Phase I Study of ^{68}Ga-HER2-Nanobody for PET/CT Assessment of HER2 Expression in Breast Carcinoma

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Human epidermal growth factor receptor 2 (HER2) status is one of the major tumor characteristics in breast cancer to guide therapy. Anti-HER2 treatment has clear survival advantages in HER2-positive breast carcinoma patients. Heterogeneity in HER2 expression between primary tumor and metastasis has repeatedly been described, resulting in the need to reassess HER2 status during the disease course. To avoid repeated biopsy with potential bias due to tumor heterogeneity, Nanobodies directed against HER2 have been developed as probes for molecular imaging. Nanobodies, which are derived from unique heavy-chain-only antibodies, are the smallest antigen-binding antibody fragments and have ideal characteristics for PET imaging. The primary aims were assessment of safety, biodistribution, and dosimetry. The secondary aim was to investigate tumor-targeting potential. **Methods:** In total, 20 women with primary or metastatic breast carcinoma (score of 2+ or 3+ on HER2 immunohistochemical assessment) were included. Anti-HER2-Nanobody was labeled with ^{68}Ga via a NOTA derivative. Administered activities were 53–174 MBq (average, 107 MBq). PET/CT scans for dosimetry assessment were obtained at 10, 60, and 90 min after administration. Physical evaluation and blood analysis were performed for safety evaluation. Biodistribution was analyzed for 11 organs using MIM software; dosimetry was assessed using OLINDA/EXM. Tumor-targeting potential was assessed in primary and metastatic lesions. **Results:** No adverse reactions occurred. A fast blood clearance was observed, with only 10% of injected activity remaining in the blood at 1 h after injection. Uptake was seen mainly in the kidneys, liver, and intestines. The effective dose was 0.043 mSv/MBq, resulting in an average of 4.6 mSv per patient. The critical organ was the urinary bladder wall, with a dose of 0.406 mGy/MBq. In patients with metastatic disease, tracer accumulation well above the background level was demonstrated in most identified sites of disease. Primary lesions were more variable in tracer accumulation. **Conclusion:** ^{68}Ga-HER2-Nanobody PET/CT is a safe procedure with a radiation dose comparable to other routinely used PET tracers. Its biodistribution is favorable, with the highest uptake in the kidneys, liver, and intestines but very low background levels in all other organs that typically house primary breast carcinoma or tumor metastasis. Tracer accumulation in HER2-positive metastases is high, compared with normal surrounding tissues, and warrants further assessment in a phase II trial.

Key Words: breast carcinoma; HER2; Nanobody; PET/CT; phase I

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One in 8 women develops breast cancer, and it remains the second leading cause of cancer death in women. Identification of cancer subtypes based on biologic markers has led to the introduction of targeted therapies, with improved survival and morbidity. Besides hormone receptor expression, human epidermal growth factor receptor 2 (HER2) is used for breast cancer classification. Breast cancers with HER2 overexpression in primary or metastatic sites will benefit from HER2-targeted therapies such as the monoclonal antibody trastuzumab, resulting in a clear survival advantage (*1*).

Because only 20% of breast cancers overexpress HER2, the decision to start HER2-targeted therapy is based on immunohistochemical assessment or demonstrated gene amplification (e.g., fluorescence in situ hybridization) on tumor tissue biopsy, usually obtained at initial diagnosis at the primary site. Only patients with strong expression of the protein (3+ on immunohistochemical assessment) or with strong amplification of the *HER2* gene (fluorescence in situ hybridization–positive) are selected for HER2-targeted therapy, as these patients gain the greatest clinical benefit from trastuzumab treatment (*1*). In most therapeutic protocols, HER2-targeted therapy is given in addition to classic chemotherapy (e.g., paclitaxel, docetaxel). Recently, this approach was optimized by linking a chemotoxin to the antibody, thereby specifically targeting it to HER2-positive tumor cells. This compound, named trastuzumab emtansine, showed improved efficacy while reducing toxicity (*2*).

