MicroPET Imaging and Biodistribution of an 18F-Labeled HER2-mimetic Peptide Tracer

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Abstract

Background: Human epidermal growth factor receptor 2 (HER2) is widely expressed in various malignant tumors and is associated with poor prognosis, particularly in breast cancer. However, HER2 expression exhibits heterogeneity, and current immunohistochemistry and fluorescence in situ hybridization methods for evaluating HER2 status have significant limitations. HER2 receptor imaging offers distinct advantages, and radiolabeled analog peptides represent a potential option for detecting HER2-positive lesions. Objective: To prepare an 18F-labeled HER2 analog peptide imaging agent, 18F-NFP-B2-S22-AFA (hereinafter referred to as 18F-NFP-TP1296), and investigate its microPET imaging and biodistribution. Methods: Using a one-step labeling method, 18F-NFP-TP1296 was applied to PET-CT imaging in HER2 breast cancer xenograft mice. In vitro studies and MicroPET imaging were performed in the SKBR-3 breast cancer model. Results: The preparation of 18F-NFP-TP1296 was completed in approximately 30 minutes, with an overall labeling yield of 1.5% and radiochemical purity >95%. MicroPET imaging showed that SKBR-3 xenograft tumors were clearly visualized. At 30, 60, and 120 min post-injection, the uptake values in SKBR-3 transplanted tumors were 5.63$±0.14±0.27±0.44±0.32, 4.08±0.73, *and*1.69±0.18; *and*1.55±0.11, 1.84±0.12, *and*3.10±$0.30, respectively. Additionally, for lung metastases, the tumor uptake values at 30, 60, and 120 min post-injection were 2.2%ID/g, 2.5%ID/g, and 2.1%ID/g, respectively. Conclusion: 18F-NFP-B2-S22-AFA offers advantages such as simple synthesis and favorable pharmacokinetics, and shows promise as a tracer for in vivo detection of HER2 status, providing certain reference value for differential diagnosis of tumors, molecular targeted therapy, and prognostic assessment.

Full Text

MicroPET Imaging and Biodistribution of 18F-Labeled HER2 Mimetic Peptide Imaging Agent

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Abstract

Background: Human epidermal growth factor receptor 2 (HER2) is widely expressed in various malignant tumors and is associated with poor prognosis, particularly in breast cancer. However, HER2 expression exhibits heterogeneity, and current assessment methods using immunohistochemistry and fluorescence in situ hybridization have significant limitations. HER2 receptor imaging offers clear advantages, and radiolabeled mimetic peptides represent a potential option for detecting HER2-positive lesions.

Objective: To prepare an 18F-labeled HER2 mimetic peptide imaging agent 18F-NFP-B2-S22-AFA (hereinafter referred to as 18F-NFP-TP1296) and investigate its microPET imaging characteristics and biodistribution.

Methods: A one-step labeling method was used to prepare 18F-NFP-TP1296, which was applied to PET-CT imaging in HER2 breast cancer xenograft mice. In vitro studies and microPET imaging were performed in the SKBR-3 breast cancer model.

Results: The preparation of 18F-NFP-TP1296 was completed in approximately 30 minutes, with an overall labeling yield of 1.5% and radiochemical purity >95%. MicroPET imaging showed that SKBR-3 xenograft tumors were clearly visualized, with tumor uptake values of $5.63\pm0.14\pm0.27\pm0.44\pm0.32, 4.08\pm0.73, and 1.69\pm0.18; and 1.55\pm0.11, 1.84\pm0.12, and 3.10\pm$0.30, respectively. Additionally, lung metastasis tumor uptake values were 2.2%ID/g, 2.5%ID/g, and 2.1%ID/g at 30, 60, and 120 minutes post-injection.

Conclusion: 18F-NFP-B2-S22-AFA offers advantages including simple synthesis and favorable pharmacokinetics, showing promise as a tracer for in vivo detection of HER2 status with potential value for tumor differential diagnosis, molecular targeted therapy guidance, and prognosis assessment.

