Indium-111-Labeled B72.3 Monoclonal Antibody in the Detection and Staging of Breast Cancer: A Phase I Study

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Sixteen patients with primary breast cancer were studied with a pancarcinoma monoclonal antibody B72.3, an IgG_1 molecule directed against tumor-associated glycoprotein (TAG-72) present in several tumors. Five millicuries of ^{111}In was used to label 0.2 mg (six patients), or 2 mg (six patients), or 20 mg using the site-directed bifunctional DTPA method (at carbohydrate moiety). Digital, planar, and SPECT images were obtained at 2, 48, 72 and 96 hr when possible. HAMA levels were obtained before the Mab infusion and at 1, 3, and 6 wk postinfusion. Fourteen of 14 known primary breast lesions were detected by imaging (100% sensitivity). Two fibrocystic lesions were negative. Seven of 14 patients had lymph node metastases by histologic methods, but all were missed by radioimmunoscintigraphy. Tumor uptake of Mab ranged 0.00054%–0.0038% of the ID/g. The tumor-to-normal breast tissue ratio was 4.3 ± 0.91 (mean ± s.e.m.). Lymph nodes localization of ^{111}In-B72.3 by tissue analysis was similar for tumor-bearing and normal nodes (0.0039 ± 0.0023 versus 0.0025 ± 0.0019). Pharmacokinetics revealed mean plasma half-life of 33.3–41.2 hr for the different doses. There was no statistical difference between any of the pharmacokinetic parameters of different doses. HAMA was positive only in 17% of the patients. The study suggests that this antibody has 100% sensitivity for primary breast cancers, but very poor detection rate of metastatic lesions in axillary lymph nodes; thus making it of questionable value in the initial staging process of this disease.

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Breast cancer is the most common cancer in women except for skin neoplasms. Approximately 44,000 women die of metastatic breast cancer (*1*) every year and its incidence is 150,000 per year. Accurate staging is extremely important in selecting appropriate treatment options. There is much interest in developing noninvasive (nonsurgical) methods for detection of regional lymph nodes and distant metastases.

In this study, we have used the monoclonal antibody (Mab) B72.3 labeled with ^{111}In to determine its safety, pharmacokinetics, and biodistribution when injected intravenously in women with breast cancer. The purpose of the study was to determine the sensitivity of the ^{111}In-B72.3 and its accuracy in detection of the primary lesion and the metastatic breast cancer in the regional lymph nodes, as well as distant metastases if any, using immunoscintigraphy and tissue analysis and to measure human antimurine antibody (HAMA) response in these patients at various doses of the conjugated antibody. In addition, pharmacokinetics of ^{111}In-B72.3 and urinary excretion studies were performed to determine whether the antibody dose affects the in vivo behavior of this reagent.

MATERIALS AND METHODS

Antibody

Mab B72.3 was developed by Schlom et al. (*2–7*) at the National Cancer Institute and found to react with gastrointestinal, breast, ovarian, and lung tumors (*8–14*). It is a murine Mab of the IgG_1 subclass, which reacts with a 200–400,000 molecular weight tumor-associated glycoprotein antigen referred to as TAG-72 (*15–19*) found in certain human colon and breast carcinoma cell lines and biopsy material (*20–26*). Mab B72.3 has been shown to react with 80% of needle-biopsy aspirates of breast cancer but not with normal tissue (*2,3,15*). However, there are several other tumors which express the TAG-72 antigen including 96% of non-small cell lung cancers, 100% of common epithelial ovarian cancers, 80% of colon cancers as well as pancreatic, gastric, and esophageal cancers (*15,27*). The antibody was prepared from hybridomas in ascites fluids or cell culture and was purified by filtration, salt fractionation, and chromatography to remove contaminants.

Conjugation and Labeling

To label the Mab B72.3 with ^{111}In, a site-specific method of Rodwell was chosen (*28*) using covalent chemistry and the carbohydrate moieties on the constant part of the antibody. The resultant Mab B72.3-GYK-DTPA solution was sterilized by filtration, aseptically filled into glass vials (Cytogen Corp.) and

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transported to our laboratory for the final step of labeling with ^{111}In.

Quality control included a rapid thin-layer chromatography of the final product. A radiochemical purity of 95% or greater was required for use. The immunoreactivity of the antibody B72.3 was not reduced.