Studies have shown a HER2 testing discrepancy between local and large reference laboratories, resulting in 14%–16% false-positive and 4% false-negative results (*3,4*). Such misclassification gives rise to unnecessary toxicity and cost for the former while denying a potentially efficacious therapy for the latter. Recently, several independent studies reported a significant discordance in HER2 expression between primary breast carcinoma and metastases ranging between 6% and 34%, as well as heterogeneity between metastases

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(*5–10*). Because of this discordance, the European guidelines now recommend that a biopsy of a metastatic lesion be obtained to reassess biologic markers (*11*). With the increasing use of trastuzumab emtansine, which affects only HER2-overexpressing cancer cells, the importance of correct HER2 assessment becomes even more important.

We here introduce the use of Nanobodies (trade name of Ablynx) directed against HER2 as probes for molecular imaging in breast carcinoma patients. Nanobodies are the smallest antigen-binding domains derived from unique heavy-chain-only antibodies that are naturally present in camelids. Nanobodies have proven to be ideal probes for SPECT and PET imaging in rodents, with high targeting potential and fast blood clearance (*12,13*). The anti-HER2-Nanobody 2Rs15 d was selected and optimized as the lead compound for clinical translation (*14,15*). In this paper we report on the safety, biodistribution, dosimetry, and tumor-targeting potential of the ^{68}Ga-anti-HER2-Nanobody (^{68}Ga-HER2-Nanobody) in breast carcinoma patients.

MATERIALS AND METHODS

This was an open-label phase I study in HER2-expressing breast carcinoma patients ($n = 20$). The supplemental data (available at http://jnm.snmjournals.org) provide details on approvals, patient selection, safety assessment, conjugation of *p*-SCN-Bn-NOTA chelator to anti-HER2 Nanobody, synthesis of ^{68}Ga-HER2-Nanobody, the PET/CT protocol, image processing and analysis, blood and urine analysis, detection of antidrug antibodies, region-of-interest definition, dosimetry, uptake in tumor lesions, and statistical analysis.

Three subgroups, receiving, respectively, 0.01 mg (patients 1–7), 0.1 mg (patients 8–15), and 1.0 mg (patients 16–20) of NOTA-anti-HER2-Nanobody were evaluated for a difference in normal biodistribution, to investigate a potential decrease in nonspecific binding in nontarget organs with increasing mass of tracer. The activity administered was similar for the different patient groups and ranged from 53 to 174 MBq.

RESULTS

Patient Characteristics

Between April 2012 and July 2014, 20 patients completed the study protocol. The patients received on average 107 ± 37 MBq (range, 53–174 MBq) of ^{68}Ga-HER2-Nanobody. Patient and study drug characteristics are summarized in Table 1.

Safety Assessment

After the administration of ^{68}Ga-HER2-Nanobody, no symptoms or signs of toxicity were reported. Clinical laboratory testing of blood, taken before and 120 min after injection, showed no significant changes that could be related to the study drug. Antidrug antibody was not detected in serum samples of 20 patients taken before and 3 mo after administration (Supplemental Fig. 1).

Pharmacokinetics and Biodistribution

Figure 1 shows images of representative patients for each subgroup. No obvious differences in biodistribution were noted between different subgroups by visual comparison.

Blood-pool activity was visible only at 10 min after injection, with weak delineation of the heart and large blood vessels. Uptake was seen mainly in the kidneys, liver, and intestines. This uptake pattern was already present on the 10-min images and decreased over time. Weak uptake was seen in glandular tissues such as the thyroid, pituitary, salivary glands, lacrimal glands, and sweat glands.

The uptake of ^{68}Ga-HER2-Nanobody in individual organs is presented in Figure 2 and Supplemental Table 1. Blood-pool activity is presented in Figure 3. A fast blood clearance was seen, with only 10% of injected activity remaining in the blood at 1 h after injection. Blood half-lives were calculated at 2.9 min (early phase) and 25.5 min (late phase). Plasma curves were identical to blood curves, indicating that the tracer was not associated with blood cells. After injection, no metabolites were detected for up to 10 min in blood or up to 2 h in urine.

All images showed uptake in kidneys and excretion of the tracer into the urine. Although liver and intestine uptake was visible, there were no signs of hepatobiliary excretion, such as an accumulation in the gallbladder or duodenum. At 1 h after injection, 50% of the tracer had been eliminated from the body, resulting in an estimated biologic half-life of 1 h (Supplemental Fig. 2).