Keywords: Breast cancer; mimetic peptide; epidermal growth factor; 18F; positron emission tomography

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Introduction

Breast cancer (BC) is the most common malignant tumor among women worldwide. According to the latest 2023 World Cancer Report [1], breast cancer has become the leading cause of cancer incidence globally. Approximately 25-30% of breast cancers exhibit overexpression of the human epidermal growth factor receptor 2 (HER2) gene. HER2, a transmembrane glycoprotein encoded by the proto-oncogene HER2/neu, belongs to the epidermal growth factor receptor family and possesses tyrosine kinase activity. The signaling pathways mediated by HER2 promote breast cancer cell proliferation, invasion, migration, and inhibit apoptosis [2-4]. HER2 is highly expressed in various malignant tumors [5], and breast cancers with heterogeneous HER2 overexpression are clinically defined as HER2-positive BC, characterized by aggressive behavior, high metastatic potential, and low survival rates.

Accurate detection of HER2 expression in lesions is critical for determining patient eligibility for HER2-targeted therapy and for prognosis evaluation [6,7]. While biopsy remains the only definitive diagnostic approach, both immunohistochemical staining (IHC) and fluorescence in situ hybridization (FISH) performed on biopsied or postoperative tumor tissues are invasive procedures with measurement error rates reaching up to 20%. Due to HER2 expression heterogeneity, partial sampling may not adequately represent the overall tumor characteristics [8]. In contrast, molecular imaging techniques such as SPECT and PET offer reliable, whole-body, non-invasive methods for detecting HER2 expression in vivo. Currently, 18F-FDG PET/CT is widely used in clinical practice, but its principle relies on elevated glucose metabolism to visualize tumor tissue, representing non-specific imaging with limited ability to discriminate HER2-positive tumors [9-10].

Recent HER2 receptor imaging radiopharmaceuticals have primarily included monoclonal antibodies and their fragments, affibodies, or small peptide molecules. Radiolabeled monoclonal antibodies such as 89Zr-trastuzumab and 89Zr-pertuzumab [11,12] have shown promising results in monitoring HER2-positive breast cancer and metastatic lesions. However, their large molecular weight (150 kDa) results in slow blood clearance, requiring 3-7 days post-injection to obtain high-contrast images, and they are prone to molecular competition for receptor binding. Radiolabeled antibody fragments of trastuzumab, such as 68Ga-DOTA-F(ab')2-trastuzumab F(ab')2 [13], are safer and allow same-day imaging, but lesion detection rates remain suboptimal. Various radiolabeled HER2 affibody probes have been developed

for tumor imaging, including 18FAl-NOTA-MalCys-MZHER2342, 68Ga-DOTA-ZHER2342, and 68Ga-NOTA-MAL-MZHER2 [14-17]. These agents rapidly accumulate in tumors, provide high image contrast, and are quickly excreted from normal organs (blood, muscle, etc.). However, these HER2-targeted affibody probes exhibit high background uptake in the liver and intestines, which may obscure abdominal visceral lesions such as liver metastases [18] and intestinal pathologies.

This study employs 18F-NFP to prepare a novel molecular probe 18F-NFP-B2-S22-AFA (abbreviated as 18F-NFP-TP1296) and investigates its pharmacokinetic performance, efficacy, and safety in mice. We anticipate that this new molecular imaging probe will demonstrate improved in vivo pharmacokinetics (such as reduced hepatic uptake) and enable prolonged imaging to achieve better tumor uptake and image quality, laying the foundation for future clinical research. Additionally, we validated a series of probes (99Tcm-TP1623 and 18F-NFP-TP1296) for detecting and evaluating HER2 status in lung metastases, comparing them with 18F-FDG PET imaging. This approach can provide comprehensive information on HER2 receptor status across all metastatic sites, which may facilitate personalized treatment for individual BC patients.

