactive retention in non-target organs and obtaining the best target/non-target value. These measures will enable the clinical use of radionuclide-labeled HER2 affibody molecular probes as soon as possible, providing a new clinical path for tumor-specific diagnosis, targeted therapy, and efficacy evaluation. The purpose of this review is to describe the application of radionuclide-labeled HER2 affibody in the imaging and treatment of ovarian cancer, including its potential clinical value and dilemmas.

Keywords: human epidermal growth factor receptor, ovarian cancer, radionuclide, HER2 affibody, molecular probes

INTRODUCTION

Ovarian cancer is the disease with the highest mortality rate after cervical cancer secondary to female reproductive tract malignant tumors; in 2020, 313,959 new patients with ovarian cancer and 207,252 new deaths from ovarian cancer were reported worldwide. Although ovarian cancer is generally more common in developed countries, studies have shown an increasing incidence in China (1, 2). Several risk factors for ovarian cancer include genetics, reproduction, sex hormones, and lifestyle behaviors (3). Specifically, a family history of ovarian cancer in first- or second-degree relatives, polycystic ovary syndrome, endometriosis, gynecological inflammation, increased estrogen/androgen, high-fat diet, etc. are associated with a high risk of cancer. However, fertility, contraception including the intrauterine device and oral contraceptives, tubal ligation, and breastfeeding can reduce the risk of ovarian cancer (4–14). Ovarian cancer has an insidious onset and lacks specific clinical symptoms in the early stage, and the clinical diagnostic process is relatively unsatisfactory. Approximately 60% of patients with ovarian cancer are in the advanced stage of the disease by the time symptoms are recognized or medical help is sought. Therefore, the prognosis of patients is poor, with a 5-year survival rate of less than 30% (15, 16).

OVERVIEW OF HER2 AND AFFIBODY

Human epidermal growth factor receptor 2 (HER2), also known as receptor tyrosine protein kinase erbB-2, is a member of the epidermal growth factor receptor family (17). The oncogenic mechanisms of HER2 include inhibiting tumor cell apoptosis, increasing tumor cell invasiveness, promoting tumor cell proliferation, and promoting tumor angiogenesis and lymphangiogenesis (18). HER2 is rarely expressed in normal ovarian epithelial cells, but is highly positive in epithelial ovarian cancers, including 45.5% of mucinous carcinomas, 41.7% of clear cell carcinomas, and 17.5% of serous carcinomas (19). Studies confirmed that the positive expression of HER2 is significantly correlated with patient prognosis (20, 21). Therefore, it is significantly important to obtain HER2-positive expression accurately and effectively for the precise diagnosis and targeted therapy of HER2-positive ovarian cancer.

The use of multiple methods to detect the presence of relevant mutations in tumor specimens and the optimal targets of targeted therapy, which are useful for prognosis and treatment, is the best strategy to strengthen and improve patients with ovarian cancer (22–24). Clinically, the state of HER2 expression is often determined by immunohistochemistry (IHC) and nucleic acid fluorescence *in situ* hybridization (FISH) on biopsy tissue (25). IHC uses the principle of specific binding of antigen and antibody, and determines the antigen in tissue cells through a chemical reaction to develop the color of the chromogenic reagent labeled with the antibody. However, the stability of this method is poor, and various technical variables, different antibody sensitivities, and resulting evaluation systems

during the operation may affect the test results (26). FISH is a molecular cytogenetic technique that uses fluorescent probes that bind only to parts of chromosomes with a high degree of sequence complementarity. DNA is analyzed under the microscope by fluorescence detection (25, 26). By using gene-specific DNA probes to determine the copy number of the HER2 gene, the detection results are reliable due to the relative stability of the DNA; however, this method is expensive and technically complicated. Furthermore, the expression of the protein is regulated by many factors; therefore, the HER2 gene is regulated by many factors and the amplification of the HER2 gene is not always consistent with the overexpression of protein (27, 28). In addition, both IHC and FISH are invasive tests, which only reflect the local situation of the biopsy tissue. These tests cannot reflect the HER2 expression of the tumor as a whole and other metastases. Hillig et al. showed that the misdiagnosis rate of HER2 expression measured by IHC or FISH is 20% (29). Single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging can obtain functional information about the organization of biological chemistry. Advantages of these methods include noninvasiveness, accuracy, and safety. become one of the hot spot of the HER2 receptor positive tumors. The positive rate of HER2 expression in ovarian cancer is low. Although the nuclear medicine molecular probe targeting the HER2 receptor is not suitable for the screening and diagnosis of ovarian cancer directly, it can be used as an important complementary means to IHC or FISH. This method can obtain a general expression of HER2 positive, including primary tumor location, the extent of the tumor invasion into the surrounding tissue, and the detection of metastasis sites. In addition, targeting the HER2 receptor helps clinicians monitor the efficacy of HER2-targeted therapy in patients and facilitates stratified patient studies, laying a solid foundation for the integration of ovarian cancer diagnosis and treatment.