Effect of Injected Mass on Liver Uptake

On the basis of the preclinical results, the injected mass of the compound was expected to have an effect on nonspecific binding. Therefore, liver uptake was assessed in the 3 subgroups receiving different amounts of Nanobody. Overall, liver uptake was quite variable among patients. There was a trend toward lower liver uptake at 90 min after injection in the 1.0-mg group, with an average uptake of 5.5% injected activity compared with 9.0% and 9.5% injected activity for 0.1 and 0.01 mg, respectively, but with overlapping 95% confidence intervals (3.3–7.6, 5.7–12.3, and 7.4–11.5, respectively). One-way ANOVA indicated no significant difference ($F_{2,15} = 3.60$, $P = 0.053$).

Dosimetry

Table 2 summarizes the individual organ doses and individual effective dose results for all subjects with normal liver and renal function. The urinary bladder wall showed the highest organ dose (0.406 mGy/MBq), followed by the kidneys (0.216 mGy/MBq), liver (0.0778 mGy/MBq), lower large intestine wall (0.0759 mGy/MBq), and upper large intestine wall (0.0619 mGy/MBq).

Uptake in Tumor Lesions

Uptake in tumor lesions could be evaluated in 19 patients, 9 of whom had only a primary lesion; 6, both a primary lesion and local or distant metastases; and 4, only local or distant metastases (Table 1).

Uptake in Primary Lesions. Tracer uptake was visible for 13 of 15 primary tumors, with SUV_{mean} ranging from 0.7 to 11.8 (Table 1). Representative images showing ^{68}Ga-HER2-Nanobody uptake in primary lesions are presented in Figure 4.

Uptake in Local and Distant Metastases. All patients with metastatic lesions showed clear tracer accumulation in at least 1 lesion, with SUV_{mean} ranging from 3.1 to 6.0. Figure 5 shows images of patients 18 and 20, with metastases in thoracic lymph nodes and the pelvis, respectively. Supplemental Video 1 shows maximum-intensity-projection PET images of patient 14 at 90 min after injection. This patient presented with a primary mammary carcinoma on the right side and an ipsilateral invaded axillary lymph node.

Heterogeneous Uptake Pattern. In patient 8, a heterogeneous uptake pattern was observed in the primary tumor (Supplemental Fig. 3A). The uptake pattern did not match the ^{18}F-FDG uptake pattern, indicating that it was not caused by necrotic tumor areas. The patient presented with diffuse metastases, of which some but not all showed uptake (SUV_{mean} range, 1.0–5.6; Supplemental Figs. 3B and 3C).

DISCUSSION

This first-in-human application of a radiolabeled Nanobody demonstrates that the procedure is safe and that tracer administration causes

TABLE 1
Patient Characteristics

Group	Patient no.	Age (y)	IA (MBq)	Tumor type	ER/PR	HER2 IHC	HER2 FISH			Range of SUV_{mean} of lesions		
							Positivity	Ratio	Copies/cell	Primary tumor	Local ADP	Distant M+ (type)
0.01 mg	1	43	77	IDC	+/+	2+	+	2.2	6.2	ISR*	A	A
	2	60	66	IDC	+/+	3+	+	>2	Macroclusters	CR	A	3.1 (bone)
	3	68	53	IDC	+/+	3+	+	12.2	20.0	3.2	A	A
	4	53	76	IDC	+/+	2+	−	1.3	3.7	2.2	A	A
	5	74	84	IDC	+/+	2+	−	1.3	3.8	2.3	A	A
	6	34	83	IMeC	+/−	3+	+	2.8	8.0	0.9	A	A
	7	34	80	IDC	+/+	2+	−	1.0	1.4	2.0	A	A
0.1 mg	8	67	92	IDC	+/+	2+	−	1.4	3.4	5.0	3.2–4.3	1.0–5.6 (bone)
	9	57	111	IDC	+/+	3+	+	1.3	6.1	2.3	A	A
	10	61	100	IDC	+/−	3+	+	9.4	15.0	SR	SR	4.1–5.7 (bone)
	11	65	90	IDC	+/+	3+	+	2.3	5.1	2.9	6.3	A
	12	46	82	IDC	+/+	3+	+	8.1	15.6	1.4	A	A
	13	32	153	IDC	−/−	2+	+	9.4	17.4	3.2	1.7	A
	14	53	103	IDC	−/−	3+	+	4.7	9.2	11.8	13.0	A
	15	78	148	IDC	+/+	2+	−	1.0	2.1	4.9	A	A
1.0 mg	16	76	96	ILC	+/+	2+	−	1.0	1.7	SR	SR	2.2–3.9 (bone)
	17	74	138	IDC	−/−	2+	−	1.2	4.3	1.8	A	A
	18	62	167	IDC	+/−	2+	+	2.6	4.5	SR	SR	3.5–6.0 (ADP mediastinum)
	19	62	174	IMiC	+/+	3+	+	2.8	8.0	4.4	5.1–5.9	3.6–3.9 (bone)
	20	48	170	IDC	+/+	3+	+	7.8	15.6	0.7†	A	4.7–5.4 (bone)†

*Patient was scanned after incomplete surgical removal but additional surgical resection could not demonstrate remaining tumor cells.

†After 4 cycles of epirubicin cyclophosphamide.

FISH = fluorescence in situ hybridization; IA = injected activity; ER = estrogen receptor; PR = progesterone receptor; IHC = immunohistochemical assessment; ADP = adenopathy; IDC = invasive ductal carcinoma; ISR = incomplete surgical removal; A = absent; CR = complete response on CT; IMeC = invasive medullary carcinoma; SR = surgically removed; ILC = invasive lobular carcinoma; IMiC = invasive mixed carcinoma.

no observable adverse reactions. The ^{68}Ga-HER2-Nanobody tracer showed a favorable biodistribution, with the highest uptake in the kidneys, liver, and intestines but very low background levels in all other organs that typically harbor primary breast carcinoma or tumor metastasis. Antidrug antibody measurements showed that no preexisting or tracer-induced antibodies against the Nanobody could be detected.

Rapid tracer clearance from the blood allows imaging at early time points (60–90 min after injection) without the risk of false-positive signal due to blood-pool activity. Tracer elimination occurs through the renal system, with high accumulation in the kidneys, similar to patterns described for other labeled peptides and small proteins (*16–18*) and as expected from Nanobody uptake patterns in rodents (*19*). Organs with substantial uptake are the liver and intestines. Although the literature describes a low expression of HER2 in the liver, the uptake most likely is of a nonspecific nature, given the high and probably supraphysiologic uptake values (*20*). Liver uptake may obscure liver metastases, but given the absence of liver metastases in this patient group, the question remains unanswered. On the basis of preclinical observations, increasing amounts of protein up to 1 mg were administered to 3 different patient subgroups in an attempt to decrease nonspecific binding, but no significant effect was observed.

Because liver uptake continues to decrease between 60 and 90 min after injection, the latter is proposed as the best time point to obtain images with an optimal signal-to-noise ratio. Later time points were not assessed in this study because of the short half-life of ^{68}Ga.

Whole-body imaging revealed weak tracer uptake in glandular tissues such as the salivary glands, pituitary, lacrimal glands, and axillary sweat glands. This pattern was also observed with ^{68}Ga- and ^{111}In-labeled anti-HER2-Affibody (*21*). The origin of this uptake remains to be determined but may be related to low levels of HER2 expression or chelator-mediated trapping mechanisms. Clinical testing of ^{18}F-anti-HER2-Nanobody may provide more insight (*22*). It is, however, noteworthy that prostate-specific membrane antigen tracers show even more pronounced glandular uptake for both ^{68}Ga- and ^{18}F-labeled compounds, suggesting a specific uptake mechanism (*17,23*).

