Pilot Study of Nectin-4–Targeted PET Imaging Agent ^{68}Ga-FZ-NR-1 in Triple-Negative Breast Cancer from Bench to First-in-Human

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Nectin cell adhesion molecule 4 (Nectin-4) is an emerging biomarker for cancer diagnosis and therapy. We developed a Nectin-4–targeted ^{68}Ga-DOTA-Sar10-Nectin-4 (^{68}Ga-FZ-NR-1) PET/CT radiotracer for detecting Nectin-4 expression in a tumor model and in triple-negative breast cancer (TNBC) patients. **Methods:** A series of Nectin-4–targeted radiotracers—^{68}Ga-FZ-NR-1, ^{68}Ga-DOTA-polyethylene glycol 5-Nectin-4 (^{68}Ga-FZ-NR-2), and ^{68}Ga-DOTA-polyethylene glycol 10-Nectin-4 (^{68}Ga-FZ-NR-3)—were synthesized, and their targeting ability and specificity were evaluated in vitro and in vivo. In vitro experiments were performed in the MDA-MB-468 (Nectin-4–positive) and MDA-MB-231 (Nectin-4–negative) cell lines. PET/CT imaging in tumor models was performed to assess the Nectin-4–targeting ability of the radiotracers. After preclinical experiments and screening, the ^{68}Ga-FZ-NR-1 radiotracer was selected for safety and efficacy evaluation in a first-in-human trial in TNBC patients. Positive lesions were biopsied and analyzed by immunohistochemistry to determine Nectin-4 expression levels. **Results:** The 3 ^{68}Ga-labeled radiotracers exhibited high radiochemical purity, stability, and strong affinity for Nectin-4. In vitro cell uptake studies showed that the radiotracers effectively targeted Nectin-4 in MDA-MB-468 cells, and ^{68}Ga-FZ-NR-1 showed the highest targeting efficacy. In the MDA-MB-468 tumor model, PET/CT imaging showed that ^{68}Ga-FZ-NR-1 was taken up at higher rates than ^{68}Ga-FZ-NR-2 and ^{68}Ga-FZ-NR-3, and it exhibited favorable pharmacokinetics and safety profiles. ^{68}Ga-FZ-NR-1 was thus selected for subsequent clinical trials. ^{68}Ga-FZ-NR-1 PET/CT effectively identified tumors in 9 patients with TNBC, which was confirmed by ^{18}F-FDG PET/CT. Biopsy samples of the tumor lesions revealed that the positive lesions identified by ^{68}Ga-FZ-NR-1 PET/CT corresponded to areas of high Nectin-4 expression. **Conclusion:** A series of Nectin-4–targeted radiotracers (^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3) was developed and evaluated. Preclinical studies demonstrated that ^{68}Ga-FZ-NR-1 can identify tumors with high Nectin-4 expression. In a preliminary clinical study, ^{68}Ga-FZ-NR-1 was used to effectively identify and visualize Nectin-4–expressing tumor lesions in patients with TNBC, which was confirmed by immunohistochemistry. This radiotracer provides a noninvasive approach to the assessment of Nectin-4 and a potential basis for the development of Nectin-4–targeted treatments for TNBC.

Key Words: triple-negative breast cancer; TNBC; Nectin-4; radiotracer; PET/CT imaging; ^{68}Ga

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Triple-negative breast cancer (TNBC) accounts for 15%–20% of breast cancer cases (*1*,*2*) and is one of the most aggressive subtypes, with a 5-y survival rate of approximately 40% (*3*–*5*). TNBC is characterized by significant heterogeneity and a high rate of recurrence, which makes treatment difficult. To address these challenges, numerous treatment strategies have been developed, of which targeted therapies show great promise. Nectin cell adhesion molecule 4 (Nectin-4), also called PVRL4, has been identified as a treatment biomarker for TNBC (*6*). It plays a crucial role in cell adhesion and tight junctions, and it affects cellular behavior via the phosphatidylinositol 3-kinase–Akt signaling pathway (*7*). It is expressed at relatively low levels in normal adult tissues, whereas high Nectin-4 expression levels are associated with tumor invasiveness and poor outcomes in several cancers, including breast cancer (*6*), urothelial carcinoma (*8*), and ovarian cancer (*9*,*10*). In TNBC, Nectin-4 expression was detected in 62% of samples, and its overexpression was correlated with a worse prognosis (*6*), suggesting that it could serve as a diagnostic and prognostic biomarker. Accurate assessment of Nectin-4 levels is thus important.

Biopsy is the clinical gold standard for diagnosing cancer and guiding treatment. However, biopsy has some disadvantages, such as an invasive nature and limited tissue samples (*6*,*11*,*12*). PET/CT is a noninvasive modality that allows visualization of tumors and provides whole-body lesion distribution data; it also provides information on tumor metabolism and molecular biology (*13*). Unlike biopsy, PET/CT can assess organs such as the brain, lungs, and kidneys (*14*), and it has the potential to overcome the limitations associated with traditional biopsy procedures (*15*). To accurately

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assess Nectcin-4 expression in vivo, Nectin-4–targeted PET/CT imaging may be a more adequate method. Therefore, the development of Nectin-4–targeted probes is important.

Nectin-4–targeted PET imaging probes have been developed and reported in recent years (*16–18*). Nectin-4–targeted monoclonal antibody-based PET imaging probes have shown potential in preclinical research, yet issues such as slow clearance and poor permeability in solid tumors have limited their use in clinical settings (*19*). In contrast, peptide-based PET/CT imaging tracers present distinct advantages. They are straightforward to synthesize and can be chemically modified to improve stability and binding affinity; in addition, their lower immunogenicity minimizes the likelihood of adverse reactions (*20*). For example, the Nectin-4–targeted radiotracer ^{68}Ga-N188, which is based on a bicyclic peptide structure, was developed for patients with advanced urothelial carcinoma and has shown good sensitivity and specificity for Nectin-4–expressing lesions, thus providing strong support for the clinical use of Nectin-4–targeted radiotracers (*21–25*). In addition, the pharmacokinetic properties of the radiotracer were optimized by incorporating polyethylene glycol (PEG) chains of different lengths (*26*). Recent research advances and the inherent benefits of the bicyclic peptide structure support the potential of peptides and their derivatives to improve imaging contrast in clinical Nectin-4 PET imaging.