Affibody is an artificial protein molecule with a single-chain structure, and is a new type of protein ligand that evolved from the B segment of the immunoglobulin binding region of *Staphylococcus* protein A, with a relative molecular weight of 6.5 kDa. Affibody has the ability to bind to other proteins (30). SPA is a cell wall protein of type A *Staphylococcus aureus*, containing 7 domains (S, E, D, A, B, C, and X), among which the B domain contains 58 amino acid residues and is the main functional fragment that mediates the binding of SPA to the FC segment of IgG (except IgG3) (31). These amino acid defects constitute three α-helix structures. In 1995, to improve the chemical stability of the affinity body, the Swedish scholar Nord et al. replaced the 29th glycine of the B domain with alanine and renamed it as the Z domain (32). The amino acids at 13 positions in the first and second helices of the Z domain are Q9 (glutamine), Q10 (glutamine), N11 (asparagine), F13 (benzene), Y14 (tyrosine), L17 (leucine), and H18 (histidine) of the first α-helix, and (alanine), E24 (glutamic acid), E25 (glutamic acid), R27 (arginine), N28 (asparagine), Q32 (glutamine), and K35 (lysine) of the second α-helix, which have no obvious effect on the higher-order structure of the Z
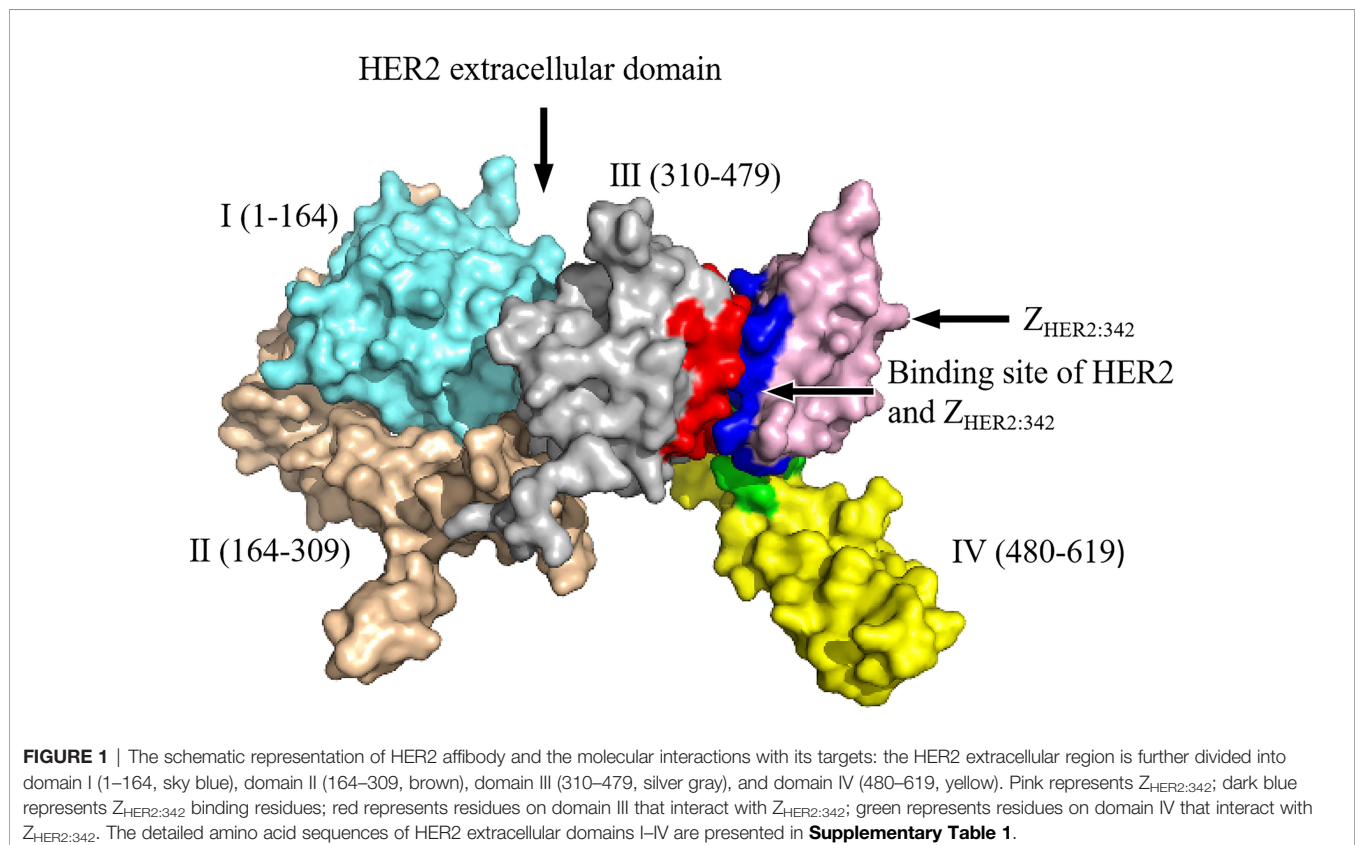
domain. The Z domain retains the binding function of the Fc segment (33). Random replacement of these 13 amino acid codons with degenerate codon NNK (K = G, Guanylate or T, Thymine, including 32 codons and 20 amino acids) can theoretically generate 3,213 gene sequences and 2,013 amino acid sequences; these constitute the affibody library. The affibody library can basically be combined with any protein molecule, and the affinity body of a certain protein molecule can be obtained after screening (32, 34). In addition to HER2, a variety of proteins such as fibrinogen, transferrin, tumor necrosis factor Q, interleukin-8, CD28, human serum albumin, IgA, IgE, IgM, and epidermal growth factor receptor (EGFR) have been discovered, and their affinity is in the range of μmol/L to pmol/L according to the characteristics of the bound proteins and the structure of the affibody (35). Due to the small size and high affinity of HER2 affibody that can specifically bind to HER2 receptor both *in vitro* and *in vivo*, rapid clearance from blood and non-targeted tissues, ease of structural modification, and selectivity for cancer-related targets, this affibody is suitable as a radionuclide molecular probe and has excellent potential for use in the diagnosis and treatment of cancer (36–41). The schematic representation of the HER2 affibody and its interaction with the target molecule, HER2, is shown in **Figure 1**.
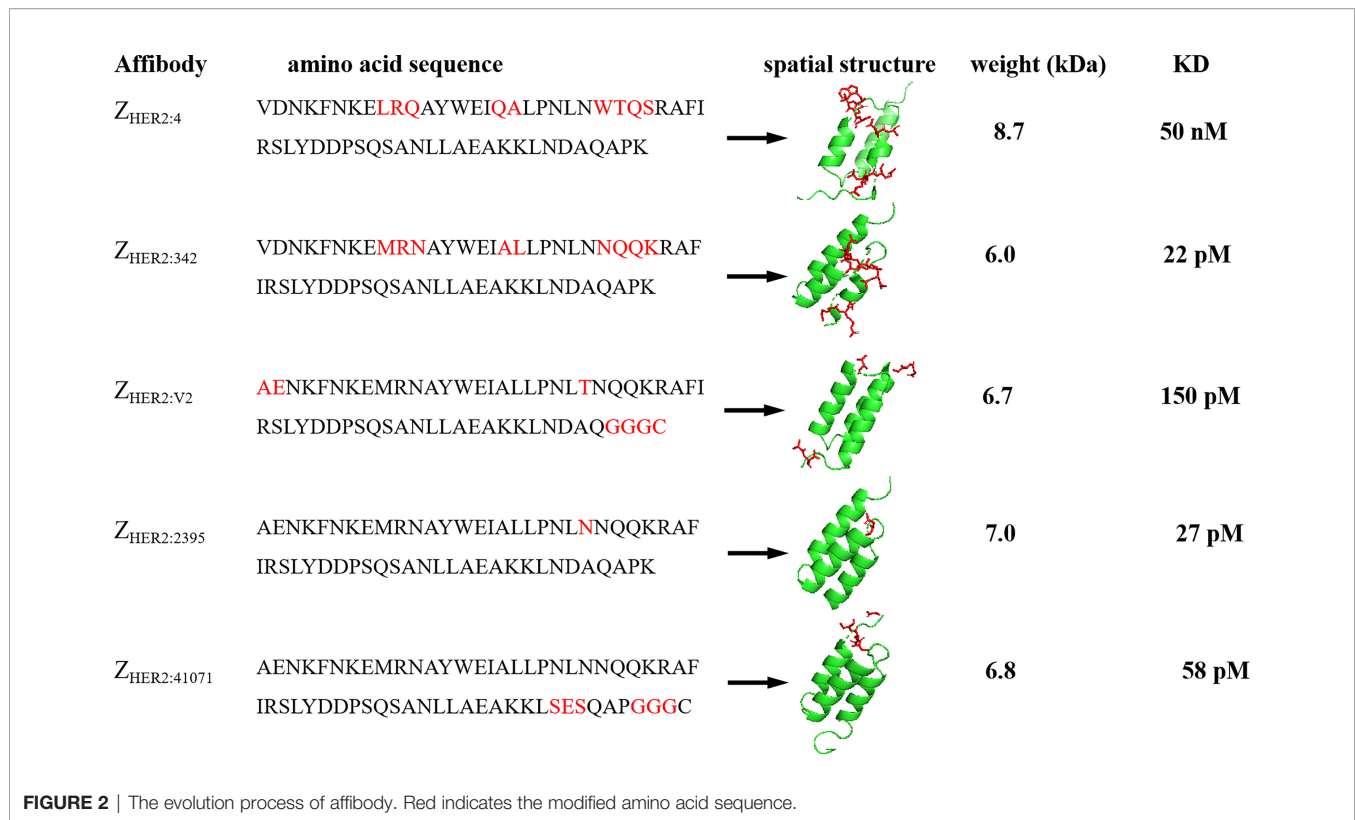
The first-generation HER2 affibody $Z_{HER2:4}$ has a low affinity; therefore, researchers combined its head and tail to form a dimer $(Z_{HER2:4})2$, and its dissociation equilibrium constant KD value changed from the original 50 nM to 3 nM, leading to obvious improvement in its affinity (42, 43). The second-generation HER2 affibody is mainly $Z_{HER2:342}$, which has a triple-helix structure through modification of the first generation; its affinity is 2,200 times that of the first generation (44). The third-generation affibody is mainly $Z_{HER2:2395}$, which is made by introducing a cysteine at the carboxy terminus of $Z_{HER2:342}$ to obtain a unique attachment site, using bifunctional chelators, linking molecules or other thiol-reactive carriers, such as maleimide or iodoacetamide, which can be directionally coupled to this particular cysteine. $Z_{HER2:2395}$ has an affinity of approximately 27 pM, which is comparable to that of the second generation (45, 46). A recent study reported the replacement of the -NDA-sequence near the C segment of the affibody $Z_{HER2:2395}$ with the -SES-sequence, which further improved the stability and hydrophilicity of the affibody, resulting in an affinity that is close to $Z_{HER2:2395}$ (47). The detailed evolution process of the affibody is shown in **Figure 2**.