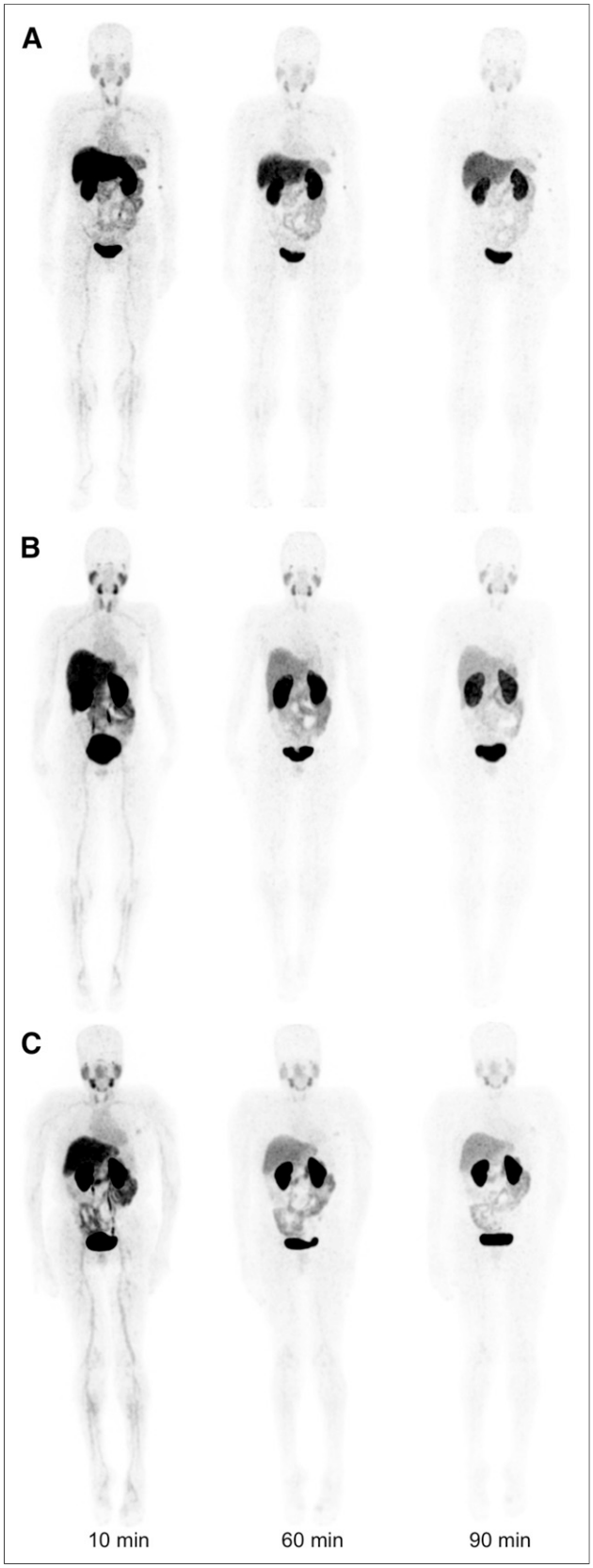

FIGURE 1. Representative maximum-intensity-projection images at 10, 60, and 90 min after injection of ^{68}Ga-HER2-Nanobody for different mass subgroups. (A) Patient 4, injected with 0.01 mg of ^{68}Ga-HER2-Nanobody. (B) Patient 12, injected with 0.1 mg. (C) Patient 17, injected with 1.0 mg.

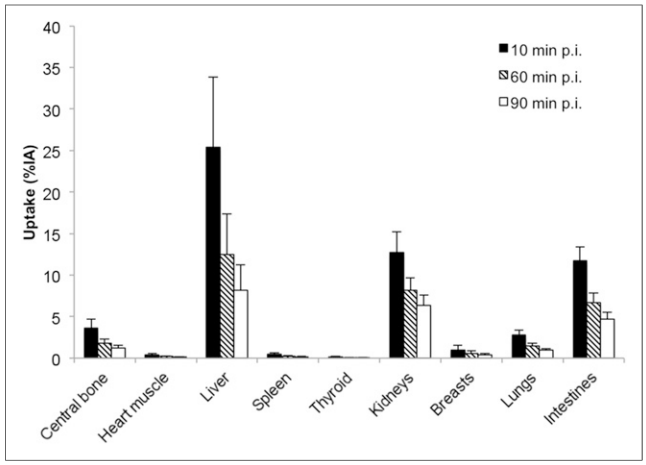

FIGURE 2. Uptake, expressed in percentage injected activity (IA), in different organs at 10, 60, and 90 min after injection (n = 18).

