Integrin Receptor Imaging of Breast Cancer: A Proof-of-Concept Study to Evaluate ^{99m}Tc-NC100692

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The present study was a proof-of-concept study to provide an initial indication of the efficacy and safety of imaging malignant breast tumors using ^{99m}Tc-NC100692. The agent is a small peptide with high affinity for integrin receptors that are upregulated and expressed preferentially on proliferating endothelial cells. **Methods:** Sixteen patients with suggestive mammographic findings and 4 patients with benign lesions were included. The "standard of truth" was based on the histopathologic diagnosis of the recruited patients. All subjects received up to 75 μg of ^{99m}Tc-NC100692 with an average ^{99m}Tc activity of 694 MBq (range, 561–747 MBq). Safety endpoints were treatment-emergent adverse events (AEs) and changes in a limited physical examination, electrocardiogram (ECG) recordings, blood biochemistry, hematology, coagulation, vital signs, and urine analysis after administration of ^{99m}Tc-NC100692 and throughout the 24-h follow-up. Static images and SPECT were acquired between 40 min and 2.5 h after injection of the agent. Two experienced nuclear medicine physicians read the images in a nonblinded fashion. **Results:** Nineteen of 22 malignant lesions were detected using ^{99m}Tc-NC100692 scintigraphy. Twenty lesions confirmed as malignant by histopathology were seen on mammography or ultrasound. Two additional lesions were identified from histopathology alone. Safety parameters evaluated through the follow-up period of 2.5 h included clinical laboratory tests, vital signs, and ECG. Five of 20 subjects experienced nonserious AEs, and all AEs were classified as mild. One subject experienced an AE (dysgeusia) possibly related to administration of ^{99m}Tc-NC100692. This AE was mild and lasted only for a few minutes. No deaths, serious AEs, or withdrawals due to AEs occurred during the study. **Conclusion:** Nineteen of 22 malignant lesions (86%) were clearly detected via scintigraphic imaging after administration of ^{99m}Tc-NC100692. Overall, the efficacy data in subjects with suspected breast lesions suggest that ^{99m}Tc-NC100692 scintigraphy may be effective in detecting malignant lesions. The use of ^{99m}Tc-NC100692 in subjects with breast cancer is safe and well tolerated. Further studies are warranted to assess the clinical potential of ^{99m}Tc-NC100692.

Key Words: breast cancer; clinical trial; integrin; angiogenesis; ^{99m}Tc-labeled peptide

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The $\alpha_v\beta_3$ integrin is a membrane-spanning protein that is expressed preferentially on proliferating endothelial cells associated with neovascularization but is absent in quiescent blood vessels (*1,2*). Integrins facilitate attachment to the extracellular matrix, differentiation, proliferation, and migration. Histology and molecular biology techniques have shown that integrins are localized on the luminal side and the abluminal side (directed away from the lumen) of nonactivated endothelial cells (*3*). It has, however, been hypothesized that the receptor is able to bind substrates only during endothelial cell activation, similar to other receptors including other integrins (GPIIb/IIIa in platelets) (*4*). Labeled synthetic ligands with demonstrated specificity for the $\alpha_v\beta_3$ integrin have proven to be successful agents for in vivo imaging of tumor-related angiogenesis (*2,5–7*) and, in particular, the agents based on the amino acid sequence Arg-Gly-Asp (RGD) have been identified as useful for imaging sites of tumor angiogenesis (*8,9*). Tumor imaging performed using radiolabeled RGD peptides has shown a correlation between tracer uptake and the level of angiogenesis (*1,2,7,8,10*). NC100692 is a cyclic synthetic ligand containing the RGD binding site with high affinity for specific integrins upregulated during angiogenesis—in particular, the $\alpha_v\beta_3$ integrin—as shown by in vitro binding assays and competitive radiolabeled ligand assays. The use of NC100692 in ischemic models shows high uptake in areas of neovascularisation with $\alpha_v\beta_3$ integrin overexpression (*11*), and binding of NC100692 has been confirmed to be localized on endothelial cells in the regions of angiogenesis (*12*).

A phase 1 study in which healthy volunteers were administered ^{99m}TC-NC100692 demonstrated uptake of the agent in the liver and intestines and excretion through the kidneys. Only minimal amounts of background activity

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were observed in the thorax and the brain. Labeling of NC100692 with ^{99m}Tc provides a potential tool for imaging sites of $\alpha_v\beta_3$ integrin expression.

Imaging by x-ray mammography (XMM) is current practice for breast cancer screening. However, XMM may be difficult to assess in several subsets of patients—that is, patients with dense breasts, prior surgical biopsies, or silicone inserts or in patients using hormone replacement therapy. In these patients additional imaging modalities are sometimes considered. One such modality is scintimammography (SMM) with sestamibi or tetrofosmin, which has been performed for many years (*13–16*). However, ^{99m}Tc-sestamibi is generally of use only for lesions >1 cm in diameter (*17–19*). Published data suggest that the sensitivity of ^{99m}Tc-sestamibi SMM is only 50% for lesions <12 mm, whereas it may be as high as 92% for lesions above this size (*20*). Consequently, SMM is used effectively as an imaging adjunct in the hands of certain specialists (*13,14*) but has failed to be accepted as a routine tool in the evaluation of difficult cases of breast cancer.

A procedure based on a different pathophysiologic mechanism than conventional methods may be of use in patients with difficult-to-interpret mammographic images.

The present study was performed to provide an initial indication of the efficacy of ^{99m}Tc-NC100692 to identify malignant breast tumors. In addition, the safety profile of ^{99m}Tc-NC100692 administration was assessed in subjects with breast cancer, up to 24 h after administration.

MATERIALS AND METHODS

Patients were recruited from the Oncology Departments at the Karolinska Institute, Stockholm, Sweden (Center 1; 8 patients), and The Norwegian Radiumhospital, Oslo, Norway (Center 2; 12 patients).

The median age was 58 y (range, 40–76 y) and the median weight was 65 kg (range, 53–150 kg). Written informed consent was obtained from all subjects. The study protocol adhered to the Helsinki Declaration and was approved by local independent ethics committees, by the institutional review boards of the 2 hospitals, and by the Norwegian and Swedish health authorities. Subject inclusion was based on a presumptive diagnosis of a malignant breast tumor based on one or more abnormalities on XMM, with additional information obtained from ultrasound (US). The subjects were scheduled to undergo breast biopsy immediately after SMM and to undergo surgery within 3 wk of inclusion. Lesion location was determined by XMM or US (or both). The "standard of truth" for malignant lesions was based on histopathologic diagnosis after surgical removal of the tumor. For benign lesions, the final clinical diagnosis was used as the standard of truth. For most of the benign lesions, cytology or histopathology was also available.

Pregnancy was a noninclusion criterion and all patients were postmenopausal, surgically sterile, or using birth control with a negative pregnancy test. Each subject participated in the study only once. The protocol outlined admission, dosing, and safety checks on the same day up to 2.5 h after administration. Imaging and safety assessments were performed during the first 2.5 h after injection, after which the subjects were allowed to leave the site. All subjects were telephoned by one of the investigators 24 h after injection to assess the occurrence of possible adverse events (AEs). A full 24-h safety follow-up was performed if abnormal safety values were obtained during the final safety measurement on the dosing day.

Safety monitoring included the recording of treatment-emergent AEs, changes in a limited physical examination (general appearance, cardiovascular, lungs, and abdomen), a limited neurologic examination, electrocardiogram (ECG) recordings, serum biochemistry, hematology, coagulation, vital signs (systolic and diastolic blood pressure, respiratory rate, heart rate, body temperature), and urine analysis after administration of ^{99m}Tc-NC100692 (^{99m}Tc-NC100692 Injection; GE Healthcare) and throughout the follow-up (Table 1).

Investigational Imaging Agent

^{99m}Tc-NC100692 is a small, cyclic peptide that contains the RGD motif in a configuration that gives the ligand a high affinity for the $\alpha_v\beta_3$ integrin. In vitro receptor binding experiments were performed using $\alpha_v\beta_3$ purified from human placenta. The dissociation constant (K_d) of NC100692 was <1.1 nmol/L, whereas a peptide control (with a single amino acid substitution in the RGD motif) had a K_d of 59 nmol/L (data not shown). A competitive radiolabeled ligand assay was performed to assess the radiolabeled ligand affinity of NC100692. High specific binding was shown for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (an integrin also expressed during angiogenesis).

^{99m}Tc-NC100692 was administered as a single bolus injection (with an NC100692 maximum content of 75 μg). The mean activity injected was 694 MBq (range, 561–747 MBq). The subjects

TABLE 1
Clinical Laboratory Parameters

Serum biochemistry	Hematology	Urine analysis
Creatinine	Hematocrit	Bilirubin
Urea nitrogen	Hemoglobin	Protein
Uric acid	RBC	Ketone
Bilirubin (total, direct, indirect)	WBC	Occult blood
Protein (total)	WBC differential count	Specific gravity
Albumin	Coagulation parameters	pH
ASAT	Protrombin time	Microscopy
ALAT	Activated partial tromboplastin time	
Alkaline phosphatases		
γ-GT		
Chloride		
Calcium		
Phosphorus		
Bicarbonate		
LD		
CPK		
Amylase		
Glucose		
Sodium		
Potassium		

RBC = red blood cell count; WBC = white blood cell count; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; γ-GT = γ-glutamyltransferase; LD = lactate dehydrogenase; CPK = creatine phosphokinase.

Physical examination included general appearance, cardiovascular, lung, and abdominal examination, motor function, cranial nerves (II–XII), and reflexes (biceps, triceps, patellar, ankle).

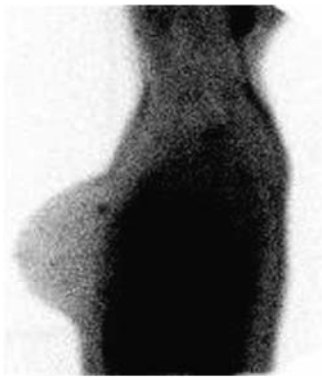

FIGURE 1. Patient 1: Infiltrative ductal carcinoma (8 mm). Lateral projection.

were administered 1 vial of radiolabeled NC100692 reconstituted in 3.5 mL of sterile saline, followed by a 10-mL saline flush. A quality control check with paper chromatography and high-performance liquid chromatography was performed before injection of the agent. A radiochemical purity of >85% was required before injection of the agent.

γ-Cameras and Imaging Protocols

A static frontal view and a lateral view were acquired (10-min acquisition time for each) at 45 min and, for selected patients, at 105 min after injection. At 1 h 15 min after injection, a SPECT image was acquired. During the acquisition of the static lateral images the subject was prone using a specially designed mattress with the breast hanging freely.

Imaging was performed using a dual-head γ-camera (ADAC Genesys in Center 1 and Siemens E-CAM in Center 2). SPECT acquisitions were performed using a 360° circular orbit, 128 steps (64 steps, 2 heads), each of 30 s.

The images were evaluated by 2 experienced on-site nuclear medicine physicians. Lesions were tracked from SMM to the standard of truth (XMM, US, and confirmatory diagnosis from histopathology, including lesions identified only by histopathology).

Contrast grading of ^{99m}Tc signal intensity in regions of interest was recorded as follows: no obvious uptake (grade 1), uncertain or heterogeneous uptake (grade 2), focal lesion uptake (grade 3). No further quantitative or semiquantitative evaluation was performed.

Surgery

All except 2 subjects with malignant lesions underwent surgery within 3 wk. These 2 subjects had locally advanced cancer, were treated with neoadjuvant chemotherapy, and underwent surgery within 3 mo of biopsy. Hence, there was a histopathologic verification of all malignant tumors. Biopsy, but no further follow-up, was performed on the patients with benign lesions. Histologic

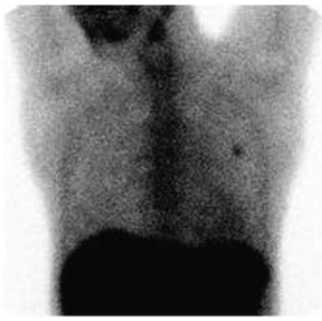

FIGURE 2. Patient 3: Infiltrative ductal carcinoma (10 mm), with surrounding ductal carcinoma in situ. Frontal projection.

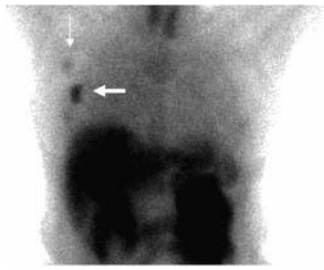

FIGURE 3. Patient 14: Large infiltrative ductal carcinoma (35 mm) (large arrow) with uptake in axillary lymph nodes (small arrow). Frontal projection.

investigation of the axillary lymph nodes (from sentinel node surgery or axillary lymph node dissection) was performed on all patients with malignant lesions.

RESULTS

Safety

Among the 20 subjects evaluated for safety there was overall stability of all parameters through the follow-up

TABLE 2
Characteristics of Malignant Lesions

Patient no.	Pathology	Lesion size (mm)	Visual uptake grading		
			US*	XMM†	SMM‡
1	IDC	8	4	5	3
2	IDC	20	5	5	3
3	IDC (+ DCIS)	10 (+ 12)	5	5	3
4	L: DCIS	10	4	1	3,
	R: IDC	25	5	5	3
	DCIS§	2.5			
5	ILC × 2	20, 23	5, 3	5, 5	3, 3
	ILC§	11	1	1	2
6	IDC (+ DCIS)	14 (+ 10)	5	5	3
7	IDC	6	5	5	1
8	IDC	7	5	5	3
9	IDC × 2 (widely DCIS)	12, 14	5, 4	5, 5	3, 3
10	IDC (+ DCIS)	40	5	5	3
11	ILC	20	5	5	3
12	IDC	28	5	5	3
13	IDC (+ DCIS)	40	5	5	3
14	IDC	35	5	5	3
15	IDC (+ DCIS)	40	5	5	3
16	IDC	40	5	5	3
	ILC	40	3	4	3

*US: 5-scale grading, where grade 3 = indeterminate and grade 5 = malignant.

†XMM: findings are graded according to BIRADS, where grade 1 = negative, grade 2 = benign finding, grade 3 = probably benign finding, grade 4 = suspicious abnormality (biopsy should be considered), and grade 5 = highly suggestive of malignancy.

‡SMM with NC100692 administration (study drug): 3-grade scale, where grade 1 = no obvious uptake, grade 2 = uncertain/heterogeneous uptake, and grade 3 = focal lesion uptake.

§Lesions were found during histopathologic examination but were not observed by any imaging modality.

BIRADS = Breast Imaging Reporting and Data System; IDC = infiltrative ductal carcinoma; ILC = infiltrative lobular carcinoma; DCIS = ductal carcinoma in situ.

period of 2.5 h. No clinically important safety signals or trends over time were noted. Postinjection outlier results for ECG, vital signs, and clinical laboratory parameters were all explained by laboratory error, exercise, or anxiety.

Five of 20 subjects experienced AEs, and all AEs were mild and resolved within 24 h. Of these 5 subjects, 1 subject experienced an AE considered by the investigator to be possibly related to ^{99m}Tc-NC100692 administration. This subject experienced a metallic taste for a short period of time during the night after the injection. No deaths, serious AEs, or withdrawals due to AEs occurred during the study.

Efficacy

A total of 22 malignant lesions in 16 patients were described in the standard of truth. Of these 22 lesions, 19 (86%) were detected with the prescribed ^{99m}Tc-NC100692 administration imaging protocol (Figs. 1–3). The size of the lesions, based on XMM, varied from 5 to 40 mm. Further characteristics are given in Table 2. Three lesions were not identified: 1 case of ductal carcinoma in situ (DCIS) and 2 cases of invasive lobular carcinoma. Components of DCIS in patients with well-defined infiltrative ductal carcinoma were generally not identified. However, a 10-mm large area of DCIS in 1 subject was clearly defined.

As axillary regions were included in the γ-camera field of view, an evaluation of uptake of the study drug in lymph nodes was made. For 9 of the 16 subjects with malignant lesions, no lymph node metastases were detected by histopathology and SMM showed no uptake in the axillary area. Seven subjects had lymph node metastases detected by histopathology. Four of these subjects had lymph nodes ≥20 mm and focal uptake in the axillary region was clearly seen by scintigraphy in 3 of the subjects. The fourth subject had focal uptake in the axilla but this could not be confirmed as lesion-specific uptake in the lymph node because the subject also had inflammatory dermatitis in the axilla. Three subjects had small, nonpalpable lymph node metastases (≤5 mm) that were not detected by scintigraphy.

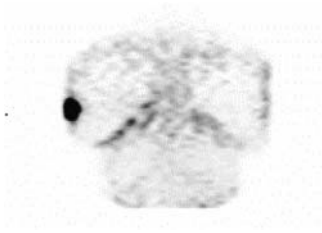

FIGURE 4. Patient 10: Diffuse, heterogeneous uptake in fibrocystic disease, with no focal pathology. Lateral projection.

Six subjects had a total of 11 benign findings (Table 3). Lesions that were not identified on SMM included 2 fibrocystic changes, 2 fibroadenomas, 1 other (where biopsy only revealed fat), and 1 lesion with unknown diagnosis.

Five benign changes were detected on SMM. These benign lesions were 4 fibrocystic changes and 1 infected cyst. All of the fibrocystic changes detected by scintigraphy were seen as heterogeneous diffuse areas of uptake and had a different appearance from the distinct focal uptake seen with malignant lesions (Fig. 4). The infected cyst showed a level of uptake similar to that of malignant lesions (Fig. 5).

In summary, the findings suggest that fibroadenomas are not visualized on SMM and that fibrocystic changes can lead to heterogeneous, diffuse uptake.

For the 19 visualized malignant lesions, adequate lesion uptake was seen in all lesions, and no apparent difference was observed in static images taken 45 min and 105 min after administration of ^{99m}Tc-NC100692.

DISCUSSION

NC100692 is an RGD-containing cyclic peptide shown, by in vitro experimentation, to have high and specific binding to angiogenesis related integrins. In addition, other workers have demonstrated a relationship between RGD tracer uptake, angiogenic status (microvessel density), and

TABLE 3
Characteristics of Benign Lesions

Patient no.	Diagnosis	Visual uptake grading		
		US*	XMM†	SMM‡
10	Fibrocystic changes, other (fat)	No lesion	No lesion	2
13	Fibrocystic changes	2	2	1
17	4 Fibrocystic changes	2	2	2 (FNAC)§, 3 others
18	Fibroadenoma	2	2	1
19	Infectious cyst	2	2	3
20	Fibroadenoma	3	2	1

*US: 5-scale grading, where grade 2 = benign finding and grade 3 = indeterminate.

†XMM: findings are graded according to BIRADS, where grade 1 = negative, grade 2 = benign finding, grade 3 = probably benign finding, grade 4 = suspicious abnormality (biopsy should be considered), and grade 5 = highly suggestive of malignancy.

‡SMM with NC100692 administration (study drug): 3-grade scale, where grade 1 = no obvious uptake, grade 2 = uncertain/heterogeneous uptake, and grade 3 = focal lesion uptake.

§FNAC = lesion had fine-needle aspiration cytology before scintigraphy.

BIRADS = Breast Imaging Reporting and Data System.

FIGURE 5. Patient 19: Intense uptake in infected cyst. SPECT, coronal projection.

integrin expression in malignant tumors (*2,7–10,21–22*). Using NC100692, such uptake has also been demonstrated in tissue recovering from ischemia (*11,12*).

Data from the present study showed that ^{99m}Tc-NC100692 scintigraphy can detect malignant lesions. However, it should be noted that no cases classified as difficult or inconclusive by XMM or US were included in this study and that no lesions <6 mm were evaluated.

All lesions ≥10 mm were clearly detected by ^{99m}Tc-NC100692 scintigraphy. Similar results have been achieved and documented for other scintigraphic agents (e.g., ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin). ^{99m}Tc-NC100692 scintigraphy using the prescribed imaging protocol detected lesions as small as 7 mm but failed to visualize a 6-mm lesion located close to the chest wall. However, this lesion was visualized using a dedicated breast imaging semiconductor γ-camera (LumaGEM; Gamma Medica Ideas, Inc. [imaging not part of this protocol]).

It is generally accepted that growth of solid cancers beyond a diameter of 2–3 mm requires new growth of blood vessels (angiogenesis) (*23*). Hence, it is unlikely that scintigraphy with ^{99m}Tc-NC100692 will be able to detect very small malignant lesions even if the spatial resolution of a γ-camera would increase.

^{99m}Tc-NC100692 distinguishes itself from contrast agents used in routine practice by potentially providing physiologic information about the lesion of interest.

Growth rate and prognosis of a lesion are associated with the microvessel density of the lesion; therefore, visualizing angiogenesis may provide additional clinical information about a given lesion.

Data from this study indicate that ^{99m}Tc-NC100692 scintigraphy is capable of detecting large lymph node metastases (≥20 mm). However, smaller lymph node metastases (≤5 mm) were not detected, suggesting that ^{99m}Tc-NC100692 scintigraphy would be of limited use as a general test for detecting lymph node metastases in the axilla.

Five of 20 subjects experienced nonserious AEs, and all AEs were mild. One subject experienced an AE considered by the investigator to be possibly related to ^{99m}Tc-NC100692 administration.

In the present study, breast cancer was chosen as a model tumor type because early diagnosis and complete surgical removal allowing histologic verification are common. The expression of angiogenically related integrins is a process coupled to the majority of cancer types. NC100692 imaging may therefore be of use in numerous cancers. In addition, the angiogenic status of a tumor is thought to be a prognostic indicator and, therefore, a suitable marker may be of clinical utility. Additional studies are required to further investigate the potential of this agent.

One area of potential interest is the use of the agent for the monitoring of response to cancer treatment with the aim of distinguishing between responders and nonresponders. Response to standard chemotherapy regimens may be monitored by assessing angiogenicity as a surrogate marker of lesion growth versus lesion death.

In addition, it may also be possible to monitor lesion response to the newer biologic anticancer agents, some of which have antiangiogenic activities.

CONCLUSION

Angiogenesis is essential for cancer growth and is a prognostic indicator of lesion malignancy. The present study demonstrates the successful imaging of malignant breast lesions using an agent that binds to a receptor expressed during angiogenesis. The agent was well tolerated without clinically important safety problems.

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