Here, ^{68}Ga-labeled radiotracers based on bicyclic peptides were designed and synthesized, and their performance was evaluated in vitro and in vivo. The most effective radiotracer was screened and tested in a pilot study that included patients with TNBC to evaluate the ability of the radiotracer to detect Nectin-4 in TNBC.

MATERIALS AND METHODS

Information on the chemical reagents, analytic methods, and experimental procedures used is provided in the supplemental materials (supplemental materials are available at http://jnm.snmjournals.org).

Synthesis and Radiolabeling

The precursors DOTA-Sar10-Nectin-4 (FZ-NR-1), DOTA-PEG5-Nectin-4 (FZ-NR-2), and DOTA-PEG10-Nectin-4 (FZ-NR-3) and the lead compound were obtained from a peptide manufacturing company (Chinese Peptide). The radiolabeling procedure was completed in a single step within 20 min, and the radiochemical purity was evaluated using radio–high-performance liquid chromatography and radio–thin-layer chromatography. A comprehensive description of the synthesis, production, and purification of the Nectin-4–targeted radiotracers is included in Supplement 3.

Cell Uptake

The uptake of the 3 ^{68}Ga-labeled radiotracers (^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3) was assessed in the MDA-MB-468 and MDA-MB-231 cell populations at 30, 60, and 120 min after exposure. Cells were treated with 37–74 kBq of radiotracer per well and incubated at 37°C to simulate in vivo conditions. The radioactivity of samples was assessed at each time point using a γ-counter, and the affinity of ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3 for cells was determined by quantification of γ-counts. The radiotracer showing the highest Nectin-4–targeted ability was used for subsequent experiments. All experiments were performed in triplicate. A comprehensive description of the cell experiments is provided in Supplement 7.

microPET CT Imaging and Biodistribution Studies

PET/CT images were acquired using a microPET CT multimodal imaging system (Biograph 6; Siemens Medical Solutions). Mice bearing MDA-MB-468 tumors were injected with 5.55–7.40 MBq (200 μL) of ^{68}Ga-labeled radiotracers through the tail vein. Mice were anesthetized with isoflurane and placed in a prone position at 30, 60, 120, and 180 min after injection, and CT scans were performed for attenuation correction and anatomic imaging (20 s, 80 kV, and 150 mAs, with a spatial resolution of 1.25). PET images were reconstructed using a filtered backprojection algorithm and combined with CT images. To evaluate binding specificity, an excess of the lead compound (400 μg) and the ^{68}Ga-FZ-NR-1 radiotracer (5.55–7.40 MBq) in 200 μL of sterilized water were injected into a tumor-bearing mouse simultaneously. PET acquisition was performed at various times after injection. The radioactivity content in the target tissue was measured, and the tissue-associated activity was reported as percentage injected dose per gram of tissue (%ID/g). The in vivo Nectin-4–targeted capabilities of the 3 radiotracers were compared by quantifying the tumor uptake of ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3. The results were expressed as %ID/g to select the most effective radiotracer for further clinical study.

Biodistribution experiments were performed using MDA-MB-468 and MDA-MB-231 tumor models. Each mouse received an injection of ^{68}Ga-FZ-NR-1 (~5.55–7.40 MBq, 200 μL) via the tail vein. The radioactivity in various target tissues (including blood, muscle, bone, liver, spleen, kidney, heart, lung, bladder, brain, small intestine, and stomach) was measured using a γ-counter. The organs were weighed, and the γ-counts were adjusted for decay to the time of injection. Tissue activity was analyzed using GraphPad Prism version 9.0 (GraphPad Software), and results were expressed as %ID/g. Additional details can be found in Supplements 8 and 10.

Patients

The human study received approval from the Ethics Committee of Fudan University Shanghai Cancer Center (number 2407299-29) and was performed in accordance with the principles outlined in the 1964 Declaration of Helsinki and its later amendments, along with comparable ethical standards. Before undergoing ^{68}Ga-FZ-NR-1 PET/CT imaging, written informed consent was obtained from all participants. Patients with a pathologic diagnosis of TNBC were included in the study. Those with severe liver or kidney dysfunction, as well as pregnant or lactating individuals, were excluded. Nine patients with TNBC were included in the study. The ability of the radiotracer to detect Nectin-4 in TNBC patients was determined by measuring SUV_{max} as an indicator of uptake by normal organs and tumor tissues. The demographic data of patients with TNBC are summarized in Supplemental Table 1, and information on the study participants is summarized in Supplemental Table 2. PET/CT scans were acquired using a Biograph mCT Flow scanner (Siemens Medical Solutions). The detailed protocols for image acquisition and reconstruction are presented in Supplements 12 and 13.

Immunohistochemistry Staining in TNBC

Paraffin sections of tumor specimens were obtained from TNBC patients. All immunohistochemical staining was performed with a BenchMark Ultra autostainer (Ventana Medical System Inc., Roche) according to the BenchMark Ultra advanced staining system operator guide. Antibody clones for estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, and Nectin-4 were SP1 (Roche), 1E2 (Roche), 4B5 (Roche), and EPR15613-68 (Abcam), respectively. Nuclear estrogen receptor or progesterone receptor staining in more than 1% of tumor cells was defined as estrogen receptor–positive or progesterone receptor–positive according to the 2020 American Society of Clinical Oncology and College of American Pathologists guideline (*27*), whereas human epidermal growth factor receptor 2 status was assessed as proposed in the 2018 guideline (*28*). Human epidermal growth factor receptor 2 was considered positive with an immunohistochemistry score of 3+ or when gene amplification was detected by fluorescence in situ hybridization (*28*). Specific Nectin-4

immunoreactivity was found and localized in the cell membrane and cytoplasm of tumor cells.

Statistical Analysis

Data are presented as the mean ± SEM of at least 3 independent experiments. The statistical significance of differences between groups was assessed using *t* test. Graphs were prepared and analyzed using GraphPad Prism (version 9.0), and a *P* value of less than 0.05 indicated statistical significance.