OVERVIEW OF RADIONUCLIDE-LABELED HER2 AFFIBODY MOLECULAR PROBES

Molecular probes targeting HER2 affibody can evaluate the expression status of tumor HER2 receptors and predict early efficacy after treatment, and significant progress has been made in the research on the integration of tumor diagnosis and treatment. The main applications of radionuclide-labeled HER2 molecular probes are summarized in **Table 1**.

FIGURE 1 | The schematic representation of HER2 affibody and the molecular interactions with its targets: the HER2 extracellular region is further divided into domain I (1–164, sky blue), domain II (164–309, brown), domain III (310–479, silver gray), and domain IV (480–619, yellow). Pink represents $Z_{HER2:342}$; dark blue represents $Z_{HER2:342}$ binding residues; red represents residues on domain III that interact with $Z_{HER2:342}$; green represents residues on domain IV that interact with $Z_{HER2:342}$. The detailed amino acid sequences of HER2 extracellular domains I–IV are presented in **Supplementary Table 1**.

FIGURE 2 | The evolution process of affibody. Red indicates the modified amino acid sequence.

HER2 Affibody Molecular Probes as SPECT Tracers

[^{111}In]: Indium-11 (^{111}In) is a pure γ-ray emitter with a half-life of 2.81 days, making it an ideal radionuclide tracer for SPECT imaging. Benzyl-dtpa,-CHX-A"-DTPA (diethylenetriaminepentaacetic acid) and DOTA (1,4,7,l0-tetraazacyclodo-decane-*N,N,N,N*-tetra acetic acid) have been used as chelating agents for affibody Z$_{HER2:342}$. When labeled with ^{111}In, which showed a good labeling yield (>95%) and a high affinity (21-65 pM), the molecular probe with specific uptake by the SKOV3 ovarian cancer xenograft tumor tissues with high HER2 receptor expression showed high tumor targeting binding properties (48–52). Unfortunately, in addition to tumor uptake of these molecular probes, renal

TABLE 1 | Overview of the major applications of radionuclide-labeled HER2 affibody molecular probes.

Radioisotope	T1/2	Diagnostic/therapy	Representative molecular probes	Study object	First posted	Phase	References
^{111}In	2.81 days	SPECT	^{111}In-ABY-002	Ovarian cancer	05/2006	Preclinical	(48–52)
				Breast cancer	07/2007	I	(53)
			^{111}In-DOTA-Z$_{HER2:342}$	Ovarian cancer	08/2008	Preclinical	(49)
			^{111}In-Z$_{HER2:2395}$	Prostate cancer	05/2012	Preclinical	(54)
^{99m}Tc	6.02 h	SPECT	^{99m}Tc-Z$_{HER2:342}$	Ovarian cancer	12/2006	Preclinical	(55–59)
			^{99m}Tc-Z$_{HER2:V2}$	Lung cancer	11/2018	Preclinical	(60, 61)
				Ovarian cancer	02/2011	Preclinical	(62, 63)
			^{99m}Tc-Z$_{HER2:41071}$	Ovarian cancer	03/2021	Preclinical	(47)
			^{99m}Tc-ABH2	Breast cancer	06/2018	I	(64)
^{125}I	60 days	SPECT/therapy	^{125}I-Z$_{HER2:4}$	Ovarian cancer	06/2005	Preclinical	(42, 43)
			^{125}I-IPEM-Z$_{HER2:2395}$	Ovarian cancer	01/2015	Preclinical	(65)
^{18}F	109.8 min	PET	^{18}F-Z$_{HER2:342}$	Breast cancer	06/2012	Preclinical	(66)
				Ovarian cancer	12/2007	Preclinical	(67, 68)
^{68}Ga	68 min	PET	^{68}Ga-DOTA-MUT-DS	Ovarian cancer	08/2009	Preclinical	(69)
			^{68}Ga-ABY-002	Breast cancer	07/2007	I	(53)
			^{68}Ga-ABY-025	Breast cancer	05/2013	I/II	(70, 71)
^{64}Cu	12.7 h	PET/therapy	^{64}Cu-Z$_{HER2:342}$	Ovarian cancer	10/2019	Preclinical	(72)
^{188}Re	17 h	Therapy	^{188}Re-Z$_{HER2:V2}$	Ovarian cancer	10/2014	Preclinical	(73, 74)
^{177}Lu	6.7 days	Therapy	^{177}Lu-Z$_{HER2:342}$	Ovarian cancer	03/2007	Preclinical	(75)

uptake is also high, increasing the potential for renal toxicity. Based on this disadvantage, Honarvar et al. (76) designed a 15-mer HP1 PNA recognition tag and a complementary HP2 hybridization probe ^{111}In-Z$_{HER2:342}$-SR-HP1 to reduce the uptake of the molecular probe in the kidney. Moreover, a number of studies have used DOTA as a chelating agent to bind to the affibodies Z$_{HER2:342min}$, Z$_{HER2:2395}$, and Z$_{HER2:2395}$-Cys, and then labeled with the radionuclide ^{111}In. This method shows good potential for targeted imaging (77–80). However, one disadvantage of using DOTA as a chelator is that the liver uptake is relatively high. Researchers then compared the results of using DOTAGA (2-[1,4,7,10-Tetraazacyclododecane-4,7,10-tris(t-butyl acetate)]-pentanedioic acid-1t-butyl ester) instead of DOTA as a chelator, and found that the change improved the biodistribution of molecular probes *in vivo*, reducing uptake by the liver (81).

[^{99m}Tc]: Technetium-99m (^{99m}Tc) emits γ-rays of 140 keV and has a half-life of 6.02 h. It can be obtained by the ^{99}Mo-^{99m}Tc generator and is inexpensive; therefore, it is widely used in clinical and basic research. An early study used the indirect labeling method to add chelating agent maGGG (mercaptoacetyl-glycyl-glycyl-glycyl) to the N-terminal of the affibody Z$_{HER2:342}$, and successfully labeled affibody Z$_{HER2:342}$ with ^{99m}Tc. The researchers further completed a series of *in vivo* biodistribution and imaging studies, and the results showed that the molecular probe was specifically absorbed by SKOV3 ovarian cancer xenograft tumor with high expression of the HER2 receptor and high targeting ability (79). However, the liver and gastrointestinal tract uptake of this molecular probe is high, thus affecting the detection rate of abdominal lesions. Subsequently, the research team replaced the chelating agent maGGG with maGSG (mercaptoacetyl-glycyl-seryl-glycyl), maG(D-S)G (mercaptoacetyl-glycyl-D-seryl-glycyl), and maSSS (mercaptoacetyl-seryl-seryl-seryl), respectively, and labeled it with ^{99m}Tc. Results showed that when the glycine residue in the chelating agent maSSS was replaced with a hydrophilic serine amino group, the hydrophilic activity of the chelating agent maGGG was increased. Therefore, the uptake of the molecular probe in the liver and gastrointestinal tract was significantly reduced, resulting in a significant increase in the detection rate of abdominal tumor lesions. However, the excretion route was shifted from the liver to the kidney, increasing the potential for kidney toxicity (80). To overcome this shortcoming, the team further optimized the study design and compared the ^{99m}Tc labeling studies using maGEG (mercaptoacetyl-Gly-Glu-Gly), maEEE (Mercaptoacetyl-Glu-Glu-G1u), maESE (mercaptoacetyl-glutamyl-seryl-glutamyl), maEES (mercaptoacetyl-glutamyl-glutamyl-seryl), maSEE (mercaptoacetyl-seryl-glutamyl-glutamyl), maSKS (mercaptoacetyl-seryl-lysyl-seryl), and maKKK (mercaptoacetyl-trilysyl) as chelators. The results showed that the use of the molecular probes ^{99m}Tc-maEEE-Z$_{HER2:342}$, ^{99m}Tc-maSKS-Z$_{HER2:342}$, and ^{99m}Tc-maESE-Z$_{HER2:342}$ with maEEE, maSKS, and maESE as chelators significantly reduced the radioactive uptake in the kidney. In addition, the radioactive uptake of ^{99m}Tc- maESE-Z$_{HER2:342}$ was found to be the most obvious, further improving the abdominal image quality (55, 56, 81).

Furthermore, other studies introduced a chelating agent at the C-terminus or/and N-terminus of the affibody Z$_{HER2:342}$ for ^{99m}Tc labeling, and obtained molecular probes ^{99m}Tc-(HE)$_3$-Z$_{HER2:342}$-GGGC, ^{99m}Tc-Z$_{HER2:342}$-GGGC, ^{99m}Tc-Z$_{HER2:V2}$, etc. The researchers performed *in vivo* biodistribution and imaging studies, and results showed that these molecular probes are stable *in vitro* and *in vivo*, with high specificity and high targeting (82–84).

[^{123}I/^{125}I]: Iodine-123 (^{123}I) has a half-life of 13.3 h and can emit 159 keV γ rays, which is suitable for SPECT imaging. ^{125}I has a long half-life (59.4 days) and is a convenient replacement nuclide usually used in the development of radioactive iodization technology instead of ^{123}I and ^{124}I (85). An early study used ^{125}I labeling for the affibody (Z$_{HER2:4}$)2, but SPECT imaging showed high radioactivity retention in the kidney and liver in addition to tumor uptake. This resulted in substandard image quality (86). To address the prevention of affibody destruction by radionuclides upon labeling, further studies were performed with iodobenzoate (PIB) and [4-isothiocyanate-amino]-undecahydro-o-dodecaborate (DABI) as chelators to label affibody molecule Z$_{HER2:342}$ with ^{125}I, and molecular probes ^{125}I-PIB-Z$_{HER2:342}$ and ^{125}I-DABI-Z$_{HER2:342}$ were prepared. Results showed that ^{125}I-PIB-Z$_{HER2:342}$ expressed high contrast imaging and low renal radioretention of HER2 in the mice bearing SKOV-3 xenografts; however, a disadvantage was that the molecular probe did not bind specifically to the tumor (44, 87). Subsequently, the team used HPEM(4-hydroxyphenyl-ethyl-maleimide) as a chelating agent to design and synthesize the molecular probe ^{131}I/^{125}I-HPEM-Z$_{HER2:342}$-C. Compared with the previous molecular probe ^{125}I-PIB-Z$_{HER2:342}$, HPEM provided a site-specific conjugate for indirect radioiodination of cysteine-containing affibody Z$_{HER2:342}$, and preserved the specificity of binding to HER2-expressing cells (88). Several studies have shown that the labeling strategy of using HPEM as a chelator further reduces renal uptake, but increases hepatobiliary and gastric uptake; thus, this strategy is detrimental to the detection of abdominal metastases (85, 87, 88). To solve this problem, the HEHEHE sequence was recently introduced into the amino terminal of the affibody, and the molecular probe ^{125}I-PIB-(HE)$_3$-G$_3$ was prepared by using PIB as a chelating agent. This process reduces the radioactive retention of the liver and kidney while maintaining the highly specific binding of tumor cells, and enables the obtainment of high contrast imaging of tumors expressing HER2 receptor *in vivo* (89).

HER2 Affibody Molecular Probes as PET Tracers

PET can provide molecular information such as function and metabolism, and has important clinical value in early diagnosis, staging and restaging of tumors, finding primary tumor or metastases, guiding tumor treatment, evaluating treatment efficacy, and predicting recurrence (90). Compared with SPECT imaging, PET imaging has the advantages of high spatial resolution, good sensitivity, and quantification (91). The radionuclide fluorine-18 (^{18}F), gallium-68 (^{68}Ga), copper-64

(^{64}Cu), rhenium-186/188 ($^{186/188}$Re), and other labeled HER2 affibodies have been used in preclinical research on ovarian cancer, showing good prospects for clinical application (54, 67, 68, 78, 92–97).

[^{18}F]: The half-life of ^{18}F is 109.8 min, and the positron energy produced by ^{18}F is low (average of 0.25 MeV). Its annihilation distance in tissues is short (approximately 2.4 mm), which enables the obtainment of high-resolution images. Therefore, ^{18}F is considered as the most ideal nuclide for PET imaging. An early study used FBO (N-[4-flfluorobenzylidene]oxime) as a chelating agent to label the affibody $Z_{HER2:477}$ to obtain the molecular probe ^{18}F-FBO-$Z_{HER2:477}$. The results of animal PET imaging showed that the molecular probe can specifically bind to the HER2 receptor *in vitro* and *in vivo*, and obvious radionuclide uptake can be seen in the SKOV3 tumor tissue. However, due to the large amount of radioactive retention in the liver and kidney, the molecular probe is not clinically applicable (94). Kramer-Marek et al. used N-[2-(4-fluoro-benzamido)ethyl] maleimide (FBEM) as a chelating agent to prepare the molecular probe ^{18}F-FBEM-$Z_{HER2:342}$, and animal PET imaging results showed that the molecular probe had high radiation uptake and high image contrast in mice bearing SKBR3 and SKOV-3 xenografts with high HER2 expression, but no obvious uptake in mice bearing MDA-MB-468 xenografts with low HER2 expression. However, the high radioactive uptake in the kidney and bone also limits its clinical application (67). Recently, researchers have used FET (fluoroethyl-L-tyrosine) as a linker to prepare a molecular probe [^{18}F]FET-$Z_{HER2:342}$, and a preclinical study has shown that the molecular probe specifically binds to tumors and has a lower radioactive uptake in the liver. Unfortunately, its radiochemical yield is relatively low (68). Moreover, different methods of ^{18}F labeling for $Z_{HER2:2395}$ and $Z_{HER2:2891}$ have been reported, but their clinical transformation has been hindered by a large amount of radioactive retention in the kidney or the complex manufacturing process and low yield of molecular probes (62, 63, 73).

[^{68}Ga]: The half-life of ^{68}Ga is 68 min, and the positron decay rate is 89%. ^{68}Ga can be prepared by a ^{68}Ge-^{68}Ga generator. It is suitable for labeling small molecules that can rapidly distribute *in vivo* and reach the target, and high-quality images can be obtained approximately 1 h after intravenous injection. An early animal study of PET imaging used DOTA as a chelating agent to label the HER2 affibody $Z_{HER2:342}$ and the small-molecule protein MUT-DS of HER2, respectively, with ^{68}Ga (69). The results showed that the tumor tissue with high expression of HER2 receptor showed obvious radionuclide aggregation, which was quickly cleared in the blood, with low background and high image quality. However, the disadvantage was that there was also a high radioactive uptake in the kidney. Recently, a number of studies have successively used DOTA, NOTA (1,4,7-triazacyclononane-*N,N,N*-triacetic acid), and NODAGA [1-(1,3-carboxypropyl)-4,7-carboxymethyl-1,4,7-triazacyclononane] as chelating agents to label the affibodies $Z_{HER2:S1}$, $Z_{HER2:2395}$, and $Z_{HER2:2891}$, respectively, and performed molecular probe research *in vivo* and *in vitro*. The results showed

that NODAGA as a chelator for ^{68}Ga-labeled synthesis of molecular probes provided the best tumor-to-organ ratio, and was the best chelator for ^{68}Ga-labeled HER2 affibody, indicating excellent prospects for clinical application (81, 92, 93).

[^{64}Cu]: ^{64}Cu can simultaneously release β$^-$ with a maximum energy of 580 keV and β$^+$ with a maximum energy of 656 keV, which can be used for PET imaging and radionuclide therapy. The half-life of ^{64}Cu is 12.7 h, and it can be produced by nuclear reactors and medical accelerators (98, 99). Researchers first used DOTA as a chelating agent to label the affibody $Z_{HER2:477}$ with ^{64}Cu and performed PET imaging studies in tumor-bearing nude mice with high HER2 receptor expression. The results showed that the molecular probe can be significantly taken up by tumor tissue. However, the disadvantage is that there is also a large amount of aggregation in the liver and kidney, and the uptake of radioactivity in liver and kidney is significantly higher than that in tumor tissue, thus affecting the detection of abdominal lesions (100). Subsequently, the research team further introduced cysteine into different positions of the affibody $Z_{HER2:342}$ for ^{64}Cu labeling to prepare molecular probes ^{64}Cu-DOTA-Cys-$Z_{HER2:342}$, ^{64}Cu-DOTA-$Z_{HER2:342}$(Cys39), and ^{64}Cu-DOTA-$Z_{HER2:342}$-Cys. All three probes showed good affinity and stability in *in vitro* studies, and ^{64}Cu-DOTA-Cys-$Z_{HER2:342}$ had the highest affinity and *in vivo* stability. However, the problem of high renal radioactivity retention remained unresolved (72). In addition, some researchers conducted *in vitro* and *in vivo* studies and compared the use of NOTA and NODAGA as chelators for ^{64}Cu labeling of affibodies. The results showed that the tumor-to-organ ratio was higher when NODAGA was used as a chelator than when NOTA was used as a chelator. The disadvantage was that the molecular probes had higher radioactive retention in the kidney and bone marrow (101). Therefore, research of ^{64}Cu-labeled HER2 affibody molecular probe needs to be further optimized and perfected.

[$^{186/188}$Re]: The two isotopes of rhenium, ^{186}Re and ^{188}Re, can simultaneously emit γ rays and β rays, enabling their use for both imaging and therapy. ^{186}Re is produced by the reactor and has a half-life of 3.72 days. ^{186}Re can emit β rays with a maximum energy of 1.08 MeV and γ rays with an energy of 155 keV at a time. ^{188}Re has a half-life of 17.0 h, can be produced by a ^{188}W/^{188}Re generator, and can emit β rays with a maximum energy of 2.1 MeV and γ rays with an energy of 137 keV. Orlova et al. (102) first used maGGG and maGSG as chelators to label affibody $Z_{HER2:342}$ with ^{186}Re and compared the resulting molecular probe with the ^{99m}Tc-labeled molecular probe. The results showed that the molecular probe ^{186}Re-maGSG-$Z_{HER2:342}$ showed obvious radioactive uptake in the tumors of mice bearing SKOV3 xenografts at 4 h after injection. Compared with the ^{99m}Tc-labeled molecular probe, the ^{186}Re-labeled molecular imaging probe significantly reduces the renal uptake; its renal uptake is only 1/4 of the ^{99m}Tc-labeled molecular probe. However, the excretion of molecular probes through the hepatobiliary tract leads to relatively high uptake in the liver, which may affect the detection of liver lesions. Subsequently, the same research team attached different amino acid sequences to the carboxy-terminus to label the affibodies with ^{188}Re and

compared them *in vitro* and *in vivo*. The results showed that the uptake of the molecular probe ^{188}Re-$Z_{HER2:V2}$ with GGGC sequence linked to the carboxyl terminus was significantly higher than that of any organ tissue (including liver and kidney) at 4 h after injection. Furthermore, the uptake of the kidney was 5 times lower than that of the tumor, indicating that this molecular probe is a promising candidate for imaging and treatment of tumors with high expression of HER2 receptor (73, 74).

In addition, preclinical studies of PET molecular probes labeled to HER2 affibody by radionuclides such as astatine-211 (^{211}At), scandium-44 (^{44}Sc), zirconium-89 (^{89}Zr), and carbon-11 (^{11}C) through different chelating agents in SKOV3 ovarian tumors with high HER2 expression have also been reported in the literature, and the results show good potential for clinical application (103–106).

Radionuclide-Labeled HER2 Affibody as Targeted Therapeutic Probes

Ovarian cancer is traditionally treated with radical tumor resection and platinum-paclitaxel chemotherapy. Most tumors eventually become resistant to cytotoxic chemotherapy, leading to disease recurrence and progression (107). Recent studies have shown significant efficacy of poly (ADP-ribose) polymerase (PARP) inhibitors in patients with epithelial ovarian cancer, especially in patients with germline breast-related cancer antigens 1 and 2 (BRCA1/2) mutations. However, its clinical application value in patients without BRCA1/2 mutation remains controversial (108–110). The HER2 proto-oncogene is closely related to the occurrence of epithelial ovarian cancer; therefore, it provides an ideal target for the treatment of ovarian cancer. Trastuzumab, pertuzumab, olaparib, and lapatinib are widely approved by the Food and Drug Administration (FDA) for the treatment of HER2-positive cancers. However, clinical studies have shown that HER2-poszitive cancers are prone to become resistant to trastuzumab and pertuzumab; resistance mainly involves crosstalk with heterologous receptors, amplification, and the destruction of binding sites to HER2 receptors (111, 112), which may be related to the abnormal activation of the PI3K-Akt signaling pathway leading to PI3KCA mutation, phosphatase, and tensin homologue loss (113–115). It is estimated that approximately half of patients with HER2-positive metastatic breast cancer do not respond to trastuzumab (116). Lapatinib has shown positive efficacy in patients with HER2-positive ovarian cancer, but its efficacy in patients with ovarian cancer is only 22% to 30% (117, 118). Therefore, many researchers have recently focused on finding new methods and approaches for the comprehensive treatment of ovarian cancer through radioimmunotherapy. To date, many studies have focused on the targeted therapy of a variety of radionuclide-labeled HER2 affibody, including lutetium-177 (^{177}Lu), ^{64}Cu, and yttrium-90 (^{90}Y). Nevertheless, studies have shown that molecular probes of the radionuclide ^{64}Cu and ^{90}Y-labeled HER2 affibody need to be further optimized due to higher radioactive uptake in kidney or bone marrow and longer retention time (100, 119). ^{177}Lu, with a half-life of 6.7 days,

can emit β$^-$ particles with three energies of 498 keV (79.3%), 380 keV (9.1%), and 176 keV (12.2%). Due to its relatively low particle energy, the normal tissue around the lesion is less damaged, making it a very suitable radionuclide for therapy (120). In 2007, Tolmachev et al. first used CHX-A"-DTPA as a chelating agent to label affibody $Z_{HER2:342}$ with ^{177}Lu; the results showed that ^{177}Lu-CHX-A"-DTPA-ABD-($Z_{HER2:342}$)2 could prevent the growth of SKOV3 ovarian cancer cells highly expressing the HER2 receptor, compared with the control group injected with PBS and simultaneously injected with non-labeled affibody, ^{177}Lu-CHX-A"-DTPA-ABD-($Z_{HER2:342}$)2 blocking group. The survival time of nude mice injected with ^{177}Lu-CHX-A"-DTPA-ABD-($Z_{HER2:342}$)2 was significantly prolonged; however, a disadvantage of this affibody is the high radioactive retention in the blood and bone marrow (75). Subsequently, the researchers further linked high-affinity ABD035 to the N-terminus of the affibody $Z_{HER2:2891}$ and linked a DOTA derivative maleimide group to the C-terminus, namely, $Z_{HER2:2891}$-ABD035-DOTA, and then labeled it with ^{177}Lu to obtain molecular probe ^{177}Lu-ABY-027. This was then compared with that of the previous molecular probe ^{177}Lu-CHX-A"-DTPA-ABD-($Z_{HER2:342}$)2. The results showed that the uptake of the former in the tumor was twice that of the latter, and the radioactive retention in the liver and kidney was significantly reduced (75). To further improve the radioactive uptake of molecular probes by tumors and reduce the radioactive retention of normal organs such as the kidneys, the research team further optimized the molecular probes in a series of studies in which they conducted comparative evaluations *in vivo* and *in vitro*. The results showed that the molecular probe [^{177}Lu]Lu-HP16, containing 9 nucleic acid bases, has the highest tumor/kidney uptake ratio and is a promising molecular probe for targeted therapy of HER2-positive ovarian cancer (121–123).

Although there are many advantages that make radionuclide-labeled HER2 affibodies particularly suitable for molecular imaging, many challenges and hurdles remain in the development of these affibodies. One disadvantage is that labeling methods can lead to increased lipophilicity, often leading to off-target interactions with normal tissues and binding to blood proteins (124). Another disadvantage may be the bacterial source of the protein scaffold, as repeated use in patient treatment leads to an increased risk of immunogenicity (125).

DISCUSSION

HER2 is found in the cytoplasm of normal cells, mainly in the mitochondrial cristae, with little amounts occurring on the cell membrane; HER2 is found only on the cell membrane in tumor cells. The expression of HER2 in normal ovarian epithelium is very low; however, the expression in epithelial ovarian tumor tissue has been reported to be high at 55 times that of normal ovarian epithelium (126). Epithelial ovarian cancer consists of histological subtypes, which include serous carcinoma (prevalence 75%), mucinous carcinoma (20%), endometrioid carcinoma (2%), and clear cell carcinoma (<1%). Regarding

HER2 positivity, serous (29%) and mucinous carcinomas (38%) had higher positivity than endometrioid (20%) and clear cell carcinomas (23.1%), while the differential expressions of HER2 among different tissue types in epithelial ovarian carcinoma were important biomarkers for prognosis in epithelial ovarian cancer (127).

Fluorodeoxyglucose (FDG) labeled with radionuclide ^{18}F is widely used in clinical practice and has been proved to be highly accurate in the differential diagnosis of benign and malignant ovarian masses (128, 129). However, a study of ^{18}F-FDG PET/CT to evaluate the expression status of HER2 receptor has not been reported. Affibody has high affinity for HER2-expressing tumors, low molecular weight, stable biological activity, and fast blood clearance time (130). Recently, the use of molecular probes from radionuclide-labeled affibody has shown promise in tumor molecular imaging research. However, there are many difficulties in the preparation of molecular probes from radionuclide-labeled HER2 affibody that need to be addressed to enable more effective use in clinical practice. Most notably, research needs to focus on the reduction of non-specific uptake in the liver, kidney, bone marrow, and gastrointestinal tract. To this end, researchers have modified the amino acid sequence to change the molecular surface charge, lipid solubility, and affinity, and used different radionuclides to optimize the labeling method, improving the specific uptake of molecular probes at the tumor

site, reducing the uptake of non-target organs, thereby optimizing the distribution *in vivo*. Moreover, different chelating agents have been introduced into the radionuclide labeling of HER2 affibody. Increasing the hydrophilicity of the chelating agent can reduce the non-specific uptake of the liver, enable the molecular probe to be excreted through the kidney, reduce the uptake of the gastrointestinal tract, and thus reduce the background of the image, increase the target/non-target ratio, and lead to an improved image. To date, more than 20 chelating agents have been introduced into molecular probes for radionuclide-labeled HER2 affibody. The advantages and disadvantages of commonly used chelators are shown in **Table 2**.

