A Practical Approach to Monoclonal Antibody Imaging

LisaAnn Trembath, B. David Collier, and William J. Schulte

Medical College of Wisconsin, Milwaukee, Wisconsin

Working together as co-investigators on the professional staff at the Medical College of Wisconsin, the authors of this article have had over five years of experience in the diagnostic use of monoclonal antibodies (MoAbs) for colorectal and ovarian cancer. We would like to share with you our approach to those MoAb imaging studies. While such agents have great potential for diagnosis and therapy, at the same time there can be problems and pitfalls. We present a practical and organized approach, which is the secret to success when undertaking MoAb imaging procedures. A complete review of MoAb technology and its application to nuclear medicine is beyond the scope of this article. However, a number of authoritative articles dealing with this topic have been published recently* (1–12).

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Radionuclide-labeled monoclonal antibodies (MoAbs) for diagnosis and therapy are now available for use in clinical nuclear medicine laboratories. The biotechnology revolution, which is responsible for the new MoAb technology, has been discussed in scientific journals and has appeared in the popular press (*13–16*). Manufacturers are now shipping diagnostic MoAb products of proven clinical efficacy, while radionuclide-labeled MoAbs for tumor therapy are undergoing clinical trials. On December 30, 1992, the Food and Drug Administration (FDA) approved a diagnostic nuclear medicine MoAb (OncoScint CR/OV, Cytogen Corp., Princeton, NJ) for clinical use in the United States. Referring physicians, including surgeons, medical oncologists, and radiation oncologists, will be sending requests to nuclear medicine departments for MoAb examinations of patients with colorectal or ovarian cancer.

MoAbs offer the opportunity to emphasize the chemical and disease-specific, as opposed to the purely anatomic, aspects of medical diagnosis. MoAbs react specifically with the antigens that are produced or exposed in morbid states. When labeled with radioisotopes, the MoAbs can be used as tracers to identify otherwise hidden sites of disease within the body.

For reprints contact: LisaAnn Trembath, CNMT, Medical College of Wisconsin, Clement J. Zablocki VA Medical Center, Nuclear Medicine Service 115, 5000 W. National Avenue, Milwaukee, WI 53295.

A variety of radionuclides have been used to label MoAbs. The first MoAb agents to be approved by the FDA for use in the United States are labeled with indium-111 (^{111}I). Some research formulations initially used iodine-131 (^{131}I). In the future technetium-99m (^{99m}Tc) may be commonly employed in clinical MoAb imaging studies. In addition, a variety of MoAb radionuclide conjugates suitable for tumor therapy undoubtedly will be introduced for clinical use: ^{131}I, rhenium-186 (^{186}Re), yttrium-90 (^{90}Y), and other particle-emitting radionuclides have been suggested for such therapeutic applications (*17*).

MOAB IMAGING OF OVARIAN AND COLORECTAL CANCER

Radionuclide-labeled MoAbs frequently are referred to as being the "magic bullet" that will search out sites of tumor anywhere in the body. Clinical research protocols for ovarian and colorectal cancer, which use a ^{111}In-labeled radioimmunoconjugate of B72.3, have been carried out at our institution. B72.3 is an immunoglobulin 1 (IgG) class MoAb, which, when conjugated with ^{111}In, is referred to as ^{111}In-CYT-103 (OncoScint CR/OV, Cytogen Corp., Princeton, NJ). B72.3 is an antibody to tumor-associated glycoprotein (TAG-72), which is found in over 90% of ovarian and colorectal carcinomas (*18*).

Following intravenous injection of ^{111}In-CYT-103, the radioimmunoconjugate circulates throughout the body with localization at vascularized sites of TAG-72-rich tumors. While localization begins within minutes following injection, a minimum of two, and as many as five, days are required before uptake at the tumor site and clearance of background activity provide favorable contrast for nuclear medicine imaging. The 2.8 day half-life of ^{111}In makes it ideal for imaging conducted between two and five days postinjection: such delayed imaging would not be possible with shorter-lived isotopes such as ^{99m}Tc, which has a half-life of 6 hr.