RESULTS

Synthesis and Characterization of the ^{68}Ga-Labeled Radiotracers

Peptides with DOTA attached at the N terminus, which aids in labeling with radioactive isotopes, were prepared using a solid-phase synthesis technique. The resulting peptide ligands, named FZ-NR-1, FZ-NR-2, and FZ-NR-3, were isolated with more than 90% purity. High-performance liquid chromatography retention times were measured at 9.47, 10.73, and 10.52 min (Supplemental Fig. 1). The structures of FZ-NR-1, FZ-NR-2, and FZ-NR-3 were validated using mass spectrometry (Fig. 1A, left; Supplemental Fig. 1). Surface plasmon resonance technology (*29*) confirmed the strong affinity of FZ-NR-1, FZ-NR-2, and FZ-NR-3 for the Nectin-4 protein, with dissociation constants of 2.81, 1.718, and 1.805 nM, respectively (Supplemental Fig. 2). The optimized FZ-NR ligands demonstrated effective chelation with the radioactive isotope $^{68}Ga^{3+}$ (Supplemental Fig. 3). Radio–high-performance liquid chromatography analysis detected radioactivity peaks at 9.52, 10.82, and 10.60 min for ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3, respectively (Supplemental Fig. 4). The consistency in the retention times indicated that the precursor peptides were successfully radiolabeled with $^{68}Ga^{3+}$ and isolated with a radiochemical purity of more than 98% for ^{68}Ga-FZ-NRs (Fig. 1A, right), providing a solid basis for further studies. The distribution coefficients (log $D_{7.4}$) for ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3 were −3.55 ± 0.10, −2.35 ± 0.07, and −2.23 ± 0.04, respectively, indicating high hydrophilicity, and ^{68}Ga-FZ-NR-1 was the most hydrophilic of the 3 radiotracers (Supplemental Fig. 5). The 3 radiotracers exhibited a high plasma protein binding rate within 180 min (Supplemental Fig. 6). After incubation in physiologic saline, fetal bovine serum, and human serum for 180 min, the radiotracers ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3 maintained a purity of more than 97% (Fig. 1B; Supplemental Fig. 7), indicating their favorable in vitro stability and suitability for preclinical and clinical applications.

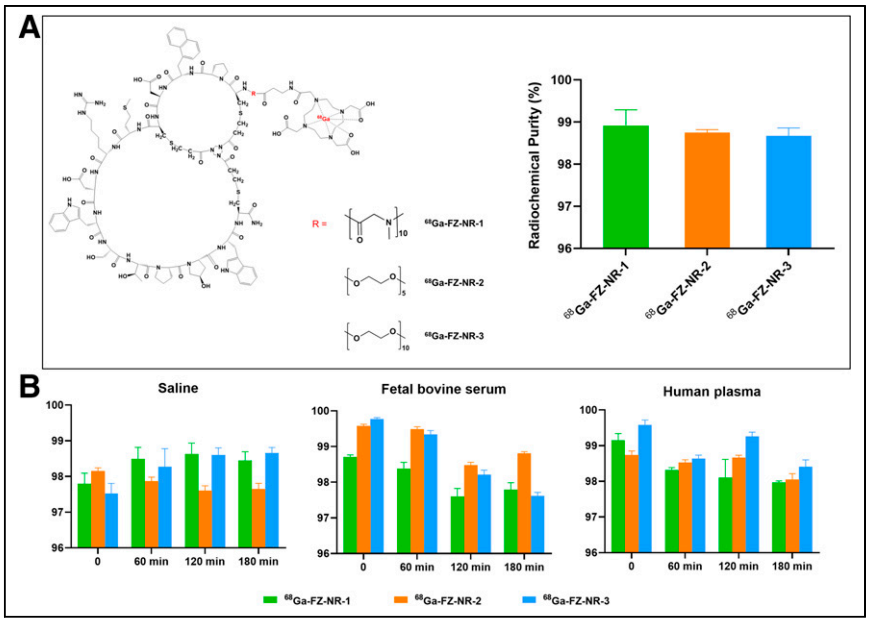

FIGURE 1. Radiochemical structure, purity, and stability of ^{68}Ga-labeled radiotracers. (A) Structure and radio–thin-layer chromatography analysis of ^{68}Ga-labeled radiotracers after purification. (B) Stability of 3 ^{68}Ga-labeled radiotracers in saline solution, fetal bovine serum, and human plasma at 0, 60, and 180 min.

Cellular Uptake of the ^{68}Ga-Labeled Radiotracers

To confirm the Nectin-4–targeted capability of ^{68}Ga-FZ-NRs, we used the MDA-MB-468 (Nectin-4–positive) and MDA-MB-231 (Nectin-4–negative) cell lines (Fig. 2; Supplemental Fig. 8). Western blot analysis showed significantly higher Nectin-4 expression levels in MDA-MB-468 cells than in MDA-MB-231 cells (Fig. 2A). To assess the specificity of ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3, the uptake of each radiotracer was measured in both cell lines. After coincubation for 60 min, MDA-MB-468 cells showed significantly greater uptake of ^{68}Ga-FZ-NRs than did MDA-MB-231 cells, consistent with the results of Western blotting (Fig. 2B). The cellular uptake of ^{68}Ga-FZ-NR-1 was higher than that of ^{68}Ga-FZ-NR-2 and ^{68}Ga-FZ-NR-3 in MDA-MB-468 cells. Blockade experiments showed a substantial decrease in the uptake of ^{68}Ga-FZ-NR-1 in MDA-MB-468 cells after coincubation with excess (500-fold) lead compound. The uptake of ^{68}Ga-labeled radiotracers in MDA-MB-468 and MDA-MB-231 cells within 120 min of incubation is shown in Supplemental Figure 8.

Preclinical Study of the ^{68}Ga-Labeled Radiotracers in TNBC by microPET CT Imaging and Ex Vivo Biodistribution

The in vivo tumor-targeting ability of ^{68}Ga-labeled radiotracers was investigated by microPET CT in TNBC tumor models (Fig. 3; Supplemental Figs. 9 and 10). Mice bearing MDA-MB-468 or MDA-MB-231 tumors were injected with 5.55–7.40 MBq of ^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3 via the tail vein and analyzed by microPET CT imaging at 30, 60, 120, and 180 min after injection. In the MDA-MB-468 tumor model (Fig. 3A, left), a notable radioactive concentration of radioactivity was detected in the tumor at 30 min. Radioactivity was also detected in nontumor regions such as the heart and liver, although radioactivity in these nontumor areas decreased significantly over time. At 60 min, the accumulation of radioactivity in the tumor was pronounced (3.53 ± 0.33 for ^{68}Ga-FZ-NR-1, 1.15 ± 0.19 for ^{68}Ga-FZ-NR-2, and 2.10 ± 0.06 for ^{68}Ga-FZ-NR-3), indicating a favorable target-to-background ratio (Supplemental Figs. 9C, 9F, and 9G). The radioactivity in the MDA-MB-468 tumor was clearly visible, whereas that in other tissues returned to nearly background levels, indicating that the tumor-targeting ability of ^{68}Ga-FZ-NR-1 was superior to that of ^{68}Ga-FZ-NR-2 and ^{68}Ga-FZ-NR-3, consistent with the cellular uptake results. The tumor uptake of ^{68}Ga-FZ-NR-1 was significantly lower in the blocking group than in

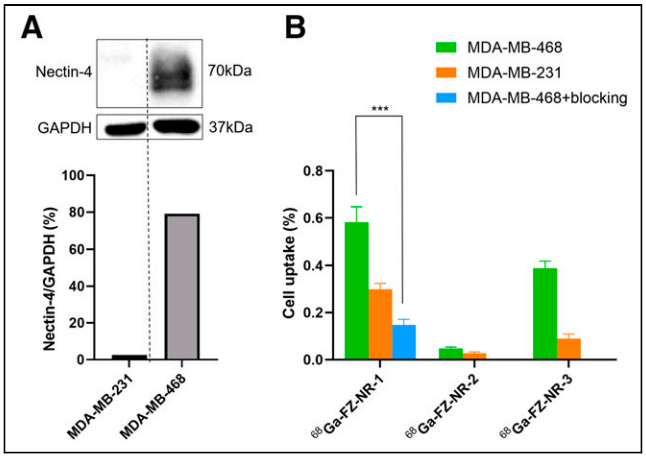

FIGURE 2. Nectin-4 expression in TNBC cell lines. (A) Expression of Nectin-4 protein in MDA-MB-468 and MDA-MB-231 cells. (B) Uptake of ^{68}Ga-labeled radiotracers in MDA-MB-468 and MDA-MB-231 cells after 60 min of incubation ($P = 0.0008$, $n = 4$). ***$P < 0.001$.

the experimental group (Fig. 3A, right). In the Nectin-4–negative MDA-MB-231 model (Fig. 3A, middle), the tumor uptake of ^{68}Ga-FZ-NR-1 was lower than that in the MDA-MB-468 model, consistent with Nectin-4 immunohistochemistry findings (Fig. 3B, right). This indicates that the expression level of Nectin-4 may affect the tumor uptake of ^{68}Ga-FZ-NR-1.

The quantitative analysis of the PET/CT imaging was supplemented with a biodistribution investigation (Supplemental Fig. 10). The uptake of ^{68}Ga-FZ-NR-1 into the MAD-MB-468 tumor was 3.53 ± 0.33 %ID/g at 60 min, which was markedly higher than the uptake rates in the blocking group, at 2.4 ± 0.18 %ID/g, and the MDA-MB-231 control group, at 2.4 ± 0.4 %ID/g. These results support the high affinity of ^{68}Ga-FZ-NR-1 for Nectin-4. The tumor-to-nontumor (muscle) ratio of ^{68}Ga-FZ-NR-1 in the MDA-MB-468 tumor model was approximately 4.5 ± 0.35 at 60 min (Fig. 3B, left). The ^{68}Ga-FZ-NR-1 radiotracer showed good pharmacokinetic properties and was rapidly cleared, resulting in an optimal contrast ratio between tumor and nontumor in the MDA-MB-468 model at the 60-min interval. Collectively, the preclinical imaging results support the use of ^{68}Ga-FZ-NR-1 for the targeted visualization of Nectin-4–expressing tumors, in addition to demonstrating an acceptable safety profile.

The biodistribution results clearly demonstrated the uptake of ^{68}Ga-FZ-NR-1 in tumors and in various organs across the groups (Fig. 4). MDA-MB-468 tumors showed a significantly higher accumulation of ^{68}Ga-FZ-NR-1 (2.39 ± 0.11 %ID/g) than did the blocked MDA-MB-468 group (1.44 ± 0.08 %ID/g) and the MDA-MB-231 group (0.89 ± 0.20 %ID/g) at 60 min (Fig. 4A; Supplemental Fig. 12A). In addition, the tumor-to-nontumor ratio was significantly higher in the MDA-MB-468 group than in the MDA-MB-231 and blocked groups at 60 min (Fig. 4B, left). The MDA-MB-468 model also showed a tumor-to-blood ratio of 27.64 ± 6.96 at 60 min (Supplemental Fig. 12B). Overall, these results confirm that ^{68}Ga-FZ-NR-1 effectively and specifically targets Nectin-4–expressing tumors in vivo; therefore, it was selected for follow-up clinical studies.

To further investigate the in vivo distribution of ^{68}Ga-FZ-NR-1, a biodistribution study in normal mice was performed. ^{68}Ga-FZ-NR-1 was gradually eliminated from the body through the urinary pathway. Most organs showed extremely low levels of radioactivity at 60 min after injection (Supplemental Fig. 12C). These findings are consistent with the pharmacokinetic data. Toxicity evaluation of ^{68}Ga-FZ-NR-1 in Bagg albino mice showed no significant differences in hematologic parameters between the experimental and the control groups (Fig. 4B, right). Histologic examination of hematoxylin and eosin–stained sections from major organs showed no abnormal changes (Supplemental Fig. 13). These results indicate that ^{68}Ga-FZ-NR-1 is safe in mice within the tested dose range.

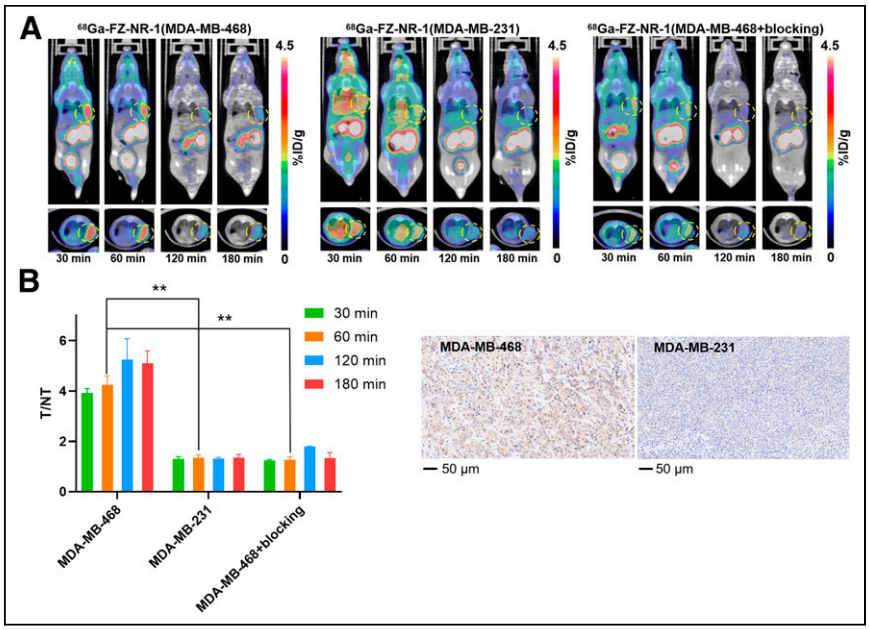

FIGURE 3. (A) Preclinical evaluation of ^{68}Ga-FZ-NR-1 radiotracer by microPET CT imaging of MDA-MB-468, MDA-MB-231, and MDA-MB-468 + blocking tumor models at 30, 60, 120, and 180 min. (B) Tumor-to-nontumor ratios (MDA-MB-468 vs. MDA-MB-231, $P = 0.0014$, $n = 3$; MDA-MB-468 vs. MDA-MB468 + blocking, $P = 0.0012$, $n = 3$) and immunohistochemistry staining of tumor slices of MDA-MB-468 and MDA-MB-231 tumor models. **$P < 0.01$. T/NT = tumor-to-nontumor.

First-in-Human PET/CT Imaging

Before the clinical trial, Nectin-4–targeting and safety assessments conducted in mice indicated that ^{68}Ga-FZ-NR-1 could target Nectin-4 without toxic effects (Figs. 3 and 4; Supplemental Fig. 13). On the basis of these favorable findings, we initiated a preliminary clinical study of ^{68}Ga-FZ-NR-1 in patients with TNBC (approval number 2407299-29). To date, 9 TNBC patients have been enrolled in the study (Supplemental Table 2). Patient 1 was diagnosed with TNBC that had metastasized to the lungs, and ^{68}Ga-FZ-NR-1 PET/CT imaging revealed multiple lung metastatic lesions with a notable uptake of ^{68}Ga-FZ-NR-1 (SUV_{max}, 10.8; Fig. 5A, left). This was confirmed by ^{18}F-FDG PET/CT imaging, which also showed multiple metastatic lesions in both lungs and increased ^{18}F-FDG metabolism (SUV_{max}, 19.3; Fig. 5A, middle). Immunohistochemical analysis confirmed the upregulation of Nectin-4 in the lung

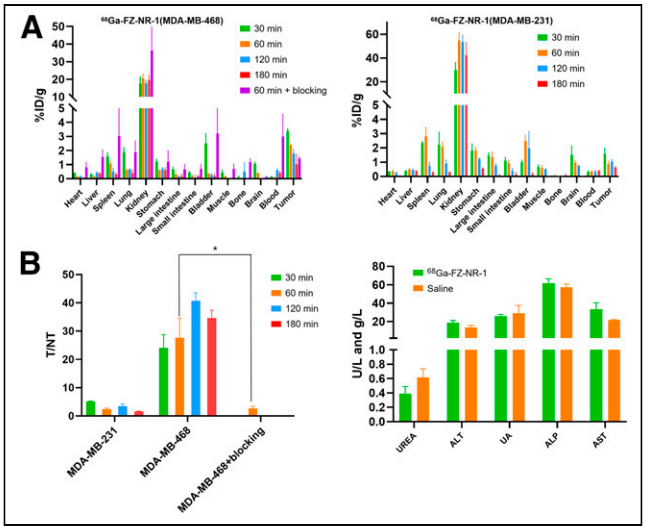

FIGURE 4. (A) Biodistribution of ^{68}Ga-FZ-NR-1 in MDA-MB-468 and MDA-MB-231 tumor models. (B) Corresponding tumor-to-nontumor ratio from biodistribution ($P = 0.0235$, $n = 3$) and main blood biochemical parameters of Bagg albino mice after injection with ^{68}Ga-FZ-NR-1 or saline. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; T/NT = tumor-to-nontumor; UA = uric acid.

metastatic lesions (Fig. 5A, right). Patient 2 was diagnosed with a local recurrence after breast-conserving surgery for TNBC. Both ^{18}F-FDG PET/MRI and ^{68}Ga-FZ-NR-1 PET/CT imaging indicated significant uptake into the tumor for both tracers (SUV_{max}, 6.2 and 5.1, respectively; Fig. 5B, left and middle). The results of immunohistochemical staining showed elevated levels of Nectin-4 in the recurrent lesions (Fig. 5B, right). The details of ^{68}Ga-FZ-NR-1 and ^{18}F-FDG uptake in the 9 patients with TNBC are showed in Supplemental Table 3. In the first-in-human trial that included 9 TNBC patients (Fig. 5; Supplemental Fig. 15), ^{68}Ga-FZ-NR-1 PET/CT allowed visualization of tumors in all patients, which was confirmed by ^{18}F-FDG PET/CT. ^{68}Ga-FZ-NR-1 PET/CT showed a higher tumor-to-background (liver) ratio than did ^{18}F-FDG PET/CT; this, combined with the results of immunohistochemistry, indicated that ^{68}Ga-FZ-NR-1 PET/CT is more effective for detecting Nectin-4–overexpressing tumors in TNBC patients. There were no reports of serious adverse events or notable changes in vital signs at 1 wk after ^{68}Ga-FZ-NR-1 administration. Despite the limited patient data (Supplemental Tables 2 and 3), the preliminary findings suggest that ^{68}Ga-FZ-NR-1 is an effective tracer for identifying patients with positive Nectin-4 expression for targeted drug therapy, as well as for monitoring and predicting the efficacy of treatment.

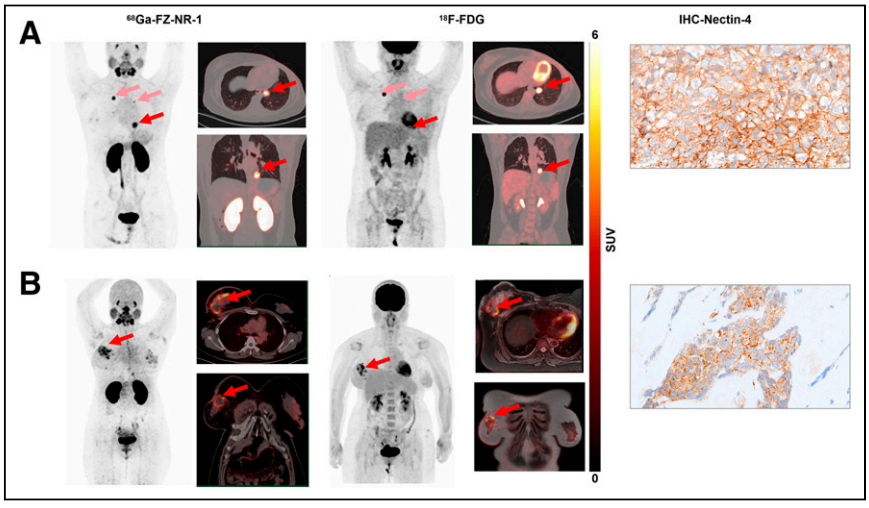

FIGURE 5. ^{68}Ga-FZ-NR-1 PET/CT images and ^{18}F-FDG PET/CT and PET/MR images in representative Nectin-4–positive TNBC patients. (A) PET/CT images and Nectin-4 immunohistochemistry (IHC) staining of patient 1. Maximum-intensity projection (MIP) images and axial and coronal PET/CT images of ^{68}Ga-FZ-NR-1 and ^{18}F-FDG both showed high uptake in lung metastases (arrow). (B) PET/CT images and Nectin-4 IHC staining of patient 2. MIP images and axial and coronal PET/CT images of ^{68}Ga-FZ-NR-1 showed high uptake in recurrence of right breast cancer after surgery (arrow), and those of ^{18}F-FDG PET/MRI showed high uptake.

DISCUSSION

Nectin-4 is a promising target for cancer diagnosis and treatment, and a U.S. Food and Drug Administration–approved drug (enfortumab vedotin) and additional options are in the developmental phase (*22*,*30*). Measuring Nectin-4 expression in tumors can help identify patients who might benefit from targeted therapies and provide a marker for monitoring the response to treatment. PET/CT using receptor-targeted tracers is a noninvasive method for measuring receptor expression, which allows real-time monitoring and the design of personalized treatments. This could increase the efficacy of treatment and minimize side effects in patients with malignant tumors (*13*). Antibody-based radiotracers that target Nectin-4 have been researched extensively, and the potential of various radiotracers, such as ^{99m}Tc-HYNIC-mAbNectin-4 and ^{89}Zr-AGS-22M6, for tumor monitoring has been evaluated in preclinical studies (*16*,*17*). However, the high molecular weight of antibodies limits their ability to penetrate tumor tissues and results in longer clearance times from the body, as well as a lower signal-to-noise ratio. These limitations hinder the imaging of antibody radiotracers shortly after injection and present challenges for clinical applications (*31*), which negatively affects the clinical translational research of these radiotracers. To address the limitations of Nectin-4–targeted antibody-based radiotracers, we developed a series of highly effective radiotracers. Three peptide radiotracers (^{68}Ga-FZ-NR-1, ^{68}Ga-FZ-NR-2, and ^{68}Ga-FZ-NR-3) were synthesized and characterized, and their low molecular weight and rapid clearance in vivo were demonstrated. Incorporating poly(sarcosine) or poly(PEG) between the targeting peptide and the chelator allows structural modifications to finely adjust the charge distribution and lipid solubility of the molecules, which may influence the pharmacokinetics and pharmacodynamics of the radiopharmaceuticals. Affinity tests indicated that these structural and ligand modifications did not alter the affinity of the bicyclic peptide for Nectin-4 (2.81 nM for ^{68}Ga-FZ-NR-1, 1.718 nM for ^{68}Ga-FZ-NR-2, and 1.805 nM for ^{68}Ga-FZ-NR-3, with 1.048 nM for the lead compound). All radiotracers demonstrated high stability and hydrophilicity. In vitro cell-based studies confirmed that ^{68}Ga-FZ-NR-1 had higher

selectivity and specificity for Nectin-4 than did ^{68}Ga-FZ-NR-2 and ^{68}Ga-FZ-NR-3, consistent with the PET/CT imaging results in tumor models.

Compared with the previously reported ^{68}Ga-N188 radiotracer (*24*), ^{68}Ga-FZ-NR-1 demonstrated superior tumor uptake and decreased background levels. It showed a longer retention time in tumors than the PEGylated bicyclic peptide radiotracer (*26*). ^{68}Ga-FZ-NR-1 showed enhanced distribution in the body, and its extended retention time at the tumor site improved imaging contrast and clarity. The capacity of ^{68}Ga-FZ-NR-1 for targeting Nectin-4, its rapid clearance from the blood, and its low nontarget organ distribution are crucial for measuring the expression levels of Nectin-4 in tumor for clinical research. microPET CT imaging in the MDA-MB-468 tumor model showed that ^{68}Ga-FZ-NR-1 could distinguish tumor tissue from muscle within 60 min, achieving a tumor-to-nontumor ratio of 4.5 ± 0.35, which further supports its potential for Nectin-4 imaging. Preclinical tests indicated that ^{68}Ga-FZ-NR-1 is more effective than ^{68}Ga-FZ-NR-2 and ^{68}Ga-FZ-NR-3 for targeting tumors. In addition, ^{68}Ga-FZ-NR-1 showed optimal in vivo pharmacokinetic properties, which are essential for its use in clinical studies. In conclusion, ^{68}Ga-FZ-NR-1 is a new radiotracer with beneficial properties and a promising radiotracer for PET/CT imaging of tumor Nectin-4 expression.

With the approval of the Ethics Committee of Fudan University Shanghai Cancer Center (number 2407299-29), we used the ^{68}Ga-FZ-NR-1 radiotracer in a preclinical study of TNBC patients. The objective was to evaluate its potential for use in Nectin-4–targeted PET/CT imaging in TNBC, as well as to evaluate its safety profile. Nine patients with TNBC were enrolled in the study, and all patients underwent PET/CT imaging approximately 60 min after the injection of the ^{68}Ga-FZ-NR-1 radiotracer. ^{68}Ga-FZ-NR-1 showed rapid blood clearance and nonspecific organ clearance, primarily being excreted through the kidneys, and it showed excellent pharmacokinetic properties. No adverse events or abnormal vital signs were observed after injection, confirming that ^{68}Ga-FZ-NR-1 is safe and well tolerated in patients. In patient 1, lung metastases were clearly visible after about 60 min with low background. Although the tumor uptake of ^{68}Ga-FZ-NR-1 (SUV_{max}, 10.8 in patient 1 and 5.1 in patient 2) did not exceed that of ^{18}F-FDG (SUV_{max}, 19.3 in patient 1 and 6.2 in patient 2), it identified the same tumor lesions as ^{18}F-FDG, indicating the overexpression of Necin-4 in the lesions. Overexpression of Nectin-4 at the tumor site was confirmed by immunohistochemistry. Preliminary clinical research results indicated that the ^{68}Ga-FZ-NR-1 radiotracer can accurately identify Nectin-4 overexpression in tumor tissues, demonstrating good sensitivity and specificity. The tumor uptake of ^{68}Ga-FZ-NR-1 is associated with Nectin-4 expression, providing support for the use of the radiotracer for the diagnosis and treatment of TNBC.

One limitation was the low number of cases included in the preclinical study. Despite evidence supporting the feasibility of PET/CT imaging technology, the clinical trial and validation of PET/CT imaging tracers for Nectin-4 require further in-depth research. This technology may allow the accurate analysis and quantification of Nectin-4 expression, which could be of value for patient diagnosis, for guiding treatment options, and for disease monitoring.

In future research, the following directions warrant further exploration and validation. First, numerous cases are needed to confirm the specificity and sensitivity of ^{68}Ga-FZ-NR-1 for PET/CT imaging of Nectin-4 expression in cancer patients. Second, the correlation between the SUV of tumors and the Nectin-4 expression levels should be investigated in patients with different types of cancer. Third, the utility of Nectin-4 PET/CT imaging for identifying patient populations most likely to benefit from targeted Nectin-4 therapies should be assessed. Fourth, the relationship between tumor SUV and patient treatment response and survival prognosis should be studied. In summary, ^{68}Ga-FZ-NR-1 showed promise as a potential Nectin-4–targeted PET/CT radiotracer in preclinical studies; however, additional research to verify its safety, efficacy, and accuracy is necessary before it can be considered a candidate for clinical application. These studies may provide more powerful tools for the precise diagnosis and personalized treatment of malignant tumors.

CONCLUSION

A series of Nectin-4–targeted radiotracers, ^{68}Ga-FZ-NRs, was developed. Among them, ^{68}Ga-FZ-NR-1 showed superior Nectin-4–targeting capability and PET/CT imaging performance in cell and tumor model studies. ^{68}Ga-FZ-NR-1 is an innovative radiotracer that showed exceptional specificity and safety in its first PET/CT imaging study of Nectin-4 targeting in patients with TNBC. It showed favorable pharmacokinetic properties and promising results in the first-in-human trials. Analysis of the SUV_{max} of lesions in 9 patients with TNBC and the immunohistochemical detection of Nectin-4 expression (patients 1 and 2) suggest a correlation between the uptake of ^{68}Ga-FZ-NR-1 and the expression levels of Nectin-4. This finding provides preliminary evidence for the use of ^{68}Ga-FZ-NR-1 as an effective tool for assessing the expression levels of Nectin-4 in tumors and confirms the ability of this radiotracer to detect Nectin-4 in patients with TNBC. The characteristics of ^{68}Ga-FZ-NR-1 confer dual clinical value: it aids in identifying patient populations most likely to benefit from Nectin-4–targeted therapies, and it can be used to monitor the occupancy of the drug target during treatment. This offers more personalized and precise methods for clinical diagnosis and therapeutic monitoring, potentially improving the clinical outcomes of patients.

DISCLOSURE

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KEY POINTS

QUESTION: Can ^{68}Ga-FZ-NR-1 visualize the Nectin-4–expressed tumor in TNBC patients?

PERTINENT FINDINGS: In a pilot study involving 9 patients with TNBC, the tracer ^{68}Ga-FZ-NR-1 accurately localized lesions, which were validated by ^{18}F-FDG PET/CT imaging. Furthermore, PET/CT imaging and immunohistochemistry staining together have preliminarily confirmed that the ^{68}Ga-FZ-NR-1 tracer can reflect the expression of Nectin-4 in TNBC.