The median survival of patients with ovarian cancer has been significantly improved recently through the introduction of novel drug treatments and minimally invasive surgical techniques (22). However, the symptomatology, diagnosis, and treatment of ovarian cancer still pose great challenges. Multidisciplinary cooperation and multiple detection methods are needed to provide gynecological oncologists with as much detailed clinical data as possible to optimally design appropriate treatment plans for each patient (22, 131). HER2 is overexpressed or amplified in various malignancies. Therefore, therapeutic drugs that target the linkage of HER2-positive tumor cells to a peptide with a high affinity for the HER2 receptor should be developed to target a variety of HER2-positive

TABLE 2 | Overview of advantages and disadvantages of commonly used chelators as molecular probes.

Chelating agent	Full name	Representative molecular probes	Metabolic pathways	Benifits	Disadvantages
-maGSG-	mercaptoacetyl-glycyl-seryl-glycyl	^{99m}Tc-maGSG-Z$_{HER2:342}$	kidney	low liver and gastrointestinal uptake	hign renal uptake
-maESE-	mercaptoacetyl-glutamyl-seryl-glutamyl	^{99m}Tc-maESE-Z$_{HER2:342}$	liver	high affinity; low image background; low renal uptake	relatively high liver uptake
-PIB-	Iodobenzoate	^{125}I-PIB-Z$_{HER2:342}$	liver	high affinity; low renal uptake	untargeted uptake
-HPEM-	4-hydroxyphenyl-ethyl-maleimide	^{131}I/^{125}I-HPEM-Z$_{HER2:342}$-C	liver	high affinity; low renal uptake	hign liver and gastrointestinal uptake
-DOTA-	1,4,7,l0-tetraazacyclodo-decane-*N,N,N,N*-tetra acetic acid	^{111}In-DOTA-Z$_{HER2:342-pep2}$ ^{68}Ga-DOTA-Z$_{HER2:342min}$	kidney	high affinity; fast blood clearance; low image background	high renal uptake
-NOTA-	1,4,7-triazacyclononane-*N,N,N*-triacetic acid	^{68}Ga-NOTA-Z$_{HER2:S1}$ ^{64}Cu-NOTA-Z$_{HER2:S1}$	kidney	high affinity; low image background	high renal uptake
-FBO-	(N-(4-flfluorobenzylidene)oxime)	^{18}F-FBO-Z$_{HER2:477}$	liver, kidney	high affinity	high liver and renal uptake
-FBEM-	N-[2-(4-fluoro-benzamido)ethyl] maleimide	^{18}F-FBEM-Z$_{HER2:342}$	kidney	high affinity; low liver uptake	high renal and bone uptake
-FET-	fluoroethyl-L-tyrosine	[^{18}F]FET-Z$_{HER2:342}$	kidney	high affinity; low liver uptake	low yield
-NODAGA-	1-(1,3-carboxypropyl)-4,7-carboxymethyl-1,4,7-triazacyclononane	^{68}Ga-NODAGA-Z$_{HER2:S1}$ ^{64}Cu-NODAGA-Z$_{HER2:S1}$	kidney	high affinity; low image background; low liver uptake	high bone marrow uptake
-maGSG-	mercaptoacetyl-glycyl-seryl-glycyl	^{186}Re-maGSG-Z$_{HER2:342}$	liver	high affinity; low renal uptake	high liver uptake
		^{99m}Tc-maSGS-Z$_{HER2:342}$	kidney	high affinity; low liver uptake	high renal uptake
-maGGG-	mercaptoacetyl-glycyl-glycyl-glycyl	^{99m}Tc-maGGG-Z$_{HER2:342}$ ^{186}Re-maGGG-Z$_{HER2:342}$	not applicable	high affinity; low liver and renal uptake	high image background

malignancies (132). To date, a variety of HER2-targeting therapeutic drugs have been developed, including HER2 addressing therapeutic monoclonal antibodies, nanobodies, and affibodies. Several of these drugs have entered the clinical trial stage, and the results of clinical studies have shown that they benefit patients with HER2-positive breast cancer. However, the study and use of radionuclide-labeled HER2 affibody have been hampered by its short residence time in the blood (133).

In conclusion, the molecular probe of radionuclide-labeled HER2 affibody can accurately evaluate the expression status of HER2 receptor in epithelial ovarian cancer, which provides favorable conditions for the localization, diagnosis of cancer foci, targeted therapy, and prognosis evaluation. The research and development of new molecular probes for nuclear medicine aims to introduce radionuclides into cancer foci in a targeted manner, display systemic lesions, and kill tumor cells through radionuclides' radioactivity. Continued progress will result in improved methods for the integration of the diagnosis and treatment of ovarian cancer. Nuclear medicine imaging technology is an important imaging method for basic and clinical research on malignant tumors. A variety of radionuclide-labeled HER2 affibody molecular probes have been successfully developed and have shown potential in preclinical experimental studies of ovarian cancer, laying a foundation for multi-center clinical trials. In the future, the research and development of HER2 affibody molecular probes will focus on optimizing the labeling method of radionuclides, using different chelating agents, or modifying the amino acid sequence to further improve the specific uptake of molecular probes in tumor sites, while reducing the uptake of non-target organs, especially in the liver, kidney, bone marrow, and gastrointestinal tract. This will optimize the distribution in the body, and the radionuclide-labeled HER2 small-molecule targeting binding protein can then be used in clinical practice as soon as possible to enable tumor-specific diagnosis, and to promote the evaluation of the therapeutic efficacy of targeted therapy drugs objectively at an early stage.

AUTHOR CONTRIBUTIONS

XH compiled/interpreted resources and was the primary author for majority of the manuscript. DL assisted with manuscript writing. YF assisted with manuscript edits. JZ and ZF developed the conception and overall design of the project/paper. JC and PW provided manuscript editing/revisions and interpretation of resources to include in the paper. All authors contributed to manuscript revision, and read and approved the submitted version.

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