Experimental Section

2.1 Instruments and Materials

2.1.1 Main Instruments Sumitomo HM-7 cyclotron (Japan); 18F-FDG synthesis module (Beijing PET); High-performance liquid chromatography (HPLC, Model 1525, Waters, USA); UV detector (Model 2487, Waters, USA); Radioactivity detector (PerkinElmer, USA); Electronic balance (Sartorius, Germany); Sep-Pak C18 column (4.6 mm × 250 mm, Waters, USA); Radioactivity calibrator (Capintec, USA); microPET scanner (Inveon, Siemens, Germany); Small animal anesthesia machine (SAR-830/P, CWE, USA); Radioactive thin-layer chromatography (TLC) scanner (Bioscan, USA).

2.1.2 Main Reagents Precursor NFP: provided by the Molecular Imaging Center, Jiangsu Institute of Nuclear Medicine; Potassium carbonate acetonitrile, formic acid, methanol: Sinopharm Chemical Reagent Co., Ltd.; Oxygen-18 water: Shanghai Chemical Research Institute; Kryptofix222 (K2.2.2): Sigma, USA; 18F- solution: provided by Jiangyuan Andyco Molecular Nuclear Medicine Research and Development Co., Ltd.; Isoflurane: Shanghai Abbott Pharmaceutical Co., Ltd.; B2-S22-AFA: purchased from Shanghai Chupeptide Biotechnology Co., Ltd., purity >95%; HE staining solution, eco-friendly dewaxing transparent solution, and other pathological staining reagents: provided by Jiangsu Institute of Nuclear Medicine; Other reagents were analytically pure and purchased from Sinopharm Chemical Reagent Co., Ltd.

2.1.3 Experimental Animals Eight female BALB/c nude mice, 4-6 weeks old, body weight (20$±2)$g, werepurchasedfromShanghaiSlackLaboratoryAnimalCo., Ltd.; Forty−twofemaleICRmice, 4 − 6weeksold, bodyweight(20±$2) g, were purchased from Changzhou Cavens Laboratory Animal Co., Ltd. All animals were housed in a specific-pathogen-free (SPF) environment. Animal experiments were approved by the Ethics Committee of Jiangsu Institute of Nuclear Medicine.

2.2 Experimental Methods

2.2.1 Synthesis of Labeling Auxiliary Group 18F-NFP (Figure 1 [Figure 1: see original paper]) 18F transferred from the cyclotron was adsorbed on a Sep-Pak QMA cartridge and eluted into a reaction vial (218.0 mCi) with a mixed solution of K222 and K2CO3. The solution was dried under nitrogen flow at 110°C, and 1.5 mL of anhydrous acetonitrile was added twice for azeotropic dehydration. Then, 2-bromopropionic acid ethyl ester acetonitrile solution (4.3 µL/400 µL) was added to the reaction vial and reacted at 110°C for 10 min. After cooling to room temperature, 50 µL of TBAH acetonitrile solution (40 mg/100 µL) was added and hydrolyzed at 110°C for 10 min. Following the reaction, nitrogen was passed through at 110°C for azeotropic dehydration. Bis(4-nitrophenyl)carbonate acetonitrile solution (40 mg/400 µL) was added to the remaining solid in the reaction vial and reacted at 110°C for 10 min. After cooling, 0.2 mL of 50% HAc acetonitrile solution was added and hydrolyzed at 110°C for 5 min (remaining activity in vial: 97.8 mCi). The crude product was injected onto a semi-preparative HPLC separation column, with fractions collected every approximately 20 seconds starting from 9 minutes. Radioactive HPLC analysis conditions: stationary phase: reversed-phase C18 column (10$×$250 mm); mobile phase: A: acetonitrile + 0.1% TFA, B: water + 0.1% TFA, isocratic elution: A/B = 40/60; flow rate: 5 mL/min, detection wavelength 254 nm. The product 4-nitrophenyl 2-[18F]-fluoropropionate (NFP) was collected (25.9 mCi product), with a radioactive peak at 10.23 min. The fractions were combined, diluted with 60 mL water, and passed through a C18 column. The radioactive auxiliary group 18F-NFP was adsorbed on the C18 column, washed with 5 mL water (activity retained on column: 7.03 mCi), and then eluted from the C18 column with 1 mL dichloromethane (4.4 mCi).