IMPLICATIONS FOR PATIENT CARE: The expression of Nectin-4 was visualized by ^{68}Ga-FZ-NR-1 in TNBC, suggesting that Nectin-4 PET might be used as a tool for the noninvasive assessment of Nectin-4 overexpression levels and has the potential to identify patients in whom Nectin-4–targeted drugs can have a clinical benefit.

REFERENCES

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74:229–263.
2. Almansour NM. Triple-negative breast cancer: a brief review about epidemiology, risk factors, signaling pathways, treatment and role of artificial intelligence. *Front Mol Biosci.* 2022;9:836417.
3. Zagami P, Carey LA. Triple negative breast cancer: pitfalls and progress. *NPJ Breast Cancer.* 2022;8:95.
4. Xiao Y, Ma D, Yang YS, et al. Comprehensive metabolomics expands precision medicine for triple-negative breast cancer. *Cell Res.* 2022;32:477–490.
5. Bianchini G, Angelis CD, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer: expanded options, evolving needs. *Nat Rev Clin Oncol.* 2022;19:91–113.
6. Rabet MM, Cabaud O, Josselin E, et al. Nectin-4: a new prognostic biomarker for efficient therapeutic targeting of primary and metastatic triple-negative breast cancer. *Ann Oncol.* 2017;28:769–776.
7. Takai Y, Miyoshi J, Ikeda W, Ogita H. Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat Rev Mol Cell Biol.* 2008;9:603–615.
8. Heath EI, Rosenberg JE. The biology and rationale of targeting Nectin-4 in urothelial carcinoma. *Nat Rev Urol.* 2021;18:93–103.
9. Chatterjee S, Sinha S, Kundu CN. Nectin cell adhesion molecule-4 (Nectin-4): a potential target for cancer therapy. *Eur J Pharmacol.* 2021;911:174516.
10. Li K, Zhou Y, Zang M, Jin X, Li X. Therapeutic prospects of Nectin-4 in cancer: applications and value. *Front Oncol.* 2024;14:1354543.
11. Dagogo JI, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol.* 2018;15:81–94.
12. Watanabe K, Nakamura Y, Low SK. Clinical implementation and current advancement of blood liquid biopsy in cancer. *J Hum Genet.* 2021;66:909–926.
13. Weber WA, Grosu AL, Czernin J. Technology insight: advances in molecular imaging and an appraisal of PET/CT scanning. *Nat Clin Pract Oncol.* 2008;5:160–170.
14. Frings L, Blazhenets G, Brumberg J, Rau A, Urbach H, Meyer PT. Deformation-based morphometry applied to FDG PET data reveals hippocampal atrophy in Alzheimer's disease. *Sci Rep.* 2024;14:20030.
15. Reinfeld BI, Madden MZ, Wolf MM, et al. Cell-programmed nutrient partitioning in the tumor microenvironment. *Nature.* 2021;593:282–288.
16. Shao F, Pan Z, Long Y, et al. Nectin-4-targeted immunoSPECT/CT imaging and photothermal therapy of triple-negative breast cancer. *J Nanobiotechnology.* 2022;20:243.
17. Campbell DO, Noda A, Verlinsky A, et al. Preclinical evaluation of an anti-Nectin-4 immunoPET reagent in tumor-bearing mice and biodistribution studies in cynomolgus monkeys. *Mol Imaging Biol.* 2016;18:768–775.
18. Wang R, Liu S, Chen B, et al. Prognostic significance of PET/CT and its association with immuno-genomic profiling in oesophageal squamous cell carcinoma treated with immunotherapy plus chemoradiotherapy: results from a phase II study. *Br J Cancer.* 2024;131:709–717.
19. Manafi FR, Ataeinia B, Ranjbar S, et al. ImmunoPET: antibody-based PET imaging in solid tumors. *Front Med.* 2022;9:916693.
20. Shabsigh M, Solomon LA. Peptide PET imaging: a review of recent developments and a look at the future of radiometal-labeled peptides in medicine. *Chem Biomed Imaging.* 2024;2:615–630.
21. McKean M, Baldini C, Verlingue L, et al. BT8009-100 phase I/II study of novel bicyclic peptide and MMAE conjugate BT8009 in patients with advanced malignancies associated with Nectin-4 expression [abstract]. *Cancer Res.* 2022(suppl 12);82:CT025.
22. Rigby M, Bennett G, Chen L, et al. BT8009: a Nectin-4 targeting bicycle toxin conjugate for treatment of solid tumors. *Mol Cancer Ther.* 2022;21:1747–1756.
23. Mudd GE, Scott H, Chen L, et al. Discovery of BT8009: a Nectin-4 targeting bicycle toxin conjugate for the treatment of cancer. *J Med Chem.* 2022;65:14337–14347.
24. Duan X, Xia L, Zhang Z, et al. First-in-human study of the radioligand ^{68}Ga-N188 targeting Nectin-4 for PET/CT imaging of advanced urothelial carcinoma. *Clin Cancer Res.* 2023;29:3395–3407.
25. Zhang J, Duan X, Chen X, et al. Translational PET imaging of Nectin-4 expression in multiple different cancers with ^{68}Ga-N188. *J Nucl Med.* 2024;65:12S–18S.
26. Wan Q, Yuan H, Cai P, et al. Effects of PEGylation on imaging contrast of ^{68}Ga-labeled bicyclic peptide PET probes targeting Nectin-4. *Mol Pharm.* 2024;21:4430–4440.
27. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *J Clin Oncol.* 2020;38:1346–1366.
28. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *J Clin Oncol.* 2018;36:2105–2122.
29. Wang X, Liu W, Li K, et al. PET imaging of PARP expression using ^{68}Ga-labelled inhibitors. *Eur J Nucl Med Mol Imaging.* 2023;50:2606–2620.
30. Wang H, Sun D, Chen J, Li H, Chen L. Nectin-4 has emerged as a compelling target for breast cancer. *Eur J Pharmacol.* 2023;960:176129.
31. Thurber GM, Schmidt MM, Wittrup KD. Antibody tumor penetration: transport opposed by systemic and antigen-mediated clearance. *Adv Drug Deliv Rev.* 2008;60:1421–1434.