Clinical Immunoscintigraphy of Ovarian Carcinoma Using Iodine-131-Labeled 145-9 Monoclonal Antibody

June-Key Chung, Soon-Beom Kang, Hyo-Pyo Lee, Myung-Chul Lee, Chang-Soon Koh, Harumi Sakahara and Keigo Endo

Departments of Nuclear Medicine and Obstetrics and Gynecology, Seoul National University Hospital, Seoul, Korea; Department of Nuclear Medicine, Kyoto University Hospital, Kyoto, Japan; Department of Nuclear Medicine, Gunma University Hospital, Gunma, Japan

The monoclonal antibody (Mab) designated 145-9 recognizes CA125 antigen but binds to a different epitope than that recognized by OC125 antibody. This is a clinical study assessing the safety, kinetics and imaging sensitivity of Mab 145-9. Two milligrams of Mab were labeled with 111 MBq (3.0 mCi) of ^{131}I and infused intravenously in 18 patients with ovarian carcinoma. Immunoscintigraphies were done at three, five, and seven days. There were no adverse reactions to the injection of this Mab. All immunoscintigraphies were considered positive. Immunoscintigraphy detected tumor lesions were confirmed in operative fields, in two patients with normal serum levels of CA125 and in four patients whose sonography and/or x-ray computed tomography showed negative findings. In five patients, immunoscintigraphy was repeated without any adverse reaction and revealed the progress of the carcinoma. Pharmacokinetic studies showed the steady-state volume of distribution (Vdss) to be 2772 ± 466 ml (mean ± s.d.), and clearance 51.3 ± 12.7 ml/hr. In summary, immunoscintigraphies using ^{131}I-labeled Mab 145-9 were done safely in patients with ovarian carcinoma. Preliminary results reveal a high sensitivity compared to radiological methods and tests currently in use.

J Nucl Med 1993; 34:1651–1655

Malignant ovarian tumor poses one of the most serious problems in gynecology (*1*). Because the disease is often metastasized and widespread by the time of surgery, the prognosis is usually poor despite rigorous treatment. Because the tumors are often in the form of peritoneal seeding or matted to bowel or omentum, use of ultrasound or x-ray computed tomography (CT) is not very successful (*2*). Diagnosis of ovarian cancer ultimately requires exploratory laparotomy. Following chemotherapy, a second-look operation is usually performed to identify remnant tumor (*3*).

To solve these problems, imaging with radiolabeled monoclonal antibodies specific for antigens present in ovarian cancer cells has been introduced (*4*). Immunoscintigraphy has been tried using several monoclonal antibodies such as human milk fat globule (HMFG) glycoprotein, SM3, OC125, OV-TL3, and B72.3, etc. (*5–11*). However, immunoscintigraphy cannot totally replace laparotomy for diagnosing ovarian cancer at the present time (*12*).

This study used 145-9, a new monoclonal antibody directed against the CA125 antigen. An IgG2b antibody, 145-9 binds to a different epitope on CA125 than that recognized by OC125 antibody (*13*). A previous report showed distinct uptake of ^{131}I-labeled 145-9 antibody in human ovarian cancer xenografts in nude mice (*14*). We evaluated the feasibility of immunoscintigraphy employing ^{131}I-labeled 145-9 antibody in patients with advanced ovarian carcinoma.

MATERIALS AND METHODS

Patients

Eighteen patients between the ages 35 and 72 entered our study from January 1991 to May 1992. Twelve patients were diagnosed with ovarian cancer and had previously undergone surgery and chemotherapy before immunoscintigraphy. The remaining six patients were hospitalized for suspicion of ovarian cancer; all six were later confirmed to have ovarian carcinoma.

The pathologic types of carcinoma in the study patients were varied: eight had papillary serous adenocarcinoma, four had endometrioid carcinoma, two had unclassified carcinoma and the remaining four each had undifferentiated, clear cell, small cell and malignant Müllerian mixed tumor, respectively. In five patients, immunoscintigraphies were repeated a few months after the initial study.

Monoclonal Antibody

The murine monoclonal antibody 145-9 is an IgG2b antibody directed against the CA125 antigen. However, it binds to a different epitope on CA125 than the epitope recognized by conventional OC125 antibody. The 145-9 antibodies were generated by fusing myeloma cells and spleen cells of mice immunized with the human lung cancer cell PC-9. The affinity constant of 145-9 is 2.75 × 10^9 liter per *M* (*13*).

Labeling of the antibody with ^{131}I was performed with the

Received Jan. 15, 1993; revision accepted May 25, 1993.

For correspondence and reprints contact: June-Key Chung, MD, Department of Nuclear Medicine, Seoul National University Hospital, 28 Yungun-dong, Chongno-ku, Seoul 110-744, Korea.

TABLE 1
In Vitro Tests of Radiolabeled Antibody

Test	Result
Labeling efficiency	90.9%–99.8%
Immunoreactivity	52.8%–71.9%
Pyrogen test	Negative
Bacteria culture	Negative
Mycoplasma culture	Negative
Virus culture	
vero cell	Negative
MRC-5 cell	Negative

chloramine T method. Two milligrams of antibody were reacted with 111 MBq (3 mCi) of ^{131}I (New England Nuclear, Boston, MA) by adding 12.5 μg of chloramine T and stopped after 2 min by adding 43.8 μg of sodium thiosulfate. A PD-10 column (Pharmacia, Piscataway, NJ) was used to separate radiolabeled antibody from free ^{131}I. More than 90% of the purified antibody was precipitable with 10% trichloroacetic acid.

The immunoreactivity of the radiolabeled antibody was determined using a serial-cell binding assay. In 200 μl, 0.25 to 10 million SNU-8 human ovarian cancer cells, which express CA125, were reacted with 5 ng of radiolabeled antibody. Nonspecific binding was measured by adding 25 μg of unlabeled antibody. After a 2 hr incubation, all tubes were centrifuged to pellet the cells, which were then counted. Cell counts were expressed as a percentage of the total count corrected for nonspecific binding. The immunoreactivity was calculated by the double inverse plot, as described by Lindmo et al. (*15*). The antibody was tested for pyrogenicity (LAL test) and sterility before being injected into patients.

Scintigraphic Technique

Immunoscintigraphy was done after obtaining informed consent from each patient. Thyroid uptake of ^{131}I was blocked by oral administration of potassium perchlorate (500 mg/day) and potassium iodide (360 mg/day) for 7 days beginning from 1 day prior to injection of the radiolabeled antibody. Three, five and seven days after injection, static planar scintigrams were obtained using a large field-of-view gamma camera (ON 410, Ohio Nuclear Inc., Solon, OH) with a medium energy collimator linked to a Gamma-11 computer.

Antibody (111 MBq, 3 mCi) was infused into each patient. The radiolabeled antibody was given intravenously over a period of 5 min in an infusion of 20 ml of normal saline. Anterior images of the chest, abdomen and pelvis were recorded, each accumulating 200,000 counts.

To evaluate the pharmacokinetics of this antibody, serial blood samples were obtained before infusion and at 5, 30 min and 1, 2, 4, 8, 24, 48, and 72 hr intervals in six patients. Serial cumulated urine collections were obtained for 0 to 2 hr, 2 to 24 hr, 24 to 48 hr and 48 to 72 hr after infusion. We also measured the serum levels of CA125 and human antimouse antibody (HAMA) using a radioimmunometric assay kit (Centocor, Pennsylvania, PA), and a ELISA kit (Immunomedics, Warren, NJ), respectively.

RESULTS

The ^{131}I-labeled antibody was tested for pyrogenicity and sterility (bacteria, mycoplasma and viral cultures); all tests were negative. The range of immunoreactivity was between 52% and 72% (Table 1). No adverse reactions or change in vital signs were seen. Repeated studies were safely performed in five patients.

Results obtained from the patient study are summarized in Table 2. All immunoscintigraphies were considered positive. In two patients, serum levels of CA125 were normal (Patients 5 and 13) and in four patients, CT tomography and/or ultrasonography showed no abnormal mass (Patients 3, 4, 5, 11). However, in all five patients, immunoscintigraphy revealed abnormal increase in the uptake of antibody; in each, the presence of tumor was confirmed surgically. The smallest tumor confirmed by operation was 2 × 2 cm (Patient 3), but almost all tumors visualized in immunoscintigraphy were above 4 cm in diameter.

A typical ^{131}I-145-9 immunoscintigraphy image of localized ovarian cancer is shown in Figure 1 (Patient 8). A hot uptake was clearly visualized in the pelvic cavity; ultrasonography showed a cystic ovarian mass. Surgery confirmed this mass to be a serous adenocarcinoma. Diffuse uptake in the pelvis and lower abdomen is shown in Figure 2 (Patient 9); this patient's serum level of CA125 was very high: 2,180 U/ml (normal range: less than 35 U/ml).

Figure 3 shows recurrence of ovarian carcinoma (Patient 3); ovarian cancer was diagnosed surgically. This patient's serum level of CA125 was higher than normal (73 U/ml). Immunoscintigraphy showed hot uptakes on the right diaphragm, liver and abdomen. The CT image revealed no abnormal mass or lesion. Six months later, a CT scan revealed masses in the liver and abdomen.

Figure 4 shows an immunoscintigraph of a patient who had surgery for ovarian cancer. After debulking, cytoreductive surgery, the serum level of CA125 decreased to normal range. The CT scan showed no abnormality in the pelvic cavity or lower abdomen. However, immunoscintigraphy showed abnormal uptake in the pelvis. Second-look surgery confirmed the presence of tumor.

Analysis of the serum clearance curves provided the mean values for clearance (Cl = 51.3 ± 12.7 ml/hr; mean ± s.d.), mean residence time (MRT = 62.2 ± 12.2 hr), half-life ($T_{1/2\alpha}$ = 2.8 ± 1.8 hr, $T_{1/2\beta}$ = 45.0 ± 10.4 hr), volume of the central compartment (Vc = 2386 ± 522 ml) and steady-state volume of distribution (Vdss = 2772 ± 466 ml) (Table 3). The 145-9 antibody showed a two-compartment clearance. The volume of the central compartment was 2.38 liters, which was the approximate plasma volume of the patients. Within 3 days after antibody infusion, about half the radioactivity was excreted in the urine (Table 4).

The level of human antimouse antibody (HAMA) in the serum was measured in 10 patients within 8 wk after antibody infusion. Three patients (30%) showed positive reactions using an ELISA test.

DISCUSSION

There is no doubt that the prognosis of ovarian cancer is determined by its staging and grading and by an accurate assessment of disease status during and after therapy. Conventional imaging modalities, such as ultrasound, x-ray,

TABLE 2
Results from Investigated Patients

Patient	Age (yr)	Serum CA125 (U/ml)	RIS	Other Image	Means of Dx
1	69	253	pelvis, abdomen	CT(+)	Surgery
2	61	269	pelvis, abdomen	MRI(+)	Surgery
3	62	73	right diaphragm, abdomen, liver	CT(−)	Surgery
4(1)	49	198	pelvis, abdomen	CT(−)	Surgery
(2)		3980	pelvis, abdomen		
5(1)	52	5	pelvis	US(−)	Surgery
(2)		139	pelvis, abdomen	CT(−)	
6	58	243	pelvis, abdomen	MRI(+)	Surgery
7(1)	50	4243	pelvis, liver	US(+)	Surgery
(2)		92	pelvis, liver		
8	35	378	pelvis, liver	US(+)	Surgery
9	42	2180	pelvis, abdomen	US(+)	Surgery
10	56	369	pelvis	CT(+)	Surgery
11(1)	43	217	pelvis	CT(−)	Surgery
(2)		250	pelvis		
12	55	78	pelvis, liver	CT(+)	Concordance
13	55	5	pelvis, abdomen, liver	CT(+)	Surgery
14(1)	61	13168	pelvis, abdomen	US(+)	Concordance
(2)		428	pelvis, abdomen, liver		
15	51	500	pelvis, liver	CT(+)	Concordance
16	54	167	pelvis, abdomen	CT(+)	Concordance
17	72	1520	pelvis, abdomen	MRI(?)	Evolution
18	60	441	pelvis, abdomen	MRI(+)	Concordance

RIS = radioimmunoscintigraphy and Concordance = concordant results of US, CT and MRI.

CT and magnetic resonance image (MRI), have not had a dramatic impact on detection or management of patients with recurrent ovarian cancer. The main difficulty in assessing these images of patients with recurrent disease is due to gross abnormality of the pelvic anatomy resulting from previous surgery; differentiating between tumor and peritoneal adhesions is difficult (*10*). Second-look surgery is usually performed. For patients not examined surgically, the diagnosis of recurrence was considered highly probable based on significant elevations in serum tumor markers, particularly elevations in CA125 levels (*9*).

Immunoscintigraphy using monoclonal antibodies against these tumor markers has been successful in visualizing ovarian carcinoma. The main role of this technique is in the follow-up of ovarian cancer and in monitoring therapeutic response. The overall sensitivity in detecting ovarian carcinoma was 70% – 100%. The specificity, however, was 33% – 83% (*12*). Chatal et al. reported that false-negative results could be attributed to the small tumor size and the high percentage of necrotic tissue (*9*). Immunoscintigraphy may demonstrate tumor masses that are as small as 8 mm in diameter; this size is undetectable by other imaging techniques (*16*).

We were able to detect ovarian cancer in four patients in whom the tumor was not demonstrated by other x-ray

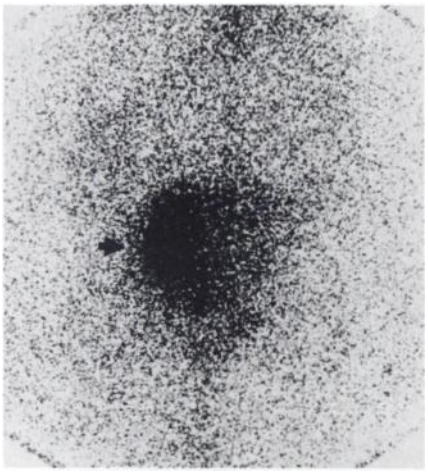

FIGURE 1.
Anterior image of the pelvis in a patient with serous ovarian carcinoma. The ^{131}I-145-9 image obtained 5 days following injection shows an abnormal concentration of radioactivity (arrow) adjacent to the bladder.

FIGURE 2.
Anterior image of the lower abdomen in a patient with multiple metastases at 3 days following antibody administration. Multiple abnormal foci of radioactivity can be seen in the abdominal cavity.

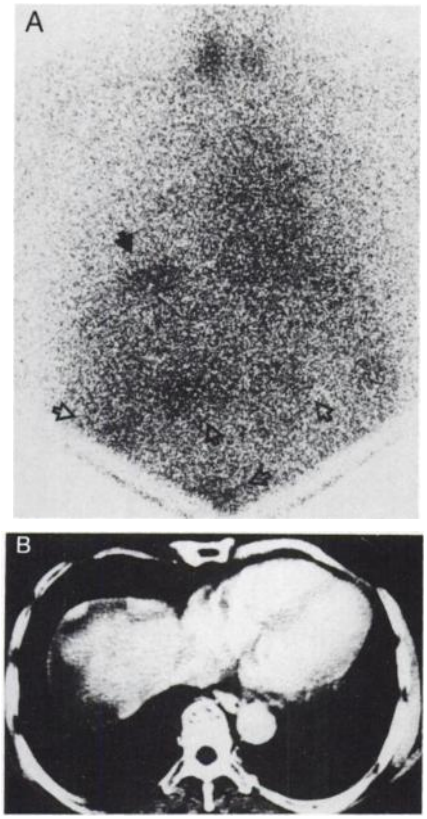

FIGURE 3.
Images of a patient with recurrent ovarian carcinoma. (A) Anterior view of the upper abdomen at 3 days postinjection shows abnormal uptake on the right diaphragm (solid arrow), liver and abdomen (empty arrows). (B) This recurrence was missed by CT scan and confirmed at laparotomy.

methods. Immunoscintigraphy findings correlated very well with subsequent laparotomy findings, when employed in established ovarian cancer patients. However, it produced more false-positive results when employed during the initial work-up of patients with pelvic mass (*6*).

Tumor sites can be demonstrated by immunoscintigraphy when rise in a serum marker is detected; however, it has been increasingly noted that tumor detection is also possible without such a rise in serum markers (*5*). We also had two cases in which the serum level of CA125 was in the normal range but the immunoscintigraphy showed positive uptake. Scan findings were confirmed to be accurate by subsequent surgery. We speculate that 1) some tumor cells express CA125 antigens on their cell surface without secreting them into the blood, or 2) CA125 antigen secreted into the circulation is diluted and is too low to be detectable as positive in serum CA125 levels.

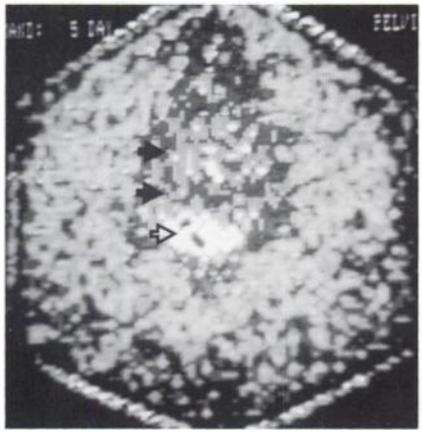

FIGURE 4.
Computer image of the pelvis in a patient with a normal level of serum CA125 at 3 days postadministration of ^{131}I-145-9. Abnormal uptake above the bladder was confirmed as a recurrence during second-look operation.

TABLE 3
Analysis of the Serum Clearance Curve

Parameter	Mean ± s.d.
Clearance	51.3 ± 12.7 ml/hr
MRT	62.2 ± 12.2 hr
T1/2α	2.8 ± 1.8 hr
T1/2β	45.0 ± 10.4 hr
Vc	2386 ± 522 ml
Vdss	2772 ± 466 ml

MRT: mean residence time; $T_{1/2}$: half-life; Vc: volume of the central compartment; Vdss: steady-state volume of distribution.

Human antimouse antibody (HAMA) was detectable in 30% of patients after infusion of 145-9 antibody, since 145-9 is a foreign mouse protein to human. Production of HAMA is also reported in patients after OC125 infusion and some are known as anti-idiotype antibody to CA125. In addition, HAMA may interfere in the determination of CA125 levels in serum.

CA125 is an ovarian cancer-associated antigen of a high-molecular-weight glycoprotein (*17*). Calcium 125 is the original antibody to this protein made by Bast et al. (*18*). The 145-9 antibody recognizes CA125 antigens but binds to determinants that are different from those recognized by OC125 antibody (*13*). The clinical utility of 145-9 antibodies might be the same as OC125 antibodies. However, 145-9 can be used as an alternative method to use of OC125. And anti-idiotype antibody to 145-9 may not interfere with determination of serum CA125 values in ovarian cancer patients after repeated infusion of 145-9 antibody.

This preliminary study shows the first clinical use of 145-9. There has been a recent evolution in the radiolabels used for immunoscintigraphy (*19*). Imaging using ^{111}In or ^{99m}Tc has shown to be of greater clinical value because of the improved image quality when compared with ^{131}I. Also, use of SPECT has the advantage of increasing contrast and providing three-dimensional localization of a focus and increasing the sensitivity of tumor detection (*20*). Antibody fragments are preferred for in vivo use since their clearance in normal tissue is faster than that of intact antibodies; however, clearance from tumors varies little using either antibody form (*21*).

We should be able to demonstrate the clinical usefulness

TABLE 4
Cumulated Excretion in Urine

Time	Mean ± s.d.
2 hr	0.90 ± 0.53% ID
24 hr	14.01 ± 4.67% ID
48 hr	33.75 ± 7.38% ID
72 hr	55.25 ± 11.30% ID

% ID: percent injected dose

of the 145-9 antibody by employing these advances in immunoscintigraphy.

SUMMARY

Eighteen patients with ovarian carcinoma showed positive uptake in immunoscintigraphy using ^{131}I-labeled 145-9 antibody. In five patients, repeated immunoscintigraphs were performed without any adverse reaction. Two patients showed normal serum levels of CA125 with positive uptakes of the antibody; tumor was confirmed in both. Immunoscintigraphy of ovarian carcinoma employing 145-9 was more sensitive in detecting tumor than CT or sonography. The 145-9 antibody has a promising potential for immunoscintigraphic detection of ovarian carcinoma. More detailed studies using ^{111}In or ^{99m}Tc labeled antibody with SPECT will follow to verify the clinical utility of 145-9 immunoscintigraphy in ovarian carcinoma.

ACKNOWLEDGMENTS

This study was supported in part by grant KOSET SRC-56-CRC-11 from the Cancer Research Center of the Korea Scientific and Engineering Foundation.

REFERENCES

1. Katz ME, Schwartz PE, Kopp DS, Luikait S. Epithelial carcinoma of the ovaries: current strategies. *Ann Intern Med* 1981;95:98–111.
2. Granowska M, Shepherd J, Britton KE, et al. Ovarian cancer: diagnosis using ^{123}I monoclonal antibody in comparison with surgical findings. *Nucl Med Commun* 1984;5:485–499.
3. Schwartz PE, Smith JP. Second-look operations in ovarian cancer. *Am J Obstet Gynecol* 1980;138:1124–1130.
4. Larson SM, Carrasquillo JA, Reynolds JC. Radioimmunodetection and radioimmunotherapy. *Cancer Invest* 1984;2:363–381.
5. Pateisky N, Phillipp K, Skooler WD, Ckerweuka K, Hamilton G, Burchel J. Radioimmunodetection in patients with suspected ovarian cancer. *J Nucl Med* 1985;26:1369–1376.
6. Shepherd JH, Granowska M, Britton KE, et al. Tumor-associated monoclonal antibodies for the diagnosis and assessment of ovarian cancer. *Br J Obstet Gynecol* 1987;94:160–167.
7. Burchell J, Genoller S, Taylor-Papadimitriou J. Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. *Cancer Res* 1987;47:5476–5482.
8. Buist MR, Roos JC, Golding RP, et al. Comparison of immunoscintigraphy ^{111}In-OVTL3-F(ab)$_2$ with other diagnostic methods in the detection of tumor deposits in ovarian carcinoma patients [Abstract]. *Antibody Immunoconj Radiopharm* 1990;3:41.
9. Chatal J-F, Fumoleau P, Saccavini JC, et al. Immunoscintigraphy of recurrence of gynecologic carcinomas. *J Nucl Med* 1987;28:1807–1819.
10. Perkins AC, Powell MC, Wastie ML, et al. A prospective evaluation of OC-125 and magnetic resonance imaging in patients with ovarian carcinoma. *Eur J Nucl Med* 1989;16:311–316.
11. Symonds EM, Perkins AC, Pimm MV, Baldwin RW, Hardy JG, Williams DA. Clinical implications for immunoscintigraphy in patients with ovarian malignancy: a preliminary study using monoclonal antibody 791 T/36. *Br J Obstet Gynecol* 1985;92:270–276.
12. Perkins AC, Pimm MV. Clinical role of immunoscintigraphy. In: Perkins AC, Pimm MV. *Immunoscintigraphy: practical aspects and clinical applications*. New York: Wiley-Liss; 1991:129–162.
13. Kunimatsu M, Endo K, Nakashima T, et al. Development of new immunoradiometric assay for CA 125 antigen using two monoclonal antibodies produced by immunizing lung cancer cells. *Ann Nucl Med* 1988;2:73–79.
14. Saga T, Ishiwata I, Endo K, et al. An antibody-tumor model for the targeting of CA 125-producing gynecologic malignancies. *Jpn J Cancer Res* 1990; 81:1141–1148.
15. Lindmo T, Boven E, Cuttitta F, Fedorko J, Bunn PA. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J Immunol Method* 1984; 72:77–89.
16. Epenetos AA, Shepherd JH, Britton KE, et al. ^{123}I radioiodinated antibody imaging of occult ovarian cancer. *Cancer* 1985;55:984–987.
17. Kabawat SE, Bast RC Jr, Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic epithelium related antigen recognized by the monoclonal antibody OC-125. *Int J Gynecol Pathol* 1983;2:275–285.
18. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 1981;68:1331–1337.
19. Granowska M, Britton KE, Mather SJ, Naeem M, Jobling T, Shepherd JH. Recent advances in the use of radiolabelled monoclonal antibodies in the management of ovarian cancer. In: Baum RP, Cox PH, Hor G, Buraggi GL, eds. *Clinical use of antibodies*. Dordrecht: Kluwer Academic Publishers; 1991:91–110.
20. Perkins AC, Whalley DR, Ballantyne KC, Pimm MV. Gamma camera emission tomography using radiolabelled antibodies. *Eur J Nucl Med* 1988; 14:45–49.
21. Wahl RL, Parker CW, Philpott GW. Improved radioimaging and tumor localization with monoclonal F(ab')$_2$. *J Nucl Med* 1983;24:316–325.