The urinary bladder wall received the highest organ dose, 0.406 mGy/MBq, which at the highest injected activity of 185 MBq is well below 100 mGy, thereby excluding potential deterministic effects. The average radiation burden was 0.04 mSv/MBq, which resulted in an average effective dose of 4.6 mSv for the 18 patients in this study. Maintaining a maximum activity of 185 MBq for future imaging studies, the highest effective dose would be 7.9 mSv. These values are acceptable for a diagnostic procedure and in line with other ^{68}Ga and ^{18}F PET radiotracers (*16,24*).

Although not the primary objective of this phase I study, tumor uptake was evaluated in these breast carcinoma patients, both in primary lesions and in metastases.

Uptake in primary lesions showed a wide range of 0.7–11.8, possibly because of the heterogeneous composition of primary lesions, with tumor cells infiltrating normal breast tissue. Moreover, patient 20, with an SUV_{mean} of 0.7, had received 4 cycles of chemotherapy, which could explain the negative result. Additionally, carcinoma in situ can coincide with infiltrating carcinoma, which can mimic uptake in the invasive carcinoma, since these carcinomas in situ can also overexpress HER2 while not correlating with HER2 expression in metastatic lesions. ^{68}Ga-HER2-Nanobody PET/CT may therefore not be ideal for assessing HER2 expression in primary breast carcinoma lesions.

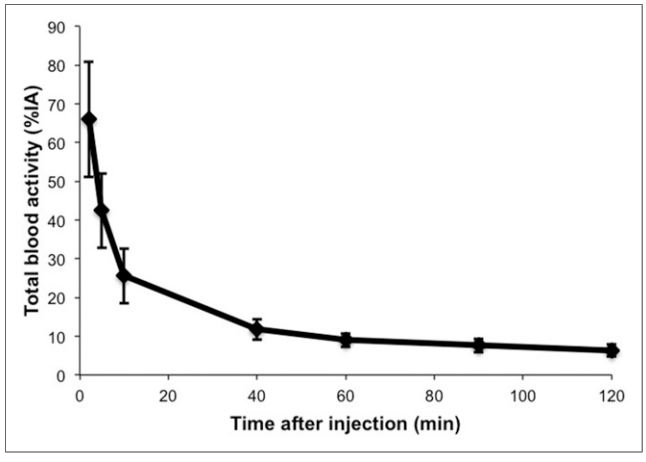

FIGURE 3. Time–activity curve of total blood activity, expressed in percentage injected activity (IA). Data are mean and SD of 12 patients.

TABLE 2
Organ Doses and Effective Dose

Patient no.	Organ dose (mGy/MBq)						Effective dose (mSv/MBq)
	UB wall	Kidneys	Liver	LLI wall	ULI wall	Thyroid	
1	0.406	0.191	0.0515	0.0843	0.0606	0.0233	0.0425
3	0.406	0.161	0.114	0.0757	0.0679	0.0326	0.0458
4	0.405	0.219	0.0788	0.0295	0.0787	0.0257	0.0371
5	0.407	0.181	0.116	0.0962	0.0566	0.0093	0.0472
6	0.406	0.297	0.0957	0.0798	0.0535	0.0282	0.0453
7	0.405	0.259	0.0788	0.0423	0.0840	0.0020	0.0371
8	0.405	0.141	0.114	0.0788	0.0715	0.0137	0.0433
9	0.406	0.273	0.0740	0.0686	0.0675	0.0200	0.0421
10	0.406	0.229	0.0922	0.0626	0.0991	0.0035	0.0425
12	0.407	0.220	0.0594	0.0816	0.0772	0.0327	0.0435
13	0.406	0.225	0.113	0.0632	0.0812	0.0496	0.0442
14	0.407	0.222	0.0849	0.0719	0.113	0.0513	0.0448
15	0.406	0.278	0.0719	0.0086	0.0071	0.0035	0.0335
16	0.407	0.192	0.0473	0.140	0.0356	0.0124	0.0485
17	0.407	0.216	0.0610	0.141	0.0518	0.0132	0.0504
18	0.406	0.193	0.0583	0.118	0.0554	0.0110	0.0469
19	0.406	0.181	0.0422	0.0087	0.0067	0.0220	0.0317
20	0.406	0.211	0.0469	0.116	0.0464	0.0177	0.0447
Mean ± SD	0.406 ± 0.001	0.216 ± 0.041	0.0778 ± 0.0252	0.0759 ± 0.0384	0.0619 ± 0.0274	0.0207 ± 0.0143	0.0428 ± 0.0050

LLI = lower large intestines; ULI = upper large intestines; UB = urinary bladder.
Patients 2 and 11 were not considered because of altered liver or kidney function.

In patients with metastatic disease, however, distinct uptake was seen in most metastases. In two of these patients, the tumor was classified as HER2-negative, on the basis of a score of 2+ on immunohistochemical assessment and fluorescence in situ hybridization–negative results in the primary tumor. The increased tracer uptake may be related to intermediate HER2 expression (score of 2+ on immunohistochemical assessment) or to discordance in HER2 expression between the primary lesion and the metastasis, as has been described in the literature for 14%–30% of cases (*7,10*). Given the absence of direct histopathologic correlation in this phase I study, a final conclusion based on the current results cannot be made. Sensitivity and specificity for the determination of HER2 status will be answered only through larger, prospective, and more clinically focused imaging trials.

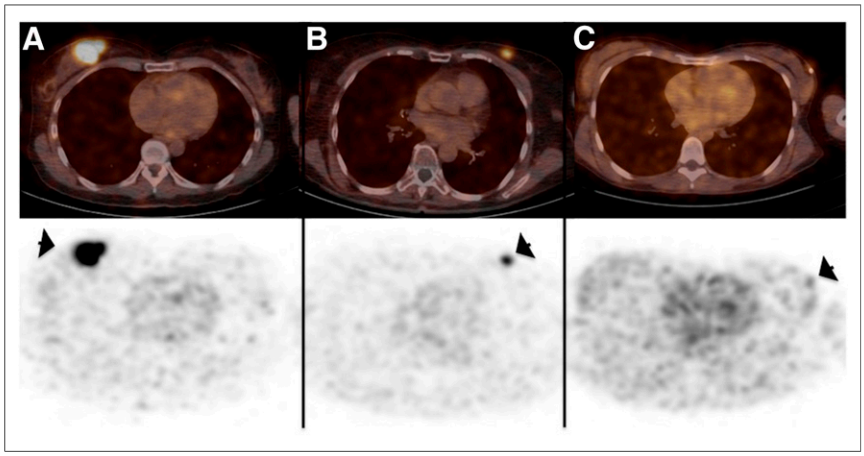

FIGURE 4. Uptake of ^{68}Ga-HER2-Nanobody in primary breast carcinoma lesions (arrows) on PET/CT images (top) and PET images (bottom). (A) Patient 14 showed highest tracer uptake (SUV_{mean}, 11.8). (B) Patient 15 showed moderate tracer uptake, which was easily discernable from background (SUV_{mean}, 4.9). (C) Patient 6 showed no uptake (SUV_{mean}, 0.9), with CT showing marker clip at tumor region.

Other research groups have developed PET/CT imaging strategies in parallel using the full therapeutic antibody trastuzumab labeled with ^{89}Zr or ^{64}Cu (*25,26*). This approach, however, has the disadvantage of slow blood clearance, resulting in a late imaging time point of 1–2 d (^{64}Cu) or 4–5 d (^{89}Zr) after tracer injection, a long scanning time of up to 1 h, and a high radiation burden of 12 mSv (^{64}Cu) or 18 mSv (^{89}Zr) for the patient (*25,27,28*). To overcome these disadvantages, antibody fragments derived from trastuzumab, such as F(ab′)$_2$, have been developed and labeled with shorter-lived isotopes. ^{68}Ga-DOTA-F(ab′)$_2$-trastuzumab showed minimal

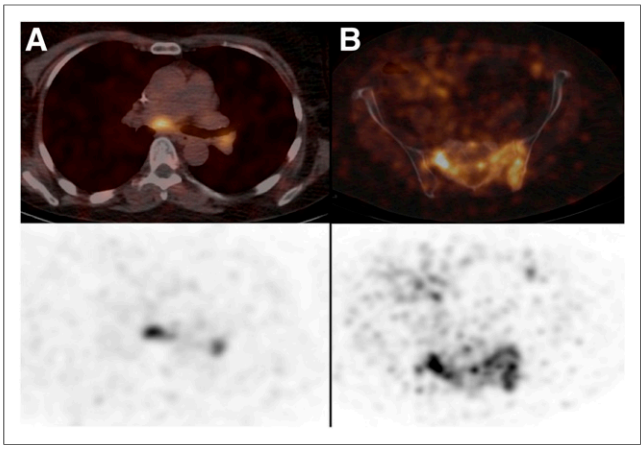

FIGURE 5. Uptake of ^{68}Ga-HER2-Nanobody in metastatic lesions on PET/CT images (top) and PET images (bottom). (A) Patient 18, with invaded lymph nodes in mediastinum and left hilar region. (B) Patient 20, with bone metastasis in pelvis.

or no tumor uptake in most cases, potentially related to suboptimal mass, lower immunoreactivity, or a blood half-life of the tracer that is too long to allow adequate PET imaging with ^{68}Ga (*29*). Moreover, contrary to ^{68}Ga-HER2-Nanobody, trastuzumab-derived tracers bind to the same epitope as the therapeutic agent, resulting in changes in uptake caused by differences in circulating therapeutic compound. The Affibody molecules labeled with ^{68}Ga and ^{111}In are also explored as tracers for HER2 imaging. The first-in-human data were published in 2010 (*21*). Meanwhile, a second compound against HER2 has been tested in a first-in-human study and showed a decrease in liver uptake (*18*). In total, 10 patients have been imaged with the different compounds, which have shown fast blood clearance and high potential for tumor targeting, similar to what is reported in this paper (*18,21*).

This first-in-human use of radiolabeled Nanobody exemplifies the translational potential of a variety of preclinically tested Nanobodies raised against a multitude of targets such as macrophage mannose receptor for assessment of the tumor microenvironment and vascular cell adhesion molecule 1 in atherosclerosis (*30,31*). In combination with improvements in radiochemical techniques for ^{18}F-labeling that allow distribution of tracers to multiple centers, this anti-HER2-Nanobody could be just the start of several exciting new PET agents. Moreover, the recent development of targeted radionuclide therapy using ^{177}Lu-labeled anti-HER2-Nanobody showed impressive preclinical results (*32*). Such a theranostic approach will soon be translated into a clinical trial.

CONCLUSION

^{68}Ga-HER2-Nanobody PET/CT is a safe procedure with a radiation dose comparable to that of other routinely used PET tracers. Its biodistribution is favorable, with the highest uptake in the kidneys, liver, and intestines but very low background levels in all other organs that typically house primary breast carcinoma or tumor metastasis. Tracer accumulation in the metastases of HER2-overexpressing patients is high, compared with normal surrounding tissues, and warrants further assessment in a phase II trial.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. Francois P. Duhoux received honoraria, was paid for consulting, or has received travel and accommodation expenses from Roche, Teva, Novartis, Pfizer, and Amgen; Tony Lahoutte, from Bayer-Schering and National Institute for Radioelements (IRE); and Marleen Keyaerts, from Biospacelab. Tony Lahoutte and Nick Devoogdt are cofounders of CamelIDs. Tony Lahoutte has received funding from Boehringer-Ingelheim and Complix. Marleen Keyaerts, Nick Devoogdt, and Tony Lahoutte have patents on Nanobody imaging and therapy. Christian Vanhove is supported by the GROUP-ID consortium of Ghent University. Research was funded by CancerPlan Action29 (Federal Public Service Health, Food Chain Safety and Environment, Belgium), Stichting tegen Kanker, Research Foundation–Flanders. No other potential conflict of interest relevant to this article was reported.

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