MATERIALS AND METHODS

^{111}In-CYT-103 Radiopharmacy

CYT-103 comes in kit form suitable for user-friendly labeling with ^{111}In-chloride. The kit provides 1 mg of antibody

complex, sodium citrate buffer, and a Millipore filter (Millipore Corp., Bedford, MA). To avoid degrading the MoAb protein, the kit must be refrigerated, but not frozen, during shipment and at the time it is received. The 5–6 mCi of ^{111}In-chloride to be used for labeling the CYT-103 must first be buffered with 1 ml of sodium acetate solution. If, through error, this buffering procedure is not performed, or if the buffer is added to the antibody complex and not to the radioisotope vial, the ^{111}In may form an insoluble species upon contact with the antibody and should not be used clinically. In addition, the antibody may be damaged by exposure to nonbuffered ^{111}In.

After buffering, the ^{111}In-chloride solution is added slowly to the antibody vial and allowed to incubate at room temperature for 30 min. The manufacturer-recommended steps for reconstitution can be performed within 45 min, including the 30-min incubation.

Note that ^{111}In-CYT-103 is a murine protein that can form particles, and, therefore, filtration of the dose before injection is required. Filtration is performed using a 0.22-μ filter that is attached to a syringe. We recommend having additional filters available. If the filter becomes air-locked, no additional liquid can be drawn through the filter, and the filter-needle attachment must be replaced with a fresh filter to continue drawing up the dose. Air-locking occurs when the bevel of the needle is raised above the liquid level. Some radioactivity is lost in the filtering process; therefore, beginning the procedure with 5–6 mCi is recommended in order to produce a dose of 4.5–5.5 mCi.

No antibacterial preservatives are included in the preparation, and the dose should be used within 8 hr of preparation. The dose may be stored at room temperature after reconstitution.

An optional instant thin layer chromatography (ITLC) procedure is available. To test radiochemical purity, one drop of the ^{111}In-CYT-103 dose is mixed with an equal amount of diethylenetriamine pentaacetic acid (DTPA). (To prepare DTPA for this procedure, add 1 ml sterile water to a commercial DTPA kit.) The DTPA-treated antibody is then used to spot an ITLC-SG strip which is placed in a beaker with normal saline. After the solution migrates for 2–4 min, the strip is cut in half, and each half is measured. Free ^{111}In will bind to the DTPA and travel to the top of the strip, while the labeled CYT-103 will remain at the bottom. Therefore, radiochemical purity is equal to the bottom net counts divided by the total net counts and multiplied by 100. Radiochemical purity must be greater than 90% for patient doses.

TABLE 1. Questionnaire for Patients Undergoing Monoclonal Antibody Imaging

1. Do you have allergies to any medication? If so, what medication?
2. Have you ever been exposed to medicine made out of mouse proteins? Have you had a monoclonal antibody scan before?
3. Do you have a colostomy?
4. Have you had any surgery of the chest, abdomen, or pelvis? If so, what type and when?

TABLE 2. Potential Adverse Reactions to Monoclonal Antibody Injections

Most Frequent Adverse Reactions (less than 4% of patients)
Fever, chills, hypotension, hypertension, itching, rash, or sweating

Infrequent Adverse Reactions
Chest pain, dizziness, headache, nausea, flushing, hypothermia angioedema, temporary joint pain, or tenderness

Potential Serious Adverse Reactions
Anaphylaxis, serum sickness with fever, urticaria, lymphadenopathy, arthralgias, or renal dysfunction.

Patient Preparation and Technique for Injection of ^{111}In-CYT-103

No special patient preparation, such as dietary or medication restrictions, are necessary during the days before commencement of a ^{111}In-CYT-103 MoAb examination. Interpretation of images, however, requires some knowledge of patient history. For example, colostomy sites are usually "hot" on ^{111}In-CYT-103 images, and should be marked on the film. Also, recent surgical scars often display increased uptake. Because the appearance of these phenomena on film may lead to an incorrect interpretation of the images, a patient interview is recommended. Table 1 gives a sample patient questionnaire. A bowel preparation, such as magnesium citrate, is recommended before the first imaging session, in order to distinguish abnormal tumor activity from normal bowel uptake.

TABLE 3. Suggested ^{111}In-CYT-103 Planar Imaging Protocol

Step	Detail
Time postinjection	Twice between 2 and 5 days postinjection
Collimator	Medium energy
Energy	173 and 247 keV photopeaks
Views	Anterior and posterior, chest, abdomen, pelvis (Laterals may be required, especially if SPECT is not performed)
Time per view	10 min
Matrix	256×256 for a 500-mm field of view 128×128 for a 400-mm field of view
Patient Preparation	Bowel cleansing prep given before first imaging session; colostomy and urine bags should be changed immediately before imaging

Hints

1. Mark colostomy sites on film with lead or ^{111}In marker.
2. Pelvis view should be acquired with only inferior edge of liver in field of view.
3. Neck should remain straight (no pillow) for chest views.
4. Position patient the same way for both imaging sessions.
5. When filming planar views, lower the upper grayscale threshold enough to see vascular structures. (Liver activity will be very intense.)

