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Preclinical evaluation of ^{99m}Tc direct labeling $Z_{HER2:V2}$ for HER2 positive tumors imaging

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Abstract. The present study aimed to label $Z_{HER2:V2}$ with technetium-99m (^{99m}Tc) using a simple method and to evaluate its clinical potential as a diagnostic probe for human epidermal growth factor receptor type 2 (HER2)-positive tumors. The $Z_{HER2:V2}$ (Affibody molecule of $Z_{HER2:2395-C}$, which is based on the $Z_{HER2:342}$ binding sequence with C-terminal engineered cysteine) with C-terminal chelating sequence GGGC was designed and labeled with ^{99m}Tc. The ^{99m}Tc-$Z_{HER2:V2}$ labeling efficiency was analyzed. The cellular uptake, retention and binding affinity, and the stability of the probe were examined *in vitro*. ^{99m}Tc-$Z_{HER2:V2}$ biodistribution analysis and imaging were performed in BALB/c nude mice bearing SKOV3 (HER2-overexpression) xenografts. Furthermore, imaging of the probe was performed in MCF-7 (HER2 low-expression) xenografts. The ^{99m}Tc-$Z_{HER2:V2}$ labeling efficiency was identified as 98.99±0.99% (n=6), and was stable in physiological saline and fresh human serum at 37˚C *in vitro*. The cellular uptake peak of SKOV3 cells at 24 h was 6.15±0.18%, the cellular retention ratio of the probe was 48.58±4.52% at 6 h following interrupted incubation, and ~70% of ^{99m}Tc-$Z_{HER2:V2}$ was membrane bound following 24 h. ^{99m}Tc-$Z_{HER2:V2}$ was blocked by excess amounts of unlabeled $Z_{HER2:V2}$ in SKOV3 cells. ^{99m}Tc-$Z_{HER2:V2}$ exhibited high distribution (10.07% ID/g) in SKOV3 xenografts at 6 h following injection. The single photon emission computed tomography (SPECT) imaging revealed clear localization of ^{99m}Tc-$Z_{HER2:V2}$ in the SKOV3 xenografts at 4 h. However, there was low uptake in MCF-7 tumors on the SPECT images. The SKOV3 xenograft imaging could be blocked by excess amounts unlabelled $Z_{HER2:V2}$. ^{99m}Tc-$Z_{HER2:V2}$ is an easy and quick labeling method, with high labeling yields, and radiochemical purity. ^{99m}Tc-$Z_{HER2:V2}$ is a promising probe for the diagnosis of HER2-overexpression tumors and the monitoring of therapy response.

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Introduction

Human epidermal growth factor receptor type 2 (HER2 also known as ErbB2, p185), in which genes located at chromosome 17q21, is one of four members of the ErbB/HER family. The HER2 modulates its activity by a tyrosine kinase signaling pathway, and is involved in the development of many cancers, such as non endocrine tumors (breast, gastric, pancreatic, esophageal, prostate, lung and colon cancers) and few endocrine tumors (thyroid, pituitary, and pheochromocytomas) (1). The HER2 over expression in tumors is responsible for tumor aggressiveness, poor prognosis, decreased survival, and is also associated with enhanced invasiveness and resistance to radiochemotherapy (2,3).

Affibody molecule (Affibody®) consists of 58 amino acids (~6.5 kDa) that contains a modified B domain of the staphylococcal protein A, and it can be obtained via chemical synthesis or produced in bacteria by the use of recombinant DNA technology (4). Because of its small molecule size and high chemical and thermal stability, there are much interested in radiolabeling these molecules and using them for the targeted imaging and treatment of HER2-overexpressing tumors (4-6). $Z_{HER2:342}$ (~7 kDa) is one of basic Affibody molecule, recently, it has been radiolabeled with ^{111}In or ^{99m}Tc and ^{177}Lu, which can be detected HER2-overexpressing tumors under single photon emission computed tomography (SPECT) (7-10). Moreover, $Z_{HER2:342}$ also could be labeled with ^{68}Ga, ^{11}C, ^{18}F and could be imaged under PET/CT (9,11). However, most of $Z_{HER2:342}$ radiolabeled results demonstrated the high abdominal accumulation in liver with prolong renal retention, which hampers its application for the detection of abdomen HER2 overexpression tumors (10).