2.2.2 Synthesis of PET Molecular Probe 18F-NFP-TP1296 (Figure 2 [Figure 2: see original paper]) After drying the dichloromethane under nitrogen flow at room temperature, 200 µg of peptide (dissolved in 400 µL DMSO) and 30 µL of DIPEA were added to the dried 18F-NFP and reacted at room temperature for 10 min. The crude product was then purified by semi-preparative HPLC.

2.2.3 Determination of Labeling Yield and Radiochemical Purity Radioactive HPLC analysis conditions: stationary phase: C18 column (10$×$250 mm); mobile phase: A: acetonitrile + 0.1% TFA, B: water + 0.1% TFA, gradient elution: from 2 min A/B = 5/95 to 35 min A/B = 65/35; flow rate: 5

mL/min; retention time (TR): 16.17 min. Fractions were collected starting from approximately 15 minutes, one tube every 20 seconds. The fractions were combined, diluted 10-fold with water, passed through a C18 column, washed with water (10 mL), and eluted with ethanol (300 µL) to obtain the product 18F-NFP-peptide (1.3 mCi). Detailed radioactive HPLC procedures are provided in the appendix. The remaining product was labeled with date and batch number, sealed, and stored in a radioactive protection lead container.

2.2.4 In Vitro Stability Study 100 µL of 3.7 MBq 18F-NFP-TP1296 was added to 1 mL of PBS and mouse plasma, mixed well, and incubated at 37°C for 30, 60, and 120 minutes. At predetermined time points, the PBS solution was directly injected into radioactive HPLC for radiochemical purity determination. The plasma mixture was first injected into a C18 column and washed twice with 15 mL deionized water, then eluted with 10 mM HCl ethanol, and the product was analyzed by HPLC.

2.2.5 Determination of Oil-Water Partition Coefficient In a 10 mL centrifuge tube (Tube 1), 1 mL of n-octanol, 1 mL of phosphate buffer (pH 7.4), and 5 µL of 18F-NFP-TP1296 (1.85 MBq) were added, mixed at room temperature, centrifuged for 5 minutes, and allowed to separate. The lower aqueous phase (0.5 mL) was transferred to another 10 mL centrifuge tube (Tube 2), supplemented with 0.5 mL buffer and 1 mL n-octanol. This process was repeated sequentially for a total of 5 tubes. Using a pipette, 100 µL of the upper n-octanol solution and 100 µL of the lower phosphate buffer solution from each tube were transferred to gamma counting tubes, weighed, and radioactivity was measured with a gamma counter. The oil-water partition coefficient logP (oil/water) was calculated as follows:

logP (oil/water) = log[(radioactivity count in n-octanol layer / mass of n-octanol layer) / (radioactivity count in buffer layer / mass of buffer layer)]

2.2.6 Establishment of Xenograft Tumor Models Human breast cancer cells SKBR-3 were cultured in a 37°C incubator using conventional methods. After reaching logarithmic growth phase, PBS was used to wash away unhealthy cells, followed by addition of trypsin solution. After incubation, culture medium was added to terminate the digestion reaction, and cells were repeatedly pipetted into a cell suspension. The passaged tumor cells were inoculated subcutaneously into the axillary region of 8 female nude mice, which were fed normally. Tumor volume was measured regularly with vernier calipers (V = length × width × width / 2). The xenograft tumor model was considered successfully established when the tumor volume reached 100-300 mm^3.

Establishment of HER2-positive lung metastasis model: Breast cancer cells SKBR-3 at a concentration of 1$×10^{6}$/mL were injected via tail vein into female nude mice to establish a breast cancer lung metastasis model. Mice were fed normally and observed regularly (every 2 days) to monitor their health sta-

tus. After approximately 3 weeks, lung tissue was dissected for pathological examination to confirm tumor identification.

2.2.7 MicroPET Imaging in Normal ICR Mice and Tumor-Bearing Nude Mice with Solid Tumors and Lung Metastases Six normal ICR mice were anesthetized with isoflurane and injected via tail vein with 3.75 MBq of 18F-NFP-TP1296 diluted in saline (0.2 mL). Dynamic scanning was performed for 120 minutes under continuous isoflurane anesthesia (1.5% volume fraction). Images were segmented and reconstructed at different time points, then processed and analyzed using ASIPRO V6.8.5.0 software. Regions of interest (ROI) were drawn for major organs to obtain time-activity curves (TAC).

Four tumor-bearing nude mice were injected with 3.75 MBq of 18F-NFP-TP1296 diluted in saline (0.2 mL). Static scans of 10 minutes were performed at 30, 60, and 120 minutes post-injection, with 3D mode acquisition, energy peak 511 keV, time window 3.432 ns. Images were reconstructed using ordered subsets expectation maximization (OSEM) algorithm. ASIPRO V6.8.5.0 software was used to delineate ROIs in tumors and major organs (muscle, liver, kidney, etc.) to obtain TACs. For blocking experiments, four tumor-bearing nude mice were pre-injected with B2-S22-AFA at 10 mg/kg body weight 30 minutes prior to injection of 3.75 MBq 18F-NFP-TP1296 solution diluted in saline. Scanning and data processing were performed as described above.

2.2.8 Pathological and HER2 Immunohistochemical Analysis After imaging, tumor tissues or lung metastasis tissues were harvested from tumor-bearing nude mice for fixation, paraffin embedding, sectioning, and pathological and immunohistochemical analysis.

2.2.9 Statistical Analysis Data were analyzed using GraphPad Prism 8.0 software. Quantitative data with normal distribution were expressed as $x \pm s$. Comparisons between two groups were performed using independent samples t-test. $p<0.05$ was considered statistically significant.

Results and Discussion

3.1 Results

3.1.1 Preparation of 18F-NFP **3.1.1.1** The synthesis route of 18F-NFP is shown in Figure 1 [Figure 1: see original paper], with a labeling yield of 4.6%.

Fig. 1 Synthesis route of 18F-NFP

3.1.1.2 The synthesis scheme of 18F-NFP-TP1296 is shown in Figure 2 [Figure 2: see original paper], with a labeling yield of 32.8%.

Fig. 2 A schematic radiosynthesis of radiotracer 18F-NFP-TP1296

3.1.2 Labeling Yield and Radiochemical Purity of 18F-NFP-TP1296 The preparation of 18F-NFP-TP1296 was completed in approximately 30 minutes, with an overall labeling yield of 1.5% and radiochemical purity >95%. Analytical HPLC of unlabeled peptide showed a retention time of 15.4 min at 218 nm. As shown in Figure 3 [Figure 3: see original paper], the probe peptide appeared as a single peak, with HPLC chromatograms showing retention times of 17.65 min for the probe and 15.4 min for the cold compound, both with high purity, indicating successful probe preparation.

Fig. 3 HPLC chromatograms of the probe (A) and cold compound (B)

3.1.3 In Vitro Stability After incubation in PBS at room temperature for 30, 60, and 120 minutes, the radiochemical purity of 18F-NFP-TP1296 solution was 96.4$±1.1±1.0±0.9±$0.7%.

Fig. 4 Stability assay of 18F-NFP-TP1296 in PBS (A) and plasma (B)

3.1.4 Specificity Studies in Tumor-Bearing Mice 3.1.4.1 MicroPET Imaging in Tumor-Bearing Mice

(1) Figure 5 [Figure 5: see original paper] shows clear tumor visualization at 30 minutes post-injection of 18F-NFP-TP1296, with peak uptake at 60 minutes. Quantitative analysis demonstrated tumor uptake values of 5.63$±0.14±0.27±$0.44%ID/g at 30, 60, and 120 minutes, respectively. Biodistribution results revealed high tracer accumulation in both liver and kidneys at 30 minutes post-injection, indicating that 18F-NFP-TP1296 is primarily excreted through hepatobiliary and renal pathways.

Fig. 5 MicroPET imaging of 18F-NFP-TP1296 in SKBR-3 tumor-bearing mice at different time points (A) and quantitative analysis (B). Arrows indicate tumor location.

(2) Figure 6 [Figure 6: see original paper] demonstrates that non-target organ uptake of 18F-NFP-TP1296 in tumor-bearing nude mice decreased after 1 hour, yielding high tumor-to-muscle uptake ratios. Clear radioactive accumulation was observed in tumor lesions, indicating specific targeting of B2-S22-AFA by 18F-NFP-TP1296 in vivo. Excess B2-S22-AFA could block tumor uptake of 18F-NFP-TP1296. Arrows indicate tumor location.

Fig. 6 MicroPET imaging in SKBR-3 tumor-bearing mice at 30, 60, and 120 minutes post-injection of 18F-NFP-TP1296 [unblocked (a) and blocked (b)], time-course of tumor and muscle uptake (B), and tumor-to-muscle uptake ratio (C). Arrows indicate tumor location.

(3) Figure 7 [Figure 7: see original paper] shows clear tumor visualization at 30 minutes post-injection of 18F-NFP-TP1296, with peak imaging at 60 minutes. Non-target organ uptake decreased after 1 hour, resulting in high tumor-to-muscle uptake ratios. Tumor uptake values at 60 minutes were 2.2%ID/g, 2.5%ID/g, and 2.1%ID/g. Arrows indicate tumor location.

Fig. 7 MicroPET imaging in non-metastasis-bearing mice at 60 minutes (A), MicroPET imaging in SKBR-3 lung metastasis-bearing mice at 30, 60, and 120 minutes post-injection of 18F-NFP-TP1296 (B), time-course of tumor and muscle uptake in lung metastasis-bearing mice (C), and tumor-to-muscle and tumor-to-blood uptake ratios (D). Arrows indicate tumor location.

(4) Figure 8 [Figure 8: see original paper] shows clear tumor visualization at 60 minutes post-injection of 18F-NFP-TP1296, confirmed by HE staining.

Fig. 8 MicroPET imaging of 18F-NFP-TP1296 in two SKBR-3 lung metastasis-bearing mice (A) and corresponding tumor pathology results (B). Arrows indicate tumor location.

(5) Figure 9 [Figure 9: see original paper] compares MicroPET imaging with 18F-NFP-TP1296 and 18F-FDG, and MicroSPECT/CT imaging with 99Tcm-TP1623 at 60 minutes post-injection, showing lung metastasis uptake values in descending order: 18F-FDG, 99Tcm-TP1623, and 18F-NFP-TP1296.

Fig. 9 MicroPET imaging of 18F-NFP-TP1296 and 18F-FDG, and MicroSPECT/CT imaging of 99Tcm-TP1623 at 60 minutes post-injection, with lung metastasis uptake values. Arrows indicate tumor location.

3.1.5 Immunohistochemical Analysis Figure 10 [Figure 10: see original paper] shows immunohistochemical analysis of HER2-positive tumor tissues from tumor-bearing nude mice, demonstrating strong HER2 expression.

Fig. 10 Immunohistochemical staining of SKBR-3 solid tumors (3+)

3.2 Discussion

The most commonly used positron-emitting tumor imaging agent is the glucose metabolism tracer fluorine [18F]deoxyglucose (18F-FDG), accounting for approximately 90% of clinical applications and often referred to as the "molecule of the century." Due to altered energy metabolism in malignant tumor cells, tumor cells exhibit significantly enhanced anaerobic glycolysis and lactate secretion compared to normal cells. When 18F-FDG is recognized by glucose transporter proteins and enters cells via transmembrane proteins, it cannot be further metabolized because key enzymes fail to recognize it, causing it to be trapped within cells. Therefore, the degree of 18F-FDG PET/CT uptake throughout the body reflects tumor glucose uptake levels, where higher SUVmax values indicate higher malignancy and poorer differentiation, enabling sensitive, whole-body visualization of tumor metabolism and function. 18F offers high positron emission efficiency, low energy of 0.64 MeV, and a longer half-life compared to 68Ga (T1/2: 18F 109.7 min vs. 68Ga 67.7 min), making it the most commonly used clinical positron-emitting radionuclide. The longer half-life of 18F allows extended imaging time and delayed scanning compared to the short half-life of 68Ga, providing more useful information for differentiating inflammatory from

neoplastic lesions. However, 18F-FDG PET/CT imaging lacks specificity as a broad-spectrum tumor imaging agent and cannot effectively reflect HER2 overexpression in tumors, only metabolic activity, thus offering limited guidance for targeted therapy. Many researchers have found no correlation between SUVmax on 18F-FDG PET/CT and HER2 expression in breast cancer metastasis or gastric cancer patients [21-23], demonstrating that 18F-FDG cannot specifically express HER2. Therefore, although 18F-FDG plays a crucial role in detecting and monitoring primary and metastatic breast cancer, its ability to selectively identify HER2-positive tumors is limited [24]. Additionally, false-positive results related to inflammation frequently occur.

18F-labeled HER2 analog molecular imaging shows great potential for tumor characterization and therapy monitoring. Kramer-Marek et al. [25] prepared 18F-FBEM-ZHER2:342 with a preparation time of 2 hours and radiochemical yield of 6.5%. Xu Yuping et al. [26,27] optimized this approach by modifying the HER2 affibody MZHER2:342 with a hydrophilic linker (GGGRDN) to prepare 18FAl-NOTA-MAL-MZHER2:342, which effectively improved pharmacokinetics, reduced hepatobiliary excretion, optimized contrast, and improved image quality without affecting specific binding to HER2. Radiochemical purity analysis demonstrated that Sep-Pak C18 cartridges could be used for purification of 18FAl-labeled HER2:342 without requiring further HPLC separation.

However, unfavorable hepatic excretion of radiolabeled affibody tracers may affect tumor-to-normal tissue ratios and reduce contrast. Small peptide molecules offer numerous advantages: unlabeled small peptide precursors can be synthesized by solid-phase methods with ease, defined chemical structures, flexible spatial modification, controllable pharmacokinetics, non-immunogenicity, strong tissue penetration, and rapid blood clearance [28,29], enabling short imaging times. HER2 expression levels in HER2-positive tumors can guide therapy, and many peptides have shown excellent application value in detecting HER2 expression in breast cancer.

We successfully prepared 18F-NFP-TP1296 with a simple method, good labeling yield, high radiochemical purity, excellent in vitro stability, and radiochemical purity >95%.

MicroPET imaging of solid tumors with 18F-NFP-TP1296 showed tumor uptake values of $5.63\$\pm0.14, 6.26\pm0.27, and 5.83\pm0.44\pm\$0.11\%$ID/g at 120 minutes. The uptake values in liver and blood at different time points (30, 60, and 120 minutes) were slightly elevated (Figure 5), suggesting that 18F-labeled peptides are primarily metabolized through the liver and have prolonged retention in blood, consistent with literature reports [30].

Although mimetic peptides have small molecular weight and rapid plasma clearance, why does connection with 18F-NFP labeling prolong in vivo retention? The reasons may be twofold: (1) The 18F-labeling auxiliary group method is a multi-step synthesis with a large auxiliary group that more easily affects peptide structure and alters pharmacokinetic properties. (2) Biodistribution shows

persistent hepatic uptake around 4.5% ID/g from 30 to 120 minutes, indicating the liver is an important organ for metabolic degradation, requiring drug decomposition through hepatic pathways. Therefore, 18F-NFP-TP1296 can be used for delayed imaging.

Blocking imaging of solid tumors with 18F-NFP-TP1296 showed uptake values in unblocked vs. blocked groups at 30, 60, and 120 minutes of 5.63$±0.14, 6.26±0.27, 5.83±0.44±0.4, 4.3±0.39, 3.53±$0.13% ID/g, respectively, demonstrating good tumor targeting. However, the blocking rates of 18F-NFP-TP1296 at each time point (30, 60, and 120 minutes) were lower than those of 68Ga-TP1580 (70.1%, 62.9%, and 69.4%), suggesting suboptimal specificity of 18F-NFP-TP1296. This may also be attributed to the multi-step 18F-labeling auxiliary group method, where the large auxiliary group affects peptide conformation and activity, altering the specificity of 18F-NFP-TP1296 binding to HER2.

To further determine whether radiolabeled HER2 mimetic peptides can play a complementary role in evaluating target expression in lung metastases, we examined the biological properties of different radiolabeled HER2 mimetic peptides in a lung metastasis model.

MicroPET imaging of HER2 lung metastases with 18F-NFP-TP1296 showed moderate uptake in pulmonary metastases, with peak imaging at 60 minutes and average uptake of 2.07$±0.17±$0.32%ID/g. This uptake was significantly lower than that in HER2 solid tumors at their 30-minute peak, suggesting heterogeneity in HER2 expression between pulmonary metastases and primary lesions. Diffuse metastatic foci were observed in gross lung tissue specimens, and histopathological analysis confirmed metastatic breast cancer to the lung (Figures 7, 8, and Table -2). These results suggest that 18F-NFP-TP1296 has potential application value for diagnosing HER2-positive breast cancer lung metastases.

Figure 9 shows that among the three probes 18F-NFP-TP1296, 18F-FDG (MicroPET), and 99Tcm-TP1623 (MicroSPECT/CT) at 60 minutes post-injection, lung metastasis uptake values in descending order were: 18F-FDG, 99Tcm-TP1623, and 18F-NFP-TP1296.

Comparing 18F-NFP-TP1296 and 18F-FDG MicroPET imaging at 60 minutes, the uptake value in lung metastases was significantly lower for the former (2.50%ID/g vs. 8.08%ID/g). The reason is that 18F-NFP-TP1296 PET imaging reflects the biological activity of HER2 expression in lung metastases, representing specific imaging, whereas 18F-FDG PET imaging reflects glucose metabolic activity in lung metastases, representing broad-spectrum tumor imaging. Therefore, 18F-FDG PET imaging often yields false-positive results for benign lesions such as pulmonary inflammation, tuberculosis, and atelectasis, which can be avoided with 18F-NFP-TP1296 PET imaging.

Comparing 18F-NFP-TP1296 MicroPET imaging at 60 minutes with 99Tcm-TP1623 MicroSPECT/CT imaging at 60 minutes, lung metastasis uptake values

were 2.50 and 4.82, respectively, indicating that the labeling method for 99Tcm-TP1623 is superior for HER2 mimetic peptides.

This study employed the auxiliary group NFP conjugated with the HER2 small molecule mimetic peptide TP1296 to prepare the HER2-targeted probe 18F-NFP-TP1296 under optimal 18F-labeling conditions. The method is simple, yields high radiochemical purity, demonstrates good in vitro stability, and shows favorable imaging properties. For diagnosing HER2-positive nude mouse lung metastases, 99Tcm-TP1623 showed the highest sensitivity, followed by 18F-NFP-TP1296. This study provides experimental exploration for further development of HER2 mimetic peptide imaging probes for breast cancer.

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Note: Figure translations are in progress. See original paper for figures.