Patients

Sixteen female patients with biopsy-proven, operable breast cancer were studied prior to total mastectomy (Stage I, II, or III disease). Patients with Stage III treated with preoperative chemotherapy were eligible only if they had the last cycle of chemotherapy at least 3 wk prior to the study. All the patients had adequate hematologic, renal, and hepatic function. Patients with collagen-vascular disease or vasculitis or cardiac or central nervous system disease were excluded, and so were pregnant patients, patients who had prior treatment with non-human antibodies, and patients who tested positive for HAMA.

All patients signed an informed consent indicating the investigational nature of the study, which was approved by the Institutional Review Board (Surveillance Committee) of the University of Texas M.D. Anderson Cancer Center.

Study Outline

Patients were divided into three groups according to the total dose of the antibody given. Group I (six patients) were given 0.2 mg and Group II (six patients) were given 2 mg and the rest were given 20 mg of antibody. Whatever the antibody dose used, the total amount was labeled with 5 mCi of ^{111}In by the site specific GYK-DTPA method (*28*). In each case, the dose was infused intravenously through an infusion pump over 1 hr in 100 ml of sodium chloride with 1% human serum albumin. Surgery was scheduled for 5–8 days following the antibody infusion.

All patients received an intradermal skin test of 0.1 μg of the unlabeled dose and observed for 30 min for hypersensitivity reaction prior to infusion of 1 ml test dose of the labeled antibody. Following an additional 30-min observation, the total dose was administered. Vital signs were monitored and recorded prior to infusion and at 30-min intervals until stable and then every 4 hr for 12 hr.

Imaging Schedule

Total-body anterior and posterior gamma camera digital images were obtained within 2 hr of completion of infusion of the radiolabeled antibody and again at 24, 48, and 72 hr, and whenever feasible, at 96 hr as well. Multiple planar digital spot views were also taken including anterior and posterior views of the chest, abdomen, and pelvis using a 256 × 256 matrix for 7 min per image and oblique views of the breast. Radioactive markers were used to identify the nipple. SPECT of the chest and upper abdomen combined was obtained for three-dimensional localization of the lesions in the breast and identification of any positive axillary lymph nodes. SPECT images were acquired for 360° at 64 × 64 matrix using 128 stops of 30 sec each.

Pharmacokinetic Studies

Blood samples were collected prior to the Mab infusion and at 5, 15, 30, 45, 60, 90, 120, and 240 min after the end of Mab infusion and then at 24 hr, 48 hr, and at 72 hr. Urine was collected at 0–2 hr, 2–24 hr, and 24–48 hr post-Mab infusion. After recording the total volume of urine, a 10-ml aliquot from each specimen was stored for analysis. For pharmacokinetic studies, duplicate 100-μl aliquots of plasma or urine were counted in a Packard gamma counter (Model 5360). In addition, duplicate 10-μl aliquots of the original infusate were counted to determine total cpm administered and to serve as a decay-correction control. The values were subjected to non-linear regression analysis for calculation of standard pharmacokinetic parameters.

Quantitation of ^{111}In-B72.3 Uptake in Tumor

Mastectomy was performed on all but one patient and exploration of axillary lymph nodes was undertaken. Tissues from the primary tumor and lymph node metastases, as well as normal breast and regional lymph nodes, were weighed and counted in the gamma counter and results were expressed as the percent of injected dose per gram (%ID/g) of tissue. Similar ratios were calculated for positive and negative lymph nodes.

Measurement of TAG-72 Antigen Expression

Biopsy specimens obtained from 10 of the 14 patients were examined for TAG-72 antigen using an immunoperoxidase technique. Freshly cut sections of breast tumor and lymph nodes were incubated for 1 hr with normal goat serum to block non-specific binding of secondary antibody, washed in Triton X-100/PBS, incubated with biotinylated goat anti-mouse serum for 20 min, rewashed, incubated with avidin-biotin horseradish peroxidase complex for 20 min, rinsed in buffer and then developed in diaminobenzidine tetrahydrochloride (DAB) for 5 min, counterstained, cleared, and mounted. Serum levels of TAG-72 were measured in all patients at the Cytogen Corporation Laboratory using an RIA method, except the one patient who was HIV-positive.

Measurement of HAMA

HAMA were measured using a "sandwich" ELISA method (IMMUSTRIP HAMA Test System) developed by Immunomedics (*31*). Values above 0.4 μg/ml were considered positive. Blood samples were taken prior to the antibody infusion, at 24 hr after the infusion, and also at 1, 3–4, and 6–7 wk after infusion.

RESULTS

Imaging Results

Fifteen of the sixteen patients underwent breast surgery. One patient did not have surgery and another patient had

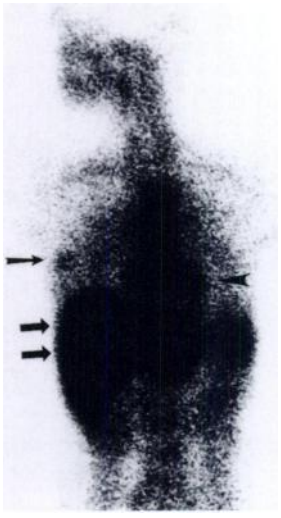

FIGURE 1. An anterior planar, digital image taken at 96 hr following intravenous injection of 5 mCi ^{111}In-B72.3 Mab showing a primary breast cancer localization on the right side (arrow). Note the significant liver uptake (double arrow) of the antibody. Blood pool was still present at 96 hr (arrow head). Bowel activity was minimal with this antibody.

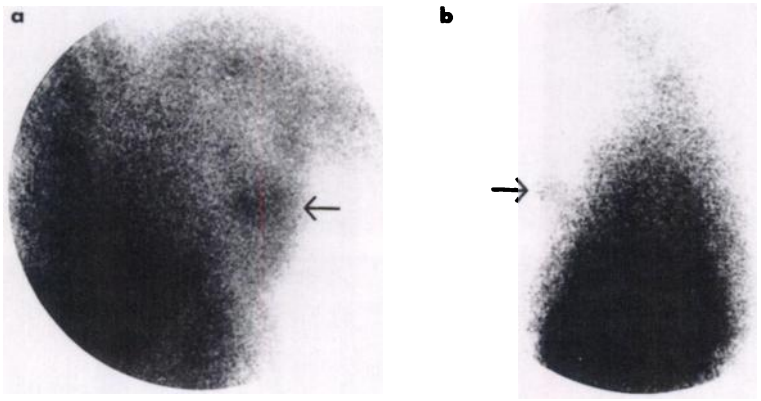

FIGURE 2. In some patients, special views were needed before the abnormal uptake by the breast lesion could be confidently identified: (a) right anterior oblique and (b) left lateral views in another patient show the abnormal uptake (arrows) of ^{111}In-B72.3 by the breast cancer.

two lesions that were not malignant. All fourteen primary breast lesions were detected with the ^{111}In-labeled B72.3 (100% sensitivity) (Figs. 1 and 2). The normal nipple was visualized in more than half of the patients (Fig. 3). The two benign lesions (fibrocystic disease) remained as true negative. Seven patients had lymph node metastases, but none were detected with radioimmunoscintigraphy. There was one false-positive lymph node uptake in the axilla (Fig. 4). This was negative for tumor histologically (Table 1). There were four other lymph nodes with histologically proven tumor, but negative scans. The sizes of the breast lesions removed at surgery ranged from 1.2 cm to 2.5 cm in diameter, except one of them which was 4.3 cm; and those for the lymph nodes ranged from 0.3 cm to 1.5 cm. We were able to detect three metastatic lesions that were previously not known to be present, one each in the skin, bone, and lymph node (Fig. 5). These turned out to be true lesions on retrospective and follow-up examinations.

Tissue Counts and Radiolocalization Index

The results of the in vitro studies are shown in Table 2. There was no significant difference ($p>0.05$) in the average tumor uptake in %ID/g between patients receiving 0.2 mg (0.0011 ± 0.0003), 2 mg (0.0025 ± 0.00075), or 20 mg (0.0014 ± 0.0001) of Mab. Despite low uptake of radiolabeled B72.3 in breast tumor, the ratio of %ID/g in tumor versus normal breast tissue was quite favorable, averaging 5.1 for patients receiving 0.2 mg and 2.9 for patients receiving 20 mg Mab. There was a wide range of Mab uptake in nodes containing microscopic tumor (0.0015 ± 0.0011 %ID/g); moreover uptake in negative nodes was equivalent to that in positive nodes (Table 2). Of 10 breast cancer specimens analyzed for TAG-72, 6 were positive and 4 were negative for an overall sensitivity of only 60%. Likewise three of five nodes (60%) that contained tumor were positive for TAG-72 antigen. On the other hand, 80% of tissues that were negative for tumor (including benign fibroadenomas and negative nodes) were negative for TAG-72. Overall, there was a weak correlation between cpm in tumor and positivity for TAG-72 antigen. Serum TAG-72 assay was negative (less than 100 U/ml) in all 14 patients tested.

Pharmacokinetic Results

The pharmacokinetic clearance of In-111 B72.3 from plasma is shown in Figure 6. The clearance profile for all patients at all dose levels closely fit ($r^2>0.95$) a one-

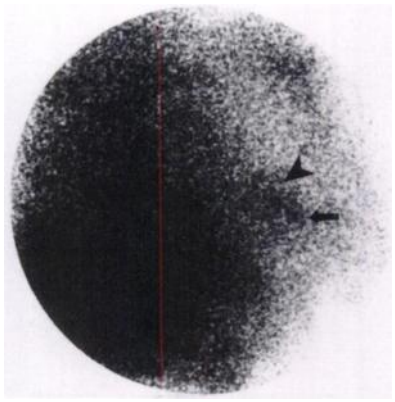

FIGURE 3. This anterior oblique view was acquired at 72 hr postintravenous injection of ^{111}In-B72.3. Normal localization is noted in the left nipple (arrow) which is separate from the abnormal tumor uptake (arrow head).

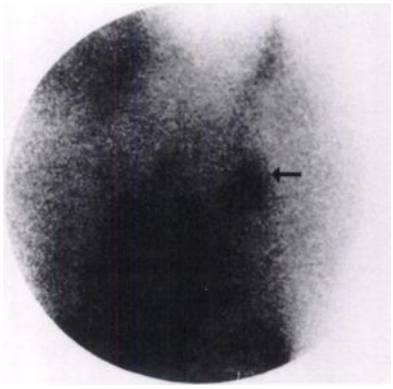

FIGURE 4. This patient manifested abnormal uptake in the lymph nodes in the left axilla (arrow). However, this was a false-positive case, since the tissue histology was negative for tumor. Unfortunately, this antibody missed on scanning axillary lymph nodes was known to be positive for tumor on histology.

TABLE 1
Radioimmunoimaging Results in All Patients

Site	Scan findings TP	TN	FP	FN	Imaging sensitivity no. (%)
Breast					
Tumor (n = 14)	14	0	0	0	14/4 (100)
Fibrocystic (n = 2)	0	2*	0	0	—
Lymph nodes					
+ for tumor (n = 4)†	0	—	0	4	0/4 (0)
− for tumor (n = 11)†	—	11	1	0	—
Occult Metastases					
Skin	1†	0	0	0	1/1 (100)
Bone	1‡	0	0	0	1/1 (100)
Lymph nodes	1‡	0	0	0	1/1 (100)

* Specificity for tumor = 100%.
† Documented by surgery/biopsy.
‡ Radiographic conformation of F/U.
TP = true-positive, TN = true-negative; FP = false-positive, and FN = false-negative.

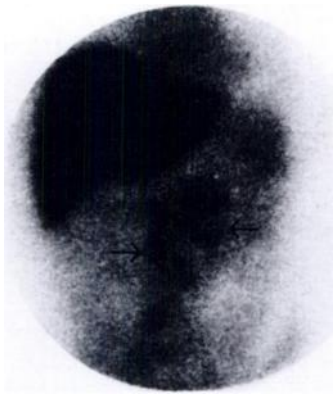

FIGURE 5. Anterior abdominal view of a patient given 20 mg of B72.3 labeled with 5 mCi ^{111}In. Note abnormal uptake by the lymph nodes in the mid-abdomen. These were confirmed to be metastatic lesions on follow-up examination within 6 mo.

compartment mathematical model. Increasing doses of antibody substantially increased the initial concentration of the radiolabel in plasma immediately after administration (Cpo) and therefore resulted in a decreased apparent volume of distribution (Vd, Table 3). The half-life of ^{111}In-B72.3 in plasma was 41.2 ± 2.6 hr at the 0.2-mg dose level and 35.6 ± .5 hr and 33.3 ± 3 hr at the 2.0- and 20-mg dose levels, respectively. Although there appeared to be a trend toward decreasing half-life with increasing antibody dose, this was not statistically significant. There was a dose-dependent increase in the area under the concentration curve (Cxt) with increasing dose. The increase in Cxt was due primarily to a decrease in the Vd, since the clearance rate of antibody from plasma (Clp) appeared to be unaffected by dose. The cumulative urinary excretion (Table 3, Fig. 7) indicated a faster excretion of the ^{111}In radiolabel at the 0.2-mg dose (1st 24 hr) and less of the ^{111}In radiolabel was excreted at the 20-mg dose level compared to the 2.0-mg or the 0.2-mg dose levels. However, these differences were not statistically significant ($p>0.05$).

HAMA Results

All patients were negative for HAMA pre-study. Only 2 of 12 patients tested for HAMA showed positive results (17%). One of them went up to 117.9 μg/ml 1 wk after the Mab and then dropped to 27.2 μg/ml at the fourth week of testing. She had received the 20-mg dose of the Mab. The second patient remained negative the first week, but became minimally positive at 0.5 μg/ml (negative if <0.4 μg/ml) at 4 wk and reverted to negative at 7 wk. This patient had received the 0.2-mg Mab dose.

DISCUSSION

The results of this study indicate that ^{111}In-labeled B72.3 has a very high sensitivity for primary breast cancer (100%), however, it has failed to detect microscopic tumor deposits in any of the tumor-positive lymph nodes in the axillae despite the use of SPECT imaging. This would suggest that this antibody may not be satisfactory for initial

TABLE 2
Summary of Tumor and Axillary Lymph Node Uptake of ^{111}In-B72.3

Mab dose	Tumor average uptake* (%ID/g × 10^{-3})	Normal breast uptake (%ID/g × 10^{-3})	Average T:N ratio†	Positive nodes‡ (%ID/g × 10^{-3})	Negative nodes (%ID/g × 10^{-3})
0.2	1.13 ± 0.30§	0.22 ± 0.04	5.1	2.8 ± 2.5	5.9 ± 5.3¶
2	2.50 ± 0.75	0.64 ± 0.08	3.9	—	2.7 ± 0.4
20	1.40 ± 0.10	0.48 ± 0.09	2.9	1.5 ± 0	1.8 ± 0.2

* $\frac{\text{cpm/g tumor}}{\text{cpm injected}} \times 100$ and %ID/g = %ID/g × 10^{-3}.
† T:N = ratio % ID/g tumor: normal breast localization.
‡ Positive nodes (i.e., those containing tumor).
§ All values are mean ± s.e.m.
¶ One patient had a high uptake of 27 × 10^3 giving the high mean.

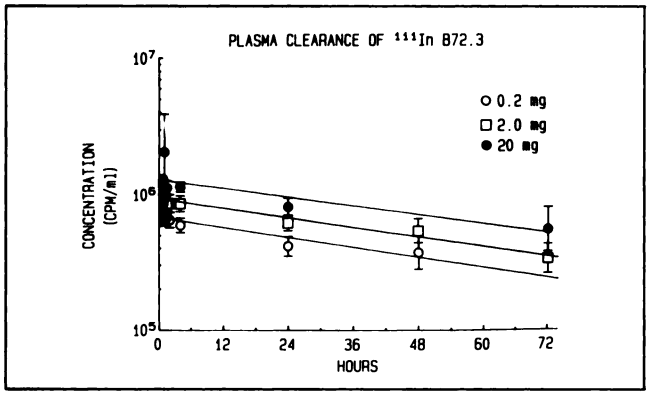

FIGURE 6. Plasma clearance of the different doses of intravenously administered ^{111}In-labeled monoclonal antibody B72.3.

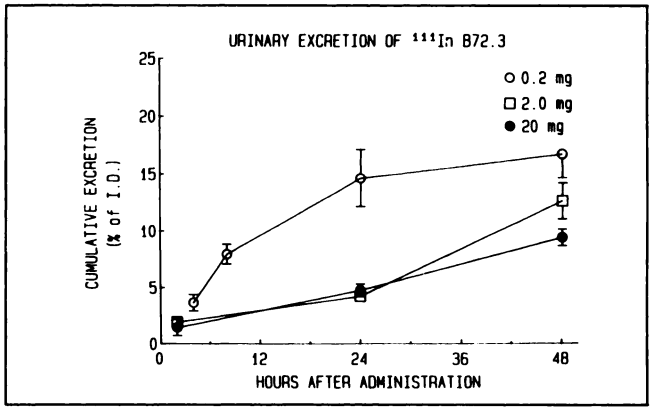

FIGURE 7. Cumulative urine excretion of the same doses of the ^{111}In-B72.3 antibody as in Fig. 6.

staging of these patients. The patient with a false-positive lymph node on immunoscanning was subsequently documented to be HIV-positive on follow-up, possibly accounting for the false-positive lymph node uptake.

The data with respect to quantitative uptake of radiolabeled B72.3 and immunohistology of tissue specimens did not necessarily complement the imaging results. Of the breast tumors imaged, 100% had uptake of Mab, which was significantly greater than that of normal breast tissue, whereas only 60% (6 of 10 patients tested) were positive for TAG-72 antigen. This finding could be due to nonspecific uptake of ^{111}In-labeled antibody or labeled catabolite by the tumor. However, since tumor samples available for analysis were only 10 of the 15, it is possible that a higher percentage of tumors may have been positive for the antigen. In contrast, there was virtually no correlation between imaging of lymph nodes and uptake of radiolabeled Mab and/or TAG-72 antigen content. It is possible that the relatively high background activity of ^{111}In in the axilla combined with the smaller size of the lymph nodes significantly hindered scan resolution. The use of F(ab')$_2$ or Fab' fragments may improve resolution due to their rapid clearance from the circulation (*31–36*).

Of interest was the finding that the %ID/g of B72.3 uptake in positive nodes was equivalent to that for negative nodes. Such a finding has been noted by other investigators (*37,38*) and may be related to nonspecific uptake and metabolism of Mabs in lymph nodes. Nonspecific localization has been observed in the nipple (Fig. 3), but there was not a clear explanation. The lack of lymph node detection is a problem that has also been noted by other investigators (*39–42*).

The plasma pharmacokinetics demonstrated no statistically significant change in the plasma half-life or clearance rate from plasma with increasing antibody concentration. Within the limitation of the methods used in this study, the clearance of this antibody appears to be a single compartment system for all three doses used. The slight decrease in the volume of distribution (Vd) with a concomitant increase in the CxT suggests that higher antibody doses prevent extravascular distribution of the radiolabel.

The HAMA results are interesting and despite following the patients up to 6–7 wk only two patients (12 tested) have developed HAMA antibodies. This may be a function of the radiolabeling technique or it may be related to other factors including the dose of Mab (*43*), differences in assay methods, differences in patient populations among studies

TABLE 3
Pharmacokinetic Summary of ^{111}In-Labeled B72.3*

Dose (mg)	Number of patients	$T_{½}$‡ (Hr)	Vd§ (l)	CxT (μCi/mlxmi)¶	Clp (ml/gxm)**	48 hr cum (%)†† urinary excretion
0.2	4	41.2 ± 2.6	4.0 ± 0.7	398 ± 55	0.009 ± 0.0047	16.6 ± 2.0
2.0	6	35.6 ± 5†	3.9 ± 0.7	550 ± 121	0.006 ± 0.003	12.5 ± 1.6
20	3	33.3 ± 3†	2.4 ± 0.3	1131 ± 216	0.007 ± 0.001	9.3 ± 0.7

* All figures are mean ± s.e.m.
† Not significantly different ($p > 0.05$) compared to 0.2-mg group.
‡ Plasma half-life.
§ Volume of distribution.
¶ Concentration curve (area under the curve).
** Plasma clearance.
†† Cumulative urine excretion over 48 hr expressed as %ID.

in the literature, and timing of serum sampling. Experience with other ^{111}In-labeled monoclonal antibodies has indicated a generally higher incidence of HAMA with intact antibodies (*44–49*), but it varies with different Mabs and lowers with the use of fragments (*50*).

In several ways, this antibody has behaved significantly different from our previous experience with other indium-labeled Mabs (*51–53*). From the experience of others with this antibody, and from our experience with other antibodies, it is possible that some form of manipulation either by modulating the antigen with Interferon (*54*), using Interferon in a combination with other antibodies (*55*), or using a different radiolabel (*56*) might improve the sensitivity for metastatic lesions.

CONCLUSIONS

The lack of sensitivity for the lymph node detection is disappointing and raises the question of whether there is any role for this antibody in the initial staging of breast cancer, despite its 100% sensitivity for primary lesions. Perhaps the low sensitivity of lymph nodes may be site-dependent and further trials of this antibody or its fragment may yield different results for patients with distant metastatic disease. The role of ^{111}In-B72.3 in the detection of occult metastatic lesions cannot be determined from this study, despite the detection of three previously unknown lesions.

ACKNOWLEDGMENT

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