The $Z_{HER2:V2}$, Affibody molecule of $Z_{HER2:2395-C}$, based on the $Z_{HER2:342}$ binding sequence, with C-terminal engineered cysteine (named $Z_{HER2:V2}$) has been studied recently (12). The structure of $Z_{HER2:V2}$ is similar with $Z_{HER2:342}$, only was replaced-AEN at the N-terminal and -GGGC as a chelator at the C-terminal (13). $Z_{HER2:V2}$ radiolabeled as a probe shows good HER2 tumor target, rapid blood clearance, low levels of stomach and salivary gland radioactivity, low levels of renal radioactivity and low levels of hepatobiliary excretion.

Therefore, the radionuclide labeled $Z_{HER2:V2}$ may be a promising imaging agent for HER2-overexpressing tumors.

The aim of the present study was to label the $Z_{HER2:V2}$ with ^{99m}Tc directly using fast and simple approach and evaluate its properties for HER2 positive tumor imaging: With an eye towards clinical translation.

Materials and methods

Preparation of ^{99m}Tc-$Z_{HER2:V2}$. $Z_{HER2:V2}$(AENKFNKEMRNA YWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEA KKLNDAQGGGC) has been assembled by which changing VEN- to AEN- at the N-terminal and the -GGGC as a chelator at the C-terminal (13). $Z_{HER2:V2}$ was synthesized and purified by Skylight Biotechnology, LLC, (Beijing, China). The labeling method referred to the paper of Tran T and co-authors, and revised the labeling method (14). Briefly, 10 μl of 0.1 M NaOH were added to 10 μl (10 μg) of $Z_{HER2:V2}$ and the solution pH were adjusted to about 12. Subsequently, 200 μl [111 MBq (3 mCi)] of fresh $^{99m}TcO_4^-$ solution obtained from ^{99}Mo/^{99m}Tc generator was added, immediately after 0.3 μl (0.3 μg) of $SnCl_2$was added. The mixture solution was incubated for 20 min at 20-25˚C room temperature. Finally the pH of the mixture was about 7~8. The labeling efficiency and radiochemical purity of the labeled conjugate were analyzed by reversed-phase high-pressure liquid chromatography (RP-HPLC) with a microbond C_{18} column (Alltech, model 305, Deerfield IL, 4.6x250 mm) connected a ultraviolet detector, an NaI (Tl) radioactivity monitor, and a rate meter. The C_{18} column was eluted with a gradient of 5-80% buffer A (0.1% trifluoroacetic acid-CH_3CN) and buffer B (0.1% trifluoroacetic acid-H_2O) in 20 min and a flow rate of 1 ml/min at 25˚C monitoring at 260 nm.

We also analyzed the labeled probe using instant thin layer chromatography (ITLC) method to determine the technetium colloids, ITLC was eluted with acetonitrile. In this eluent, the technetium colloids remained at the origin, while the radiolabeled probe and pertechnetate migrated with solvent front.

In vitro stability analysis. To assess the stability of the radiotracer *in vitro*, ^{99m}Tc-$Z_{HER2:V2}$ was incubated in physiological saline or human fresh serum at 37˚C, and the stability of the radiotracer *in vitro* was evaluated at 1, 2, 4, 6 and 8 h respectively. The same RP-HPLC conditions were used as those for measuring the release of free pertechnetate.

Cell cultures. The HER2-overexpressing ovarian carcinoma cell line SKOV3 and HER2 low expression breast carcinoma cell line MCF-7 were purchased from the Institute of Cell Biology of the Chinese Academy of Science (Shanghai, China). All cells were cultivated in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% foetal bovine serum under standard conditions (37˚C, humidified atmosphere containing 5% CO_2). Cell growth was monitored under inverted microscope with phase contrast. When the cell density reached 90%, the cells were harvested.

Cellular uptake, retention, binding affinity and blocking studies. Sixtuplicate cell wells were used for each data point. The SKOV3 cells were washed twice with phosphate-buffered saline (PBS), then 2 ml of fresh RPMI 1640 medium containing 10% FBS were added, followed by 37 kBq of ^{99m}Tc-$Z_{HER2:V2}$ (0.0001 μg/μl) directly into each well. Cells were harvested by the same trypsin-EDTA solution at 1, 2, 4, 6, 8, 12, and 24 h respectively as described above. Before cell lyses, the medium containing 10% FBS was removed, and the wells were washed twice with PBS. The collected fractions radioactivity was measured using an automated γ counter. Counts the containing radioactive medium and PBS were defined as C_{up}. After being lysed with 0.5 ml of trypsin-EDTA, 0.5 ml of 10% FBS was added to each well, and then cells were washed twice with PBS. Radioactivity of the lyses solution and PBS were designated as C_{down}. The collected fractions radioactivity was measured by an automated γ counter. The cellular uptake ratio was calculated by the formula $C_{down}/(C_{down} + C_{up})$ x100%.

To determine cellular retention, ^{99m}Tc-$Z_{HER2:V2}$ (37 kBq 0.0001 μg/μl) was directly added into 6-well plates that containing 6x10^5 SKOV3 cells and incubated at 37˚C for 4 h. Then, the medium was removed, and all wells were washed twice with PBS solution. After 2 ml of fresh RPMI 1640 medium containing 10% FBS was added into each well, the plates were incubated at 37˚C for 1, 2, 4, 6, 8, 12, and 24 h respectively. The medium containing 10% FBS of the wells was removed before cell lyses, and the wells were washed twice with PBS. The collected fractions radioactivity was measured by an automated γ counter. The cellular retention ratio was calculated by the same formula for calculating cellular uptake kinetics given above. In this way, the radioactivity counts of C_{down} contain ^{99m}Tc-$Z_{HER2:V2}$ which have been internalized and membrane-bound.

For internalization studies, ^{99m}Tc-$Z_{HER2:V2}$ uptake was performed as described. Several additional cell plates were used during the binding study to separate the membrane-bound fraction of the conjugate from externalized radioactivity. The wells were washed twice with PBS. Then the isolated cell pellet was washed using 0.2 M acetic acid/0.5 M NaCl, pH 2.5, to remove the cell surface-bound radioactivity at each time point. The radioactivity that was removed from cells by an acidic buffer was defined as membrane-bound.

For blocking studies, 1.5 μg (500 times) or 3 μg (1,000 times) of unlabeled $Z_{HER2:V2}$ and the ^{99m}Tc-$Z_{HER2:V2}$ were simultaneously added to the cell well. After incubation for 4 h, the ^{99m}Tc-$Z_{HER2:V2}$ uptake of HER2-overexpression SKOV3 cells was measured as the same method that was mentioned above.

Biodistribution studies. All animal studies were performed in accordance with the guidelines of local animal care and use committee. Biodistribution studies of ^{99m}Tc-$Z_{HER2:V2}$ in BALB/c nude mice bearing SKOV3 xenografts were performed. For biodistribution studies, 0.1 ml 1,110 kBq dose of ^{99m}Tc-$Z_{HER2:V2}$ was injected via the tail vein into the BALB/c nude mice bearing SKOV3 cells (n=4/group), which were sacrificed at 1, 2, 4, or 6 h after the ^{99m}Tc-$Z_{HER2:V2}$ injection. The organ or tissue samples of interest (blood, heart, liver, spleen, kidney, lung, stomach, small intestine, brain, bone, muscle and tumor) were harvested by dissection and weighed. A gamma well counter (CAPRAC®; Capintec Inc., Ramsey, NJ, USA) was used to measure radioactivity uptake in organs or tissues, which was defined as the percentage of injected dose per gram of tissue (%ID/g).

In vivo SPECT imaging studies. Three nude mice were used for (SPECT) imaging. Two mice which bearing SKOV3

xenografts and MCF-7 xenografts were respectively injected 37 MBq (100 μl) of ^{99m}Tc-$Z_{HER2:V2}$ (0.003 μg/μl) via the tail vein. The other SKOV3 xenografts nude mice was injected 30 μg unlabeled $Z_{HER2:V2}$via tail vein 1 h before the ^{99m}Tc-$Z_{HER2:V2}$ (0.003 μg/μl) was injected 37 MBq (100 μl). After 4 h of injection, animals were anesthetized and placed supine near the centerfield of the view of the SPECT detector and imaged using the e.cam^{duet} SPECT equipped with a pinhole collimator. One static anterior image (300,000 counts) was obtained with a zoom 1.78, matrix 128x128 and energy window 140 keV, 15%.

Statistic analysis. Variables were expressed as mean value plus or minus standard deviation ($\bar{x} \pm s$). Analysis of variance (ANOVA) was used to analyze the variation of cellular uptake in the blocking experiment. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Radiolabeling of $Z_{HER2:V2}$. The labeling efficiency was 98.99±0.99% (n=6). ^{99m}Tc-$Z_{HER2:V2}$ using RP-HPLC analysis showed that the retention time was 12 min, with a single, high and sharp peak, which was consistent with its single absorbance peak at 260 nm (Fig. 1) whereas that of the free ^{99m}Tc was about 4.5 min (Fig. 2). Even 6 h later the labeling efficiency of ^{99m}Tc-$Z_{HER2:V2}$ was still 97.23%. The result of ITLC showed that the origin has little counts, and almost radioactivity at the front, indicating most of ^{99m}Tc have been bound with $Z_{HER2:V2}$, and almost no technetium colloids existence.

In vitro stability analysis. The results showed that the ^{99m}Tc-$Z_{HER2:V2}$ incubated in physiological saline or human fresh serum at 37˚C for 1, 2, 4, 6 and 8 h was very stable *in vitro*. RP-HPLC analysis demonstrated that the radiochemical purity of ^{99m}Tc-$Z_{HER2:V2}$ was (98.17±1.42%, 98.08±0.94%, 97.81±0.75%, 97.54±0.75%, 96.71±0.51%) and (98.89±0.76%, 98.09±0.57%, 97.87±0.31%, 97.81±0.73%, 96.84±0.69%) respectively. The radiochemical purity of the probe was still as high as 96% even after 6 h, indicating that no significant degradation and off-labeling occurred in human fresh serum or physiological saline at 37˚C.

Cellular uptake, retention, binding affinity and blocking studies. The results of cellular uptake ^{99m}Tc-$Z_{HER2:V2}$ studies are presented in Fig. 3. With the interval of time, the cellular uptake was rising slowly, and the peak of cellular uptake ratio appeared at 24 h was 6.15±0.18%. After the peak, the cellular uptake ratio of ^{99m}Tc-$Z_{HER2:V2}$ showed a little decline.

The cell membrane binding rate was showed in Fig. 4, indicating that the cell membrane binding is a rapid process in SKOV3 cells, greater than 85.5% of the radioactivity was bound on the cell membrane after incubating ^{99m}Tc-$Z_{HER2:V2}$ with SKOV3 cells, and after 24 h later, there were almost 70% ^{99m}Tc-$Z_{HER2:V2}$ sill binding on cell membrane. As a result, the ^{99m}Tc-$Z_{HER2:V2}$ has a slowly internalizing course characterize.

The result of retention kinetics analysis was shown in Fig. 5. The results demonstrated good ^{99m}Tc-$Z_{HER2:V2}$ retention in SKOV3 cells, with 48.58±4.52% at 6 h, even after 24 h, the retention ratio of ^{99m}Tc-$Z_{HER2:V2}$ still reached 35.16±11.23%.

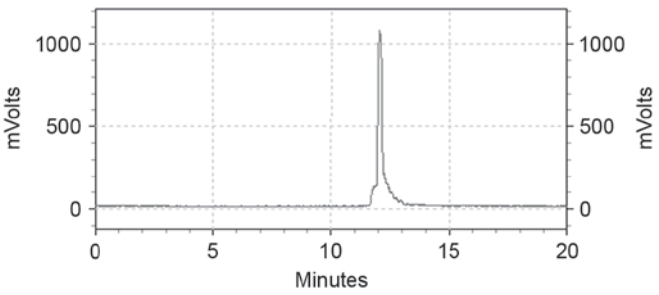

Figure 1. HPLC retention time of ^{99m}Tc-$Z_{HER2:V2}$.