The 4.5–5.5 mCi of ^{111}In-CYT-103 is slowly infused over 5 min. A physician prepared to handle allergic reactions should be in attendance or readily available, and appropriate medications (i.e., epinephrine) should be available. At our institution, we perform the injection using a butterfly I.V. setup equipped with a stopcock and saline flush. This allows for ready venous access in the unlikely event of an allergic reaction. To allow for slow progressive infusion over 5 min, we dilute the ^{111}In-CYT-103 dose with saline from the other stopcock port.

In the initial clinical trial of ^{111}In-CYT-103, there were no serious life-threatening reactions, and minor allergic reactions or other responses occurred in less than 4% of patients (Table 2) (*19*). Nonetheless, as is the case with all injections of diagnostic products that may produce a potential serious allergic response, appropriate medical precautions are warranted. We view injection of ^{111}In-CYT-103 in much the same light as the potential for serious complication from injection of iodinated X-ray contrast agents. Physician supervision, medications for treatment of allergic reactions, and equipment for patient support are always readily available.

TABLE 4. Suggested ^{111}In-CYT-103 SPECT Protocol

Acquisition

Step	Detail
Time postinjection	48–120 hr
Collimator	Medium energy
Energy	173 and 247 keV photopeaks
Orbit	Elliptical stepwise, 360°
Time per frame	40 sec
Number of frames	64
Matrix	128×128 for a 500-mm field of view (64×64 can be used with smaller field of view)

Hints

1. Time per frame can be reduced to 30 sec/frame for patients who are agitated or in pain.
2. Use pillows and blankets for patient comfort; avoid patient motion.

Processing

Step	Detail
Filter	Prereconstruction Butterworth filter (cut-off frequency of 0.3 to 0.4 cycles/pixel and power factor of 10)
Attenuation Correction	None
Slice thickness	3 pixels for 128×128 matrix and 500-mm field of view, or 2 pixels for 64×64 matrix and 400-mm field of view
Planes	Transaxial, sagittal, and coronal tomograms are reconstructed and filmed

Hints

1. When filming SPECT of the pelvis, adjust the upper threshold to visualize vascular structures. It may be helpful to determine intensity on coronal slices first.
2. When first doing monoclonal imaging, try several filters in the 0.3 to 0.4 cycles/cm range. Processing parameters are dependent on count density and equipment performance.

TABLE 5. Common Technical Imaging Problems

Problem	Solution
Colon activity	To identify colon activity, compare early and late, e.g., 48- and 96-hr images; bowel prep before initial image is recommended
Activity at colostomy site or urine collection bag site	Mark site on planar images with a ^{111}In or lead marker; collection bags should be changed before each imaging session; acquire lateral images
Bladder activity obstructing possible pelvic lesions	Have patient void completely before imaging
High liver activity	Inherent to ^{111}In antibody imaging; acquire pelvic view with only bottom edge of liver in view; threshold images to view vascular structures
High body background	Delayed imaging required; recommended times are 48–96 hr postinjection; images have been obtained out to 144 hr
Renal activity	Delayed imaging reduces amount of blood-pool activity in kidneys
Long imaging times	Make patient as comfortable as possible; use pillows under knees to relieve strain on lower back; support the arms with straps and arm boards if necessary; allow patient to sit up or walk around between planar and SPECT imaging
Poor-quality SPECT images	Patient motion is the most frequent cause of poor-quality SPECT; ask patients to lie very still on the table; explain the importance of remaining motionless
Uptake at sites of recent surgical incision	Obtain patient history with emphasis on previous surgeries; if SPECT imaging is not performed, acquire lateral view

Protocol for Imaging with ^{111}In-CYT-103

At 2–5 days following injection, ^{111}In-CYT-103 patients are imaged twice (Tables 3–5). Using a large field of view gamma camera, 10-min planar images of the anterior and posterior chest, abdomen, and pelvis are obtained. With a 500-mm field of view camera, the chest, abdomen, and pelvis can usually be adequately visualized with two planar views. If a smaller field of view is used, three separate images are

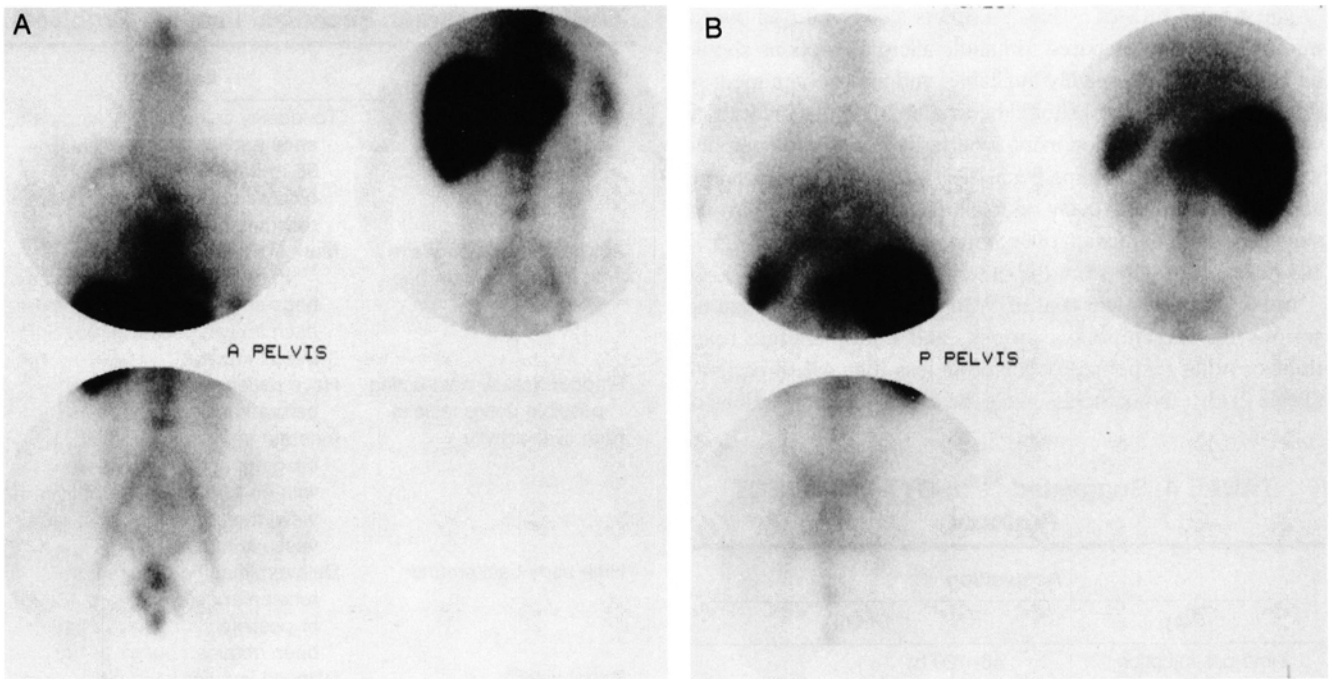

FIG. 1. Normal (A) anterior and (B) posterior ^{111}In-CYT-103 planar images of chest, abdomen, and pelvis in 40-yr-old man with history of colorectal cancer. Note normal physiologic uptake of tracer in liver, spleen, bone marrow, cardiac blood pool, iliac vessels, and genitalia.

recommended. The pelvic view is considered to be the "highest yield" image, i.e., most recurrences of ovarian and colorectal cancer occur in the pelvis and lower abdomen. ^{111}In-CYT-103 images are characterized by a much higher count density in the liver than in extrahepatic regions.

We recommend positioning the pelvic view with only the bottom edge of the liver in the field of view and adjusting the intensity enough to see vascular structures. The planar examination is supplemented with at least one single-photon emission computed tomography (SPECT) study, usually of the pelvis. Figure 1 shows planar images in a normal patient, 72-hr postinjection of ^{111}In-CYT-103. Figure 2 is an example of a positive ^{111}In-CYT-103 scan, using both planar imaging and SPECT.

Acquisition and processing parameters for SPECT are dependent on the type of imaging equipment that is being used. Regardless of the equipment used, SPECT images are characterized by a relatively low count density and therefore the need for long imaging times. In addition, it is important to add 2 or 3 slices together and adequately filter the data.

DISCUSSION

Clinical Utility of ^{111}In-CYT-103

For patients with colorectal cancer, ^{111}In-CYT-103 imaging may be of particular value in the following situations.

- A rising CEA tumor marker and no other imaging studies which localize the recurrent tumor
- Equivocal computed tomography (CT) or magnetic resonance imaging (MRI) findings of recurrent disease, which may, in fact, represent fibrosis, edema, or tumor
- Suspected isolated disease that will not be treated surgically if a ^{111}In-CYT-103 scan shows additional metastatic sites
- Occult widely disseminated disease

For patients with ovarian cancer, ^{111}In-CYT-103 imaging may be of value in the following circumstances.

- Detection of diffuse "miliary" disease in the abdominal cavity, which cannot be imaged using CT or ultrasound
- A rising CA-125 tumor marker and no other imaging studies which localize the recurrent tumor
- Suspected isolated disease that will not be treated surgically if a ^{111}In-CYT-103 scan shows additional metastatic sites
- Occult widely disseminated disease

Reports in the scientific literature indicate that ^{111}In-CYT-103 frequently will detect sites of disease that were not clinically obvious and could not be imaged using CT (*10,19,20*). Thus, the ability of radionuclide-labeled MoAbs to detect malignancies, such as colorectal and ovarian cancer, based on the presence of tumor antigens converts oncologic diagnosis from a radiographic study in anatomy into a nuclear medicine study in tumor chemistry.

Human Anti-Murine Antibody (HAMA)

CYT-103 and virtually all other MoAbs under investigation for medical diagnosis are produced by cells that come from mice. Because the antibody is based on murine rather than human proteins, the immune system in the human recipient of the MoAb injection may recognize the injected

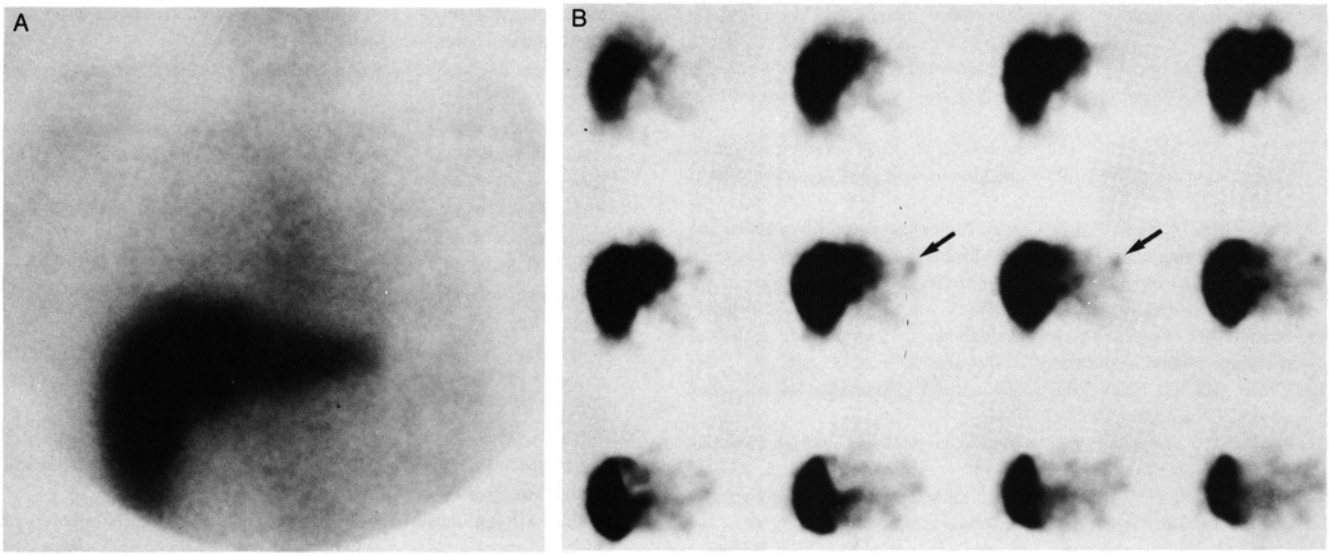

FIG. 2. Images of 42-yr-old man, status post transverse colectomy and splenectomy for colon carcinoma. Computed tomography (CT) scan 1 yr after surgery shows new finding of 3-cm metastasis centrally located in right lobe of liver. ^{111}In-labeled CYT-103 study was undertaken to identify any extrahepatic metastases in patient who was candidate for resection of hepatic lesions. (A) Anterior view planar image of chest and upper abdomen at 120 hr in addition to all other planar views was normal. (B) Sequential transaxial SPECT images through liver and upper abdomen show faint but definitely abnormal uptake in left upper quadrant (arrows). CT did not identify left upper quadrant tumor, but extrