^{99m}Tc-Labeled Antihuman Epidermal Growth Factor Receptor Antibody in Patients with Tumors of Epithelial Origin: Part III. Clinical Trials Safety and Diagnostic Efficacy

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Monoclonal antibody (moAb) ior egf/r3 is an IgG_{2a} that recognizes the epidermal growth factor receptor (EGF-R). The aim of this study was to evaluate the diagnostic efficacy of the ^{99m}Tc-labeled moAb ior egf/r3 for the detection of epithelial-derived tumors, their metastases and recurrences. **Methods:** One hundred forty-eight adult patients (51 women, 97 men; mean age 53 ± 13 y) who were suspected of having cancer of epithelial origin were administered 3 mg/50 mCi (1.85 GBq) ^{99m}Tc-labeled moAb ior egf/r3 by intravenous bolus injection. Planar anterior and posterior images of the lesion sites and suspected metastases were acquired at 2, 4, 6 and 24 h after injection, and SPECT images were scanned at 5 h postinjection, using a 360° circular orbit with 64 images. The backprojection method was used for image reconstruction with a Hamming-Hann filter. **Results:** Labeling efficiency was always greater than 98.5% ± 2.1%. No adverse reactions or side effects were observed. Results of the biopsy specimens showed that 85.1% (126/148) of the patients had tumors of epithelial origin, 14.2% (21/148) were negative and 0.7% (1/148) had non-Hodgkin's lymphoma. The sensitivity rate by organ was as follows: brain (8/8, 100%), digestive tract (10/11, 90.9%), head and neck (17/23, 73.9%), lung (52/62, 83.9%) and breast (16/18, 88.9%). Overall sensitivity, specificity, accuracy, and positive and negative predictive values of the immunoscintigraphic imaging were 84.2% (106/126), 100.0% (22/22), 86.5% (128/148), 100% (106/106) and 52.4% (22/42), respectively. New metastases not identified previously by other diagnostic methods were detected in the 50% of the patients. **Conclusion:** Immunoscintigraphy with ^{99m}Tc-labeled moAb ior egf/r3 could be a useful procedure for the diagnosis and follow-up of the patients with tumors of epithelial origin.

Key Words: anti-epidermal growth factor receptor antibody; radioimmunoscintigraphy; diagnostic efficacy; epithelial tumors

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Despite the advances in antitumoral therapy, cancer of epithelial origin is one of the leading causes of death worldwide (*1*). The second-leading cause of death in Cuba in 1996 was malignant tumors, with a rate of 137.3 per 100,000 people (*2*). Tumors of epithelial origin, such as non-small cell lung cancer, digestive tract, breast and others, have 100-fold more expression of the epidermal growth factor receptor (EGF-R) than normal tissues (*3–5*). This fact is often related to malignancy and poor prognosis of the disease (*6–9*). It is thought that the upregulated EGF-R expression allows epidermal growth factor (EGF) and transforming growth factor-α to act as autocrine growth factors, contributing to the progression of the disease (*10*). Several clinical trials with radiolabeled antiEGF-R antibodies have shown that these monoclonal antibodies (moAbs) could be useful for immunoscintigraphic diagnosis of tumors of epithelial origin (*11–14*).

MoAb ior egf/r3 developed at the Center of Molecular Immunology (Havana, Cuba) is a murine IgG_{2a} directed against human EGF-R. It inhibits the binding of EGF to its receptor. When bound to the membrane-receptor complex, it becomes internalized with the receptor causing downregulation of the EGF-R, without stimulation of tyrosine kinase activity (*15,16*). These characteristics have been considered in the radioimmunodiagnosis of tumors of epithelial origin. ^{99m}Tc-labeled moAb ior egf/r3 has shown to be of value for the detection of epidermoid carcinoma cells in patients (*17,18*). Pharmacokinetics, biodistribution and internal radiation dosimetry studies of this radiopharmaceutical have been performed previously (*19,20*).

The aim of this study was to evaluate the safety and diagnostic efficacy of immunoscintigraphy with ^{99m}Tc-labeled moAb ior egf/r3 for the detection of epithelial-derived tumors, their metastases and recurrences.

MATERIALS AND METHODS

Patient Selection, Evaluation and Clinical Trial

One hundred forty-eight adult patients (51 women, 97 men; age range 18–82 y, mean age 53 ± 13 y) were studied. All patients were suspected of having either primary or recurrent cancer of epithelial origin and had undergone CT scanning, radiography and other determinations.

All patients had complete and differential blood cell and platelet counts, liver and kidney function tests and urine analyses between 1 and 7 d before the antibody administration.

To test for sensitivity to mouse protein, patients 1–64 received an intradermal injection of 1.0 μg unlabeled moAb ior egf/r3 in a total volume of 0.1 mL saline 0.9%. The test was read 20 min later, and only patients with negative skin test results (absence of induration, skin elevation of less than 20 mm) were included in the study. Skin testing was discontinued after the first 64 patients, because no adverse reaction was observed.

The ability of immunoscintigraphy with ^{99m}Tc-labeled moAb ior egf/r3 to detect cancer of epithelial origin was assessed by a multicenter phase I/II clinical trial. The protocol of the study was approved by the Institutional Review Board of the National Institute for Oncology and Radiobiology, the Center for Clinical Researches, Center for Medical-Surgical Researches and the National Regulatory Agency of Cuba: The State Center for Quality Control of Drugs (Havana, Cuba). Written informed consent was obtained from all patients. Table 1 summarizes patient distribution according to tumor localization.

Monoclonal Antibody

MoAb ior egf/r3 is a murine IgG_{2a} isotype antibody that recognizes human EGF-R with a high affinity (K_{aff} = 10^9–10^{10} mol/L) and specificity. It is secreted by hybridoma A24/15/128, obtained by fusion of murine myeloma cells SP2/Ag14 with splenocytes of immunized Balb/c mice with a partial purified fraction of the EGF-R from human placental extract. Its generation and properties have been reported by Fernandez et al. (*15,16*). MoAb ior egf/r3 was supplied by the Center of Molecular Immunology in vials containing 1 mL of a 5 mg/mL sterile and pyrogen-free neutral solution.

Antibody Labeling, Quality Control and Biologic Activity

MoAb ior egf/r3 was directly labeled according to the method of Schwarz and Steinstraber (*21*), which was described by Iznaga-Escobar et al. (*22*) and Morales et al. (*23*). Briefly, the antibody at 5 mg/mL in neutral phosphate buffered saline (PBS) was reduced by reaction with a 2000-mol/L excess of 2-mercaptoethanol (2-ME) at room temperature for 30 min. Reduced antibody was purified to eliminate the excess of 2-ME through a PD-10 sephadex gel filtration column (Pharmacia Biotech, Uppsala, Sweden), using cold PBS (pH 7.4) purged with nitrogen as mobile phase. Two-milliliter fractions were collected, and protein concentration determined; and the optical density was measured at 280 nm on an ultraviolet visible spectrophotometer.

A methylene diphosphonate (MDP) bone-scanning kit (Amerscan Medronate II, Amersham, UK) was reconstituted with 5 mL 0.9% saline solution purged with nitrogen, and 50 μL medronate solution per milligram of reduced antibody was added, followed by 50 mCi (1.85 GBq) pertechnetate from a ^{99}Mo/^{99m}Tc generator (Amertec II, Amersham, UK). The eluate was used within 2 h of elution and was obtained from a sterile generator eluted within the previous 24 h. The radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. The glassware, plastics and solutions used were sterile and pyrogen free.

Labeled product was subjected to ascending paper chromatography on Whatman 3 MM paper as stationary phase and 0.9% saline and acetone as mobile phases. Radioactivity bound to antibody remained at the origin, whereas free pertechnetate and ^{99m}Tc-MDP migrated with the solvent front (*22*).

Human serum albumin (1%)-impregnated instant thin-layer chromatography (ITLC)-silica gel (Gelman Science Inc., Ann Arbor, MI) strips were used as stationary phase and ethanol-to-NH_4OH-to-water (2:1:5 v/v) as the mobile phase to separate radiocolloids, which remained at the base, while the radiolabeled MoAb and free pertechnetate moved away (radiocolloid, R_f = 0.0; other labeled compounds, R_f = 1.0) (*22*). Radiochemical purity higher than 90% was considered satisfactory for patient administration.

Immunoreactivity of the reduced antibody was assessed by homogeneous radio receptor analysis (*24*), measuring its ability to interact with the EGF-R expressed in a human placenta microsomal fraction. Reduced antibody was compared with nonreduced (native) antibody for the ability to compete with radioiodinated murine EGF (^{125}I-EGF). Data from three to five independent experiments were averaged and plotted.

TABLE 1
Histopathologic Classification of Tumor

Histopathologic variety	No. of patients
Epidermoid carcinoma	75
Adenocarcinoma	25
Ductal carcinoma	12
Lobular carcinoma	2
Astrocytoma	3
Glioma	5
Another kind of epithelial tumor	6
Negative*	22
Total	148

*1 non-Hodgkin's lymphoma.

Radiopharmaceutical Administration

Patients were administered 3 mg/50 mCi (1.85 GBq) ^{99m}Tc-labeled moAb ior egf/r3 by intravenous bolus injection. To detect any adverse reaction, all patients were monitored up to 24 h after radiopharmaceutical administration. The appropriate dose was measured with a dose calibrator (Compucal II, Nuclear Associates, Division of Victoreen Inc., New York, NY).

Data Acquisition and Patient Imaging

The images were acquired by using either a gamma camera ZLC 3700 (Siemens, Hoffman Estates, IL) interfaced to an Imagamma system-based computer or a Sophy DS-7 (SMVi, Buc, France). A low-energy, all-purpose, parallel-hole collimator was used. Imaging was performed using a 20% window centered on the 140-KeV photopeak.

Planar Images. Anterior and posterior planar images of the lesion site and suspected metastases were acquired at 2, 4, 6 and 24 h after injection, with a matrix size of 128 × 128 and 500 kilocounts per view.

SPECT Images. Patients were scanned at 5 h postinjection, using a 360° circular orbit with 64 images, each lasting 30 s. The backprojection method was used for image reconstruction with a Hamming-Hann filter. No attenuation correction was used. Orthogonal tomographic slices of transaxial, sagittal and coronal images were reconstructed with a 64 × 64 matrix.

Interpretation of the images was based on asymmetry and retention of activity, especially on late images.

Studies were classified as true-positive when there was uptake in the tumor and its metastases. Studies were classified as true-negative when there was no uptake in the absence of tumor. Abnormal uptake was categorized as false-positive. Studies were classified as false-negative when there was no uptake, despite the presence of tumor or metastasis. Histopathologic findings were considered a confirmation criterion (gold standard).

Three qualified specialists analyzed all images, and the final diagnosis was determined by consensus.

Human Antimouse Antibody Assay

For human antimouse antibody (HAMA) response, blood samples were obtained from the first 30 patients at timed intervals before moAb infusion and at 2, 4, 8 and 12 wk after ^{99m}Tc-labeled moAb ior egf/r3 administration. A qualitative direct in-house HAMA assay was developed as follows: Briefly, high-binding enzyme link immunosorbent assay plates (Maxisorb; Costar, Cambridge, MA) were coated with 10 μg/mL cold moAb ior egf/r3 in a total volume of 50 μL per well and incubated overnight at 4°C. Then the plates were washed three times with PBS pH 7.4.

Fifty microliters of serum samples of the patients' serial diluted from 50 to 1500 times were then incubated for 1 h at 37°C. The plates were washed three times again with PBS and incubated with 1/2000 dilution of the antihuman IgG alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO). Pretreatment serum samples of each patient were used as controls. Irrelevant serum samples from untreated patients were used as negative controls, whereas samples from HAMA-positive patients previously treated with moAb ior egf/r3 were used as positive controls. The HAMA assay was considered negative when post-treatment-to-pretreatment ratio was less than 1.5; otherwise, the sample was considered positive.

Statistical Analysis

The immunoscintigraphic and histopathologic findings were compared using the McNemar test; $P < 0.05$ was considered statistically significant. The kappa coefficient, as well as the sensitivity, specificity, accuracy and predictive values, of the immunoscintigraphy was calculated with the statistical package SPSS for Windows version 3.1 (SPSS Inc., Chicago, IL).

RESULTS

Radiolabeling

A mean of 98.5% ± 2.1% of labeling was determined by quality control. Instant paper chromatography of labeled moAb in acetone showed about 1.2% free pertechnetate (R_f = 1.0). When the chromatogram was developed in saline, more than 98.8% of the activity stayed at the origin, indicating that ^{99m}Tc was transchelated from MDP to the moAb ior egf/r3. The colloid formation, determined by albumin-impregnated ITLC strips, was <1.5% in all preparations.

TABLE 2
Distribution of Primary Tumors by Sex and Organs According to Biopsy Results

	Female		Male		Total	
Organ	+	−	+	−	+	−
Lung	13	2	49	8	62	10
Head and neck	0	0	23	1	23	1
Breast	17	3	1	0	18	3
Brain	7	4	1	2	8	6
Digestive tract	2	0	9	0	11	0
Other organs	3	0	1	2	4	2
Total	42	9	84	13	126	22

At the same protein concentration, reduced and native antibodies showed similar binding capacity to EGF-R.

Patients Studied

Biopsy results showed that 126 of 148 patients included in the clinical trial had tumors of epithelial origin (85.1%), 21 were negative of cancer (14.2%) and 1 had non-Hodgkin's lymphoma (0.7%). Of 148 patients in the study, 109 were in stages III or IV of the disease. Table 1 shows the histopathologic classification of the tumors. The most frequent tumors within this population were epidermoid carcinoma (75 patients) and adenocarcinoma (25 patients). Table 2 shows the distribution of the tumors by sex and organs, according to the biopsy outcomes.

Radioimmunoscintigraphy

Radioimmunoscintigraphy (RIS) identified 106 of 126 patients who had cancer of epithelial origin and 22 of the 22 true-negative patients. The overall sensitivity, specificity, accuracy, and positive and negative predictive values of the immunoscintigraphic imaging were 84.1%, 100%, 86.5%, 100% and 52.4%, respectively. These values were calculated from the data summarized in Table 3. Diagnostic efficacy was computed for the organs of primary focus; the results are shown in Table 4. The sensitivity of the study increased slightly when only the 109 patients in an advanced stage (III–IV) of the disease were analyzed (Table 5). Nevertheless, this increase was not statistically significant at $P <$ 0.05.

TABLE 3
Correlation Between Results of Diagnosis by Histopathology and Radioimmunoscintigraphy Finding of ^{99m}Tc-Labeled Monoclonal Antibody ior egf/r3 by Organ

Localization	True-positive	False-positive	True-negative	False-negative	Total
Lung	52	0	10	10	72
Breast	16	0	3	2	21
Brain	8	0	6	0	14
Head and neck	17	0	1	6	24
Digestive system	10	0	0	1	11
Other	3	0	2	1	6
Total	106	0	22	20	148

TABLE 4
Diagnostic Efficacy of ^{99m}Tc-Labeled Monoclonal Antibody ior egf/r3 as Result of Immunoscintigraphic Images Obtained in Patients with Tumors for Organs of Primary Study

Localization (Kappa coefficient)	Sensitivity % (rate)	Predictive values positive/negative % (rate)/(rate)	Accuracy % (rate)	Specificity % (rate)
Lung (0.6)	83.9 (52/62)	100/50 (52/52)/(10/20)	81.7 (62/72)	100.0 (10/10)
Breast (0.7)	88.9 (16/18)	100/60 (16/16)/(3/5)	90.5 (19/21)	100 (3/3)
Brain (1.00)	100.0 (8/8)	100/100 (6/6)/(8/8)	100.0 (14/14)	100.0 (6/6)
Digestive system	90.9 (10/11)	100 (10/10)	90.9 (10/11)	—
Head and neck (0.2)	73.9 (17/23)	100/14.3 (17/17)/(1/7)	75 (18/24)	100.0 (1/1)
Other side (0.67)	75.4 (3/4)	100/66.7 (3/3)/(2/5)	83.3 (5/6)	100.0 (2/2)
Total (0.61)	84.1 (106/126)	100/52.4 (106/106)/(22/42)	86.5 (128/148)	100.0 (22/22)

Numbers in parentheses indicate true-positive patients out of total patients evaluated in the localization.

Different kinds of epithelial tumors do not have similar expression of EGF-R on the cell surface. Thus, the sensitivity of the RIS with ^{99m}Tc-labeled moAb ior egf/r3 was calculated depending on the histopathologic classification (Table 6). The sensitivity for the diagnosis of adenocarcinomas decreased, compared with epidermoid carcinomas for all localizations studied ($P < 0.05$).

Multiple images were obtained in all patients. Whole-body images up to 24 h postinjection showed decreasing blood-pool activity during the first hours with the visualization of the liver, and there were no hints of free pertechnetate by visualization of thyroid or stomach. The liver showed the highest uptake among all the organs, as a result of different reasons: (a) the high number of EGF-Rs present on its cell surface and (b) the liver is the main organ for catabolism and metabolism of the radiolabeled proteins. It was immediately perceptible after administration of ^{99m}Tc-labeled moAb ior egf/r3 and, at 24 h postinjection, it still remained visible. No selective accumulation of the radioactivity was observed in any other normal tissue, except those of the excretion pathway. Figure 1 shows planar images obtained at 4 and 24 h after administration of ^{99m}Tc-labeled moAb ior egf/r3 to a patient with lung epidermoid carcinoma. It illustrates the uptake of ^{99m}Tc-labeled moAb in the lesion on the left lung compared with the contralateral lung, and Figures 2 and 3 show the high accumulation of the radiopharmaceutical in the lymph-node metastases from the head and neck epithelial tumor and hepatic metastasis of a lung adenocarcinoma, initially identified by immunoscintigraphy.

TABLE 5
Diagnostic Sensitivity of ^{99m}Tc-Labeled Monoclonal Antibody ior egf/r3 Scintigraphic Images of 109 Patients in Stages III–IV of Disease

Stage III–IV	Rate	Sensitivity (%)
Overall patients	93/109	85.3
Lung	43/49	87.8
Breast	14/16	87.5

Rate indicates true-positive patients out of total patients.

Normal maxillofacial biodistribution of the moAb ior egf/r3 made the diagnosis of the head and neck tumors difficult within the first hours. In the later planar images, it was possible to identify these tumor lesions. Lymph-node metastases were observed starting from 3 to 4 h after administration of radiolabeled moAb for this localization. Figure 4 shows the uptake of ^{99m}Tc-labeled moAb ior egf/r3 in brain lesions not only in the case of primary tumors such as astrocytomas and glioblastomas multiforme but in the case of brain metastases of other tumors such as in the breast and lung.

Breast tumor lesions were difficult to detect in anterior planar images, and it was necessary to acquire serial lateral images. Most of the patients (14/16) had undergone surgery previously, but in these studies, the radiolabeled antibody was able to detect metastases and relapses in some patients. Figure 5 shows a ductal carcinoma operated on previously, with recurrent and metastatic disease in the other breast.

SPECT images of the lesions improved the quality of the diagnostic test, principally the detection of head and neck primary tumors and metastatic nodes.

Ovarian carcinoma, previously confirmed by biopsy specimen, was also identified by RIS with radiolabeled moAb ior egf/r3 (Fig. 6).

TABLE 6
Sensitivity and Accuracy of Radioimmunoscintigraphy with ^{99m}Tc-Labeled Monoclonal Antibody ior egf/r3

Tumor localization	Sensitivity (%)			Accuracy (%)		
	Epidermoid carcinoma	Adenocarcinoma	Other tumors	Epidermoid carcinoma	Adenocarcinoma	Other tumors
Overall	85.3 (64/73)	76.0 (19/25)	90.5 (19/21)	86.1 (68/79)	76.9 (20/26)	94.6 (35/37)
Lung cancer	87.5 (35/40)	77.8 (14/18)	—	88.1 (37/42)	78.9 (15/19)	—
Other organs except lung	82.9 (29/35)	71.4 (5/7)	—	83.8 (31/37)	—	—

Numbers in parentheses indicate true-positive patients out of total patients evaluated in the localization.

Clinical Toxicity and Adverse Reactions

The skin test was negative for all tested patients. No adverse reactions or side effects were observed after the administration of ^{99m}Tc-labeled moAb ior egf/r3. No clinically significant changes in bone marrow, liver or kidney function were detected in any of the patients.

Human Antimouse Antibody Response

Development of the HAMA response after moAb ior egf/r3 administration was assessed. Baseline preinfusion levels were available for all patients studied. No significant response was detected in the serum of most of the patients tested. The HAMA response developed in only 20% (6/30) of the patients, and 1 of 6 patients maintained measurable positive values 12 wk after radiopharmaceutical administration.

DISCUSSION

Cancer of epithelial origin is the most common malignancy worldwide. This type of tumor is the leading cause of death by neoplasm in Cuba (*2*). Early diagnosis and accurate staging before therapy, as well as the early detection of recurrences, may improve the prognosis and survival of the patients. Thus, recognition of molecular abnormalities that participate in the development and progression of these tumors provides new opportunities to improve the diagnostic and therapeutic procedures.

It is known that the aberrant expression of the EGF-R system is particularly important in the development of epithelial-derived malignancies. This fact has been associated with poor survival (*25*). The development of moAbs against human EGF-R has broadened new horizons of diagnosis and therapy of neoplasms of epithelial origin (*11–14*).

The first study about labeling and clinical application of ior egf/r3 was performed by Zayas et al. (*26*). Twenty-one

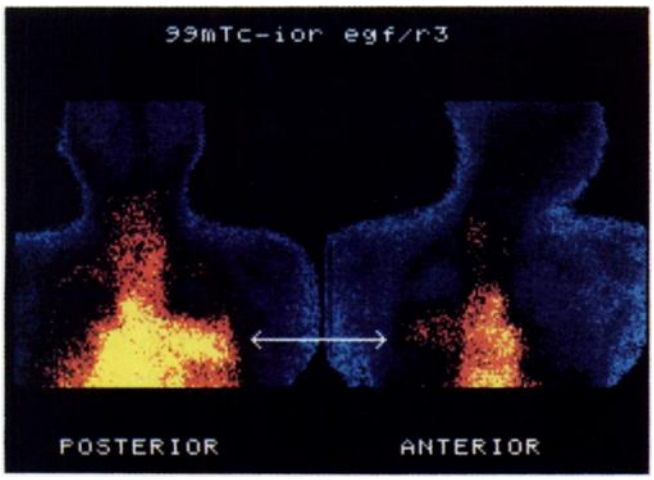

FIGURE 1. Planar anterior and posterior images taken at 24 h postadministration of 3 mg/50 mCi (1.85 GBq) ^{99m}Tc-labeled moAb ior egf/r3 show tumor uptake of lung epidermoid carcinoma.

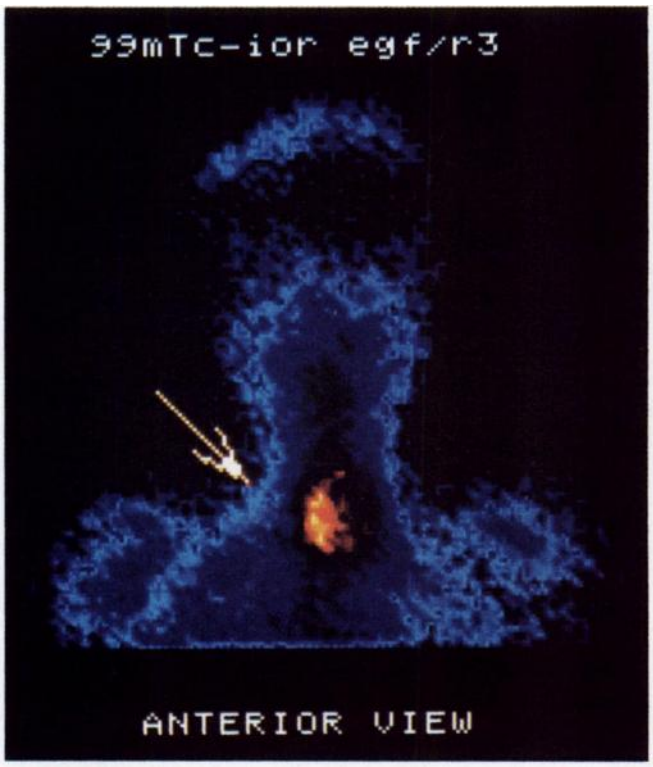

FIGURE 2. Planar anterior view of patient with primary lung adenocarcinoma taken 24 h after administration of ^{99m}Tc-labeled moAb ior egf/r3 shows lymph node metastasis in laryngeal region.

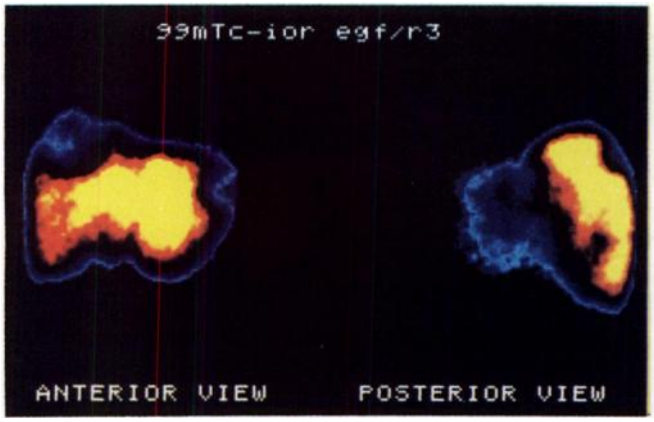

FIGURE 3. Anterior and posterior immunoscintigraphic images show metastasis in right hepatic lobe in patient with primary lung adenocarcinoma.

patients were studied, and a good correlation with CT and biopsy findings was obtained. The results of phase I clinical trial, with freeze-dried kit formulations and those in solution, were reported by Iznaga-Escobar et al. (*19,20*).

The outcomes of this clinical trial, as well as of previous studies, demonstrated the lack of toxicity in patients who received intravenous administration of 3 mg murine moAb ior egf/r3 labeled with 50 mCi (1.85 GBq) ^{99m}Tc. Despite the high uptake of radiopharmaceutical in the liver, laboratory analysis showed no evidence of hepatic damage. No adverse reaction was detected after a single intravenous administration. All patients tested had a negative result on the skin test, which was performed 20 min before moAb infusion. Similar results have been obtained by Abdel-Nabi et al. (*27*), who considered skin test outcomes not helpful in predicting or screening for the development of adverse reactions after intravenous administration of moAb. For these reasons, the skin testing was stopped after the first 64 patients.

A rapid clearance of radiopharmaceutical from the blood pool was observed. It was previously described by Iznaga-Escobar et al. (*19,20*). They reported that 12.9% ± 1.1% (mean ± SD) of the injected dose remained in circulation at 3 h after administration. At that time, there was a large concentration in the spleen and liver. The significant pres-

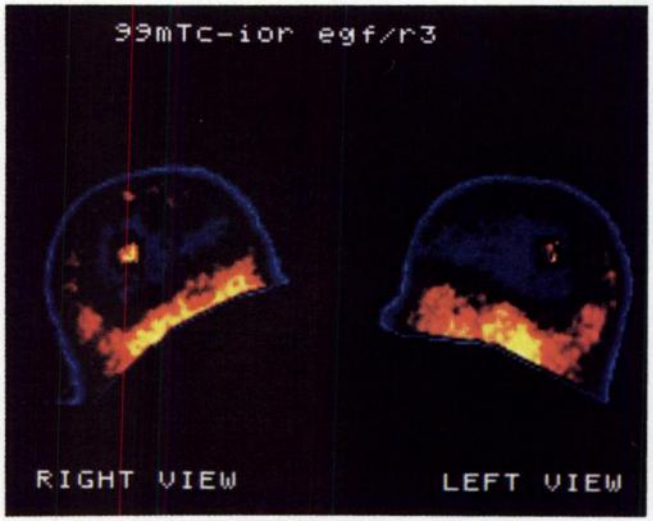

FIGURE 4. Planar right and left views of brain metastasis in region of temporal lobe.

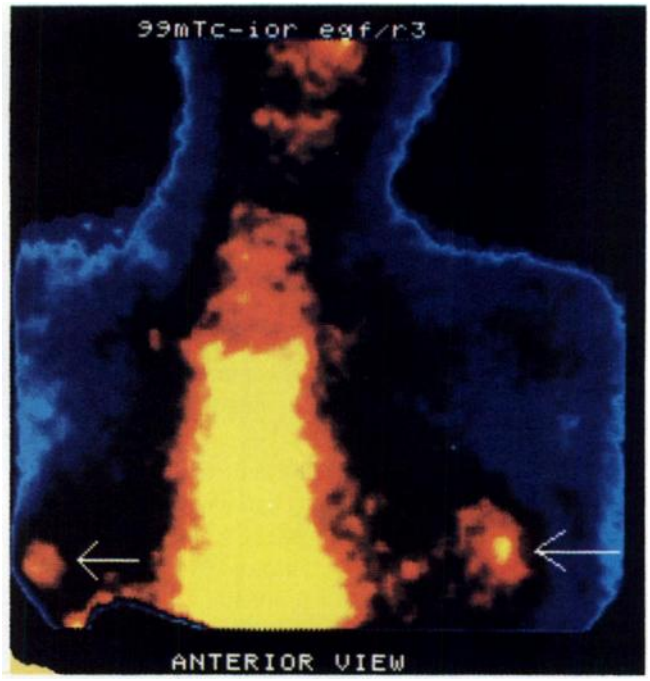

FIGURE 5. Anterior view taken 24 h after administration of radiopharmaceutical shows breast ductal carcinoma operated on previously. Recurrence and second primary breast tumor can be seen.

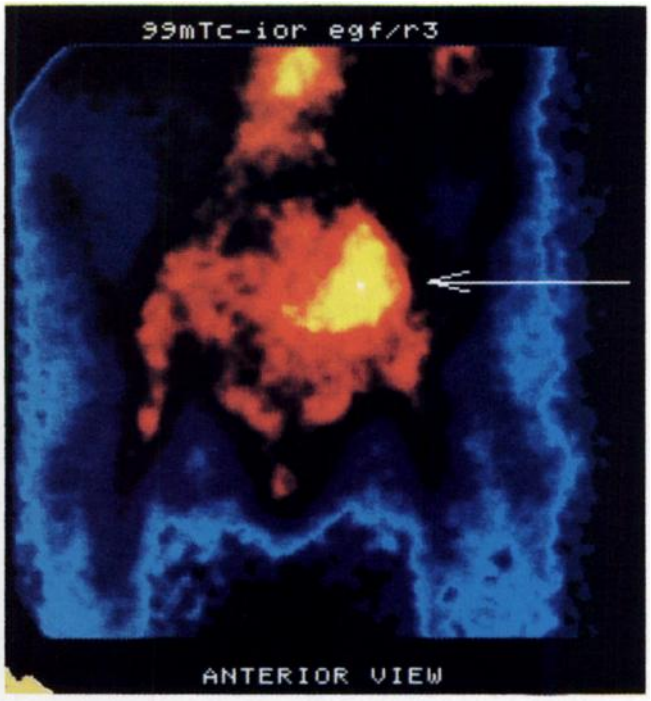

FIGURE 6. Anterior view of ^{99m}Tc-labeled moAb ior egf/r3 tumor uptake in patient with ovarian carcinoma previously confirmed by biopsy specimen.

ence of antigen (EGF-R) in the liver cells and other organs contributed to the rapid removal of the labeled moAb from the blood.

HAMA response was detected in 6 of 30 patients studied. This reaction was transient and disappeared before 8 wk postinjection. Only 1 patient developed high titer, which remained up to 12 wk after radiopharmaceutical administration. Previously used antiEGF-R moAbs have shown a higher HAMA response (*11,27,28*), except the moAb ICR62 (*29*), but it was a rat moAb. This low HAMA response could be related to the small amount of moAb administrated to the patients (3 mg), its rapid plasma clearance, the immunosuppression (secondary to the disease in advanced stages) and prior therapy.

Divgi et al. (*11*) used a labeled antiEGF-R moAb (^{111}In-225) to detect squamous cell lung carcinomas and indicated that antibody doses lower than 20 mg ($\approx 1.33 \times 10^{-7}$ mol) were ineffective for tumor localization. These authors suggested using even an additional amount of cold moAb to improve the sensitivity of the immunoscintigraphic diagnosis of the tumors due to plasma and normal tissue saturation. Modjtahedi et al. (*29*) also reported a relationship between the amount of free antibody in serum and the administrated dose of moAb ICR62. Nevertheless, the outcomes of this study showed the high capacity of the ^{99m}Tc-labeled moAb ior egf/r3 to detect primary tumors of epithelial origin, their metastases and recurrences (overall sensitivity of 84.1% and specificity of 100%), even though the amount of administrated antibody was relatively low (3 mg, $\approx 2 \times 10^{-8}$ mol). It could be related to the high affinity of this moAb to the EGF-R, the rapid internalization of the ^{99m}Tc-labeled moAb ior egf/r3-EGF-R complex and its translocation from the cytoplasm to the nucleus of cells.

EGF-R overexpression has been reported to be a frequent feature in breast (*30*), gastric (*31*), colorectal (*32*), laryngeal (*33*), thyroid (*34*), lung cancer (*8*) and other cancers. The increase of this receptor on the cell surface has been related to the malignancy of the disease, poor differentiation, degree of invasion and subsequently to the bad prognosis and short survival (*25,31,33,35*).

On the other hand, an interesting fact was increasing the sensitivity for the detection of epidermoid carcinomas with regard to adenocarcinomas. This feature is related to the different expression of EGF-R on the tumor cell membrane. Fontanini et al. (*8*) have described variability in the presence of this receptor on the cell surface according to the histologic classification of the malignancy.

Twenty patients with false-negative findings (Table 3), primarily those patients with lung cancer (n = 10) and with head and neck neoplasms (n = 6) were identified. Some authors (*31–33*) have reported an important heterogeneity in the EGF-R expression in different tumors of epithelial origin with immunohistochemical methods. However, some tumors do not express EGF-R (*31–33*). The presence of the receptor also depends on the staging of the disease and the histologic type as discussed previously. In addition, normal tissues (liver, heart, spleen, kidneys and others) near the tumor lesions could take up labeled antibody, and the detection of the neoplasms would be difficult. Unfortunately, it was not possible to determine immunohistochemically the expression of EGF-R in the tumors of false-negative patients. The observed specific uptake into tumor metastasis could be related to the high specificity and good internalization properties of this moAb. Macias et al. (*5*) observed a higher expression of EGF-R in metastases than in primary breast cancer. This fact could improve the diagnosis of occult metastatic lesions. The results showed a satisfactory correlation between sensitivity of the immunoscintigraphic imaging with ^{99m}Tc-labeled moAb ior egf/r3 and the expression of the EGF-R in tumors.

CONCLUSION

This clinical trial revealed the safety of the intravenous administration of ^{99m}Tc-labeled moAb ior egf/r3 for the patients studied. No adverse reactions and low HAMA response were noted.

The overall sensitivity was 84.1% and the specificity was 100%. There was a good correlation between histopathologic characterization of tumors through the biopsy specimens and the results of the clinical trial.

The murine moAb ior egf/r3 will be a useful radiopharmaceutical for the diagnosis of tumors of epithelial origin overexpressing EGF-R, their metastases and recurrences with immunoscintigraphic methods.

A controlled clinical trial will be necessary to establish the prognostic value of immunoscintigraphy with ^{99m}Tc-labeled moAb ior egf/r3 and the dependence of clinical sensitivity with the immunohistopathological classification of neoplasms of epithelial origin.

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