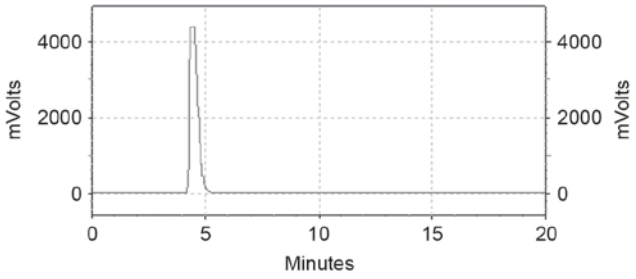

Figure 2. HPLC retention time of free ^{99m}Tc.

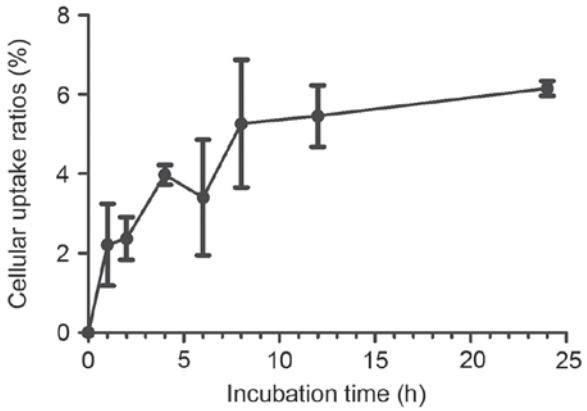

Figure 3. Cellular uptake kinetics of ^{99m}Tc-$Z_{HER2:V2}$ in SKOV3 cells.

The specific binding of ^{99m}Tc-$Z_{HER2:V2}$ in SKOV3 cells was shown in Fig. 6, the ^{99m}Tc-$Z_{HER2:V2}$ was blocked by an excess amount (500 or 1,000 times) of unlabeled $Z_{HER2:V2}$ in SKOV3 cells ($F=351.232$, $P<0.05$). In addition, ^{99m}Tc-$Z_{HER2:V2}$ was significantly blocked when 1,000 times of unlabeled $Z_{HER2:V2}$ were added as compared with that of 500 times of unlabeled $Z_{HER2:V2}$ in SKOV3 cells, demonstrating the character of HER2-specific binding.

Biodistribution studies. ^{99m}Tc-$Z_{HER2:V2}$ biodistribution analysis was performed in nude mice bearing SKOV3 xenografts at 1, 2, 4, and 6 h after intravenous injection. Data on the biodistribution of the SKOV3 xenografts are presented in Table I. The results revealed low uptake of radioactivity in all organs except kidneys (8.68±2.68% ID/g) and tumor. The other samples were <2% ID/g except the liver (2.70±0.17% ID/g). The radioactive uptake in SKOV3 xenografts was higher than that for organs other than the kidneys. The tumor accumulation

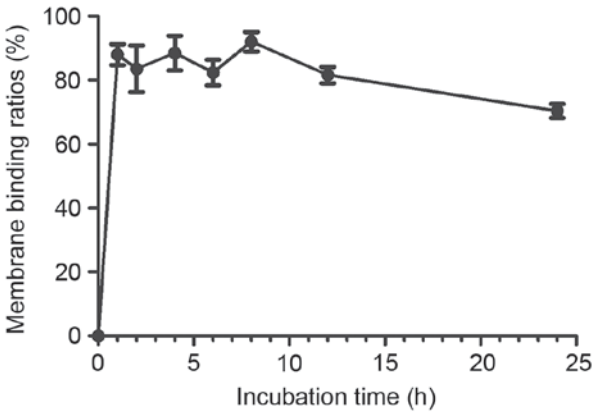

Figure 4. Membrane binding ratio of ^{99m}Tc-$Z_{HER2:V2}$ in SKOV3 cells.

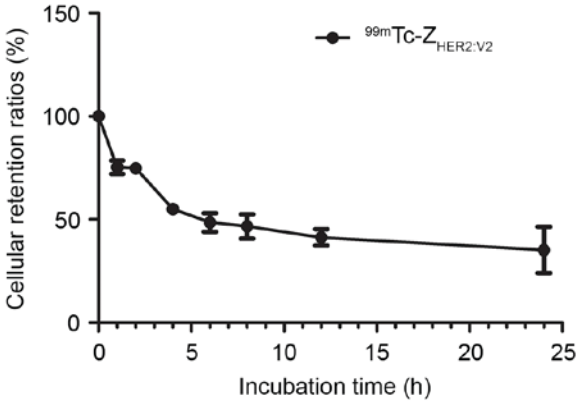

Figure 5. Cellular retention kinetics of ^{99m}Tc-$Z_{HER2:V2}$ in SKOV3 cells.

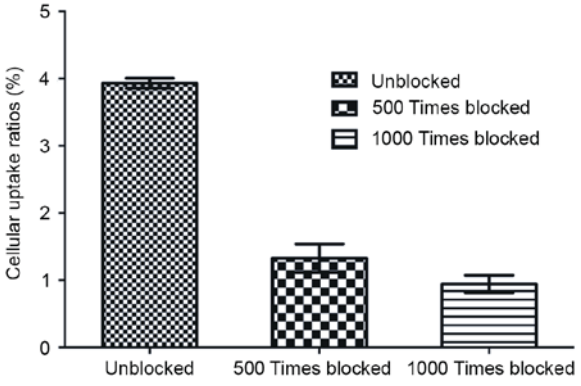

Figure 6. Specificity of ^{99m}Tc-$Z_{HER2:V2}$ to SKOV3 cells. Cell cultures were incubated with ^{99m}Tc-$Z_{HER2:V2}$ for 4 h. HER2 binding can be blocked by excess amount of unlabeled $Z_{HER2:V2}$. Data are expressed as mean ± SD (n=6). HER2, human epidermal growth factor receptor type 2.

was 4.14±1.61, 6.47±2.09, 5.04±2.58, 10.07±0.33% ID/g at 1, 2, 4 and 6 h, respectively. The radioactivity in all organs was reduced as time gone by other than the tumor, demonstrating good specificity and sensitivity of ^{99m}Tc-$Z_{HER2:V2}$ in the detection of HER2 receptors.

In vivo SPECT imaging studies. After injection of ^{99m}Tc-$Z_{HER2:V2}$ in nude mice bearing SKOV3 xenograft via the tail vein, the images revealed intense tracer uptake in the tumor (Fig. 7) and the T/NT ratios was 9.98 at 4 h after injection, while the MCF-7 xenograft has low tracer accumulated (Fig. 8) and the T/NT ratios was 3.79, indicating ^{99m}Tc-$Z_{HER2:V2}$ could be highly uptake by HER2-overexpression tumors, and further proved the probe HER2-specific binding. The kidneys also accumulated a lot of ^{99m}Tc-$Z_{HER2:V2}$ radioactivity, however, little radioactivity stay in the liver, indicating that the ^{99m}Tc-$Z_{HER2:V2}$ radioactivity was removed mainly through the renal routes and decreased gradually throughout the body. And the SKOV3 xenografts imaging can be blocked by excess amount unlabelled $Z_{HER2:V2}$ at 2 h after injection of the tracer (Fig. 9).

Discussion

Recently, HER2-receptor imaging has been developed as a method to detect tumor HER2-overexpression, and it can revealed important molecular clues for cancer patient management.

Molecular imaging is an advance imaging that can visually characterize, represent, and quantify biologic processes at the cellular or subcellular level. In previous studies, researchers used ^{64}Cu-MM-302 (15) and ^{18}F-$Z_{HER2:342}$ (11) proved to be potential for PET HER2 imaging. But these radiotracers have some limitations: ^{18}F labeling peptide requires an in-house cyclotron system, a complex infrastructure, a time-consuming synthesis procedure and tedious purification; ^{64}Cu-DOTA conjugates generally display high liver accumulation because of the possible off-labeling of ^{64}Cu from the chelator (15).

^{99m}Tc has been successfully labeled on Affibody molecule and its derivatives. As is widely known, it is a complicated process that ^{99m}Tc was labeled on peptides, 'incubate in boiling water' was used in most of previous studies (10,16,17). In this study, direct labeling method was performing: NaOH was added to adjust the PH and $SnCl_2$ as the reluctant of ^{99m}Tc, not incubated in boiling water but in room temperature. As a result, the labeling efficiency was exceeding 98%. This labeling approach is similar with Tran and co-authors (14), and the labeling efficiency has no difference (98.99% vs. 98.1%). These results indicated that this revised labeling method is very good.

Direct labeling has a disadvantage of yielding technetium colloids, to avoid this situation, how many $SnCl_2$ were added is the important. In this study, ^{99m}Tc were almost labeled on $Z_{HER2:V2}$ and technetium colloids were yielded hardly. And from the ITLC test, outcome showed that no technetium colloids were yielded.

At present, lots of studies on the ^{99m}Tc labeled HER2 molecular probes to image the HER2-overexpression tumors *in vivo* have been performed. But from the biodistribution studies, the results demonstrated that there were a high level hepatic accumulation of radioactivity and a prolong renal retention (16-19). A high level of hepatic accumulation reduces the imaging sensitivity and may obstruct the detection of liver metastases, and an elevated renal uptake was hindering the detection of metastases in the lumbar and abdomen area. Thus, it is very important to improve the probe tumor uptake and decrease liver and kidneys uptake as much as possible.

Some of previous studies demonstrated that the amino acid consists of the N-terminal was significant for the biodistribution in terms of liver accumulation and the extent of hepatobiliary excretion. Scholars agreed on that N-terminal chelator contains amino acids with hydrophilic (polar or charged) side chains

Table I. Biodistribution of ^{99m}Tc-$Z_{HER2:2891}$ in SKOV-3 xenografts.

Organ	1 h	2 h	4 h	6 h
Blood	2.26±1.20	1.55±0.59	1.04±0.45	0.75±0.10
Heart	0.40±0.15	0.29±0.27	0.43±0.36	0.61±0.46
Liver	2.83±2.23	2.72±0.00	2.45±1.30	2.80±0.16
Spleen	1.91±0.70	0.96±0.61	0.85±0.39	0.86±0.53
Kidney	12.17±3.26	9.26±2.92	6.04±4.69	7.25±1.63
Lung	0.66±0.27	0.38±0.05	0.34±0.00	0.60±0.23
Stomach	2.03±0.59	1.16±0.84	0.87±0.76	0.34±0.26
Intestine	1.17±0.44	0.71±0.53	0.59±0.44	0.51±0.38
Brain	0.32±0.06	0.08±0.02	0.11±0.01	0.13±0.01
Bone	1.02±0.75	0.69±0.27	0.47±0.37	0.21±0.18
Muscle	0.85±0.35	0.37±0.10	0.10±0.07	0.56±0.22
Tumor	4.14±1.42	6.47±2.09	5.04±2.58	10.07±0.33

Values are mean ± SD ($\bar{x}$ ± SD) and expressed as %ID/g (n=4).

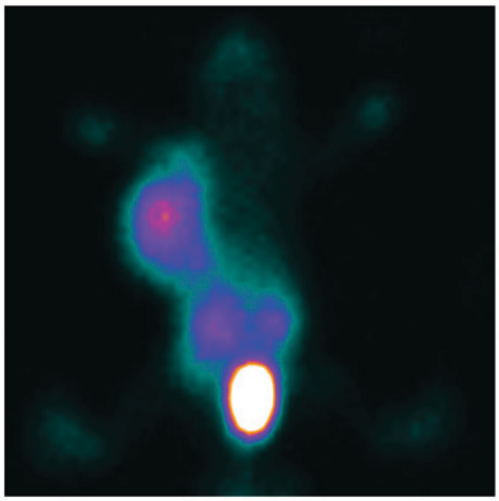

Figure 7. Imaging of HER2 over-expression in SKOV3 xenografts. Tumor was clearly visualized after injection of ^{99m}Tc-$Z_{HER2:V2}$, while liver was hardly seen. HER2, human epidermal growth factor receptor type 2.

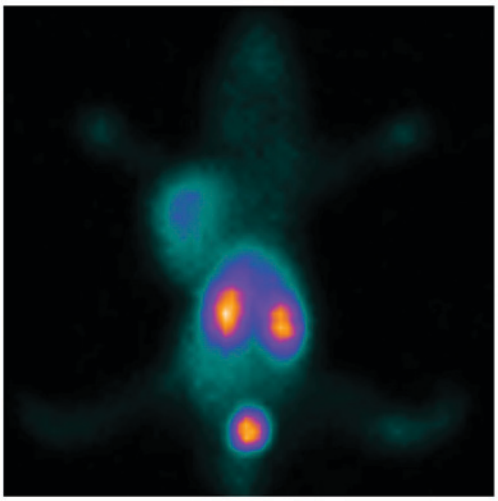

Figure 9. The SKOV3 xenografts imaging can be blocked by excess amount unlabelled $Z_{HER2:V2}$ at 2 h after injection of ^{99m}Tc-$Z_{HER2:V2}$ compared with Fig. 8.

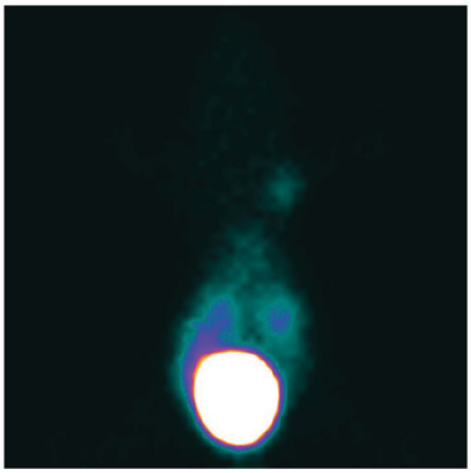

Figure 8. Imaging of HER2 low-expression in MCF-7 xenografts. Tumor was weakly imaged after injection of ^{99m}Tc-$Z_{HER2:V2}$, meanwhile liver was hardly seen. HER2, human epidermal growth factor receptor type 2.

could reduced liver accumulation and had low hepatobiliary excretion (18,20-23), whereas the consists of the C-terminal was shown to be less influential in that aspect (24). Furthermore, validation experiments showed that Ala, Glu at the N-terminal was associated with low hepatic uptake and low hepatobiliary excretion (16,22,23,25). In the present study of biodistribution, the results showed the low hepatic uptake of ^{99m}Tc-$Z_{HER2:V2}$, and the liver hardly seen from molecular image.

Our intriguing findings were further confirmed by the *vivo* research outcome. The images of nude mice bearing HER2-overexpression SKOV3 xenograft was seen clearly and the tumor showed a significant contrast compared to other organs, on the contrary, the images of nude mice bearing HER2 low expression MCF-7 xenograft showed few of ^{99m}Tc-$Z_{HER2:V2\ uptake}$. These demonstrated that the ^{99m}Tc-$Z_{HER2:V2}$ was significantly uptake by HER2-overexpression tumors. The *vitro* and *vivo* blocking study showed that HER2-overexpression SKOV3 cells uptake ^{99m}Tc-$Z_{HER2:V2}$ can be successfully

blocked using excess amount unlabeled $Z_{HER2:V2}$. These results further revealed binding specificity of HER2-recptors of ^{99m}Tc-$Z_{HER2:V2}$. Moreover, radioactivity accumulation in liver was limited which illustrates that using-GGGC as a chelator at C-terminal could be reduced the hepatic uptake.

In conclusion, ^{99m}Tc-$Z_{HER2:V2}$ can be labeled simply and quickly in directly labeling method, with good labeling yield and radiochemical purity. It is easy for ^{99m}Tc-$Z_{HER2:V2}$ kit formulation. ^{99m}Tc-$Z_{HER2:V2}$ can specifically and efficiently target HER2-overexpressing tumors with promising sensitivity and specificity. We strongly anticipate that it may be a promising probe for clinical translation to detect HER2-overexpression tumors.

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Competing interests

The authors declare that they have no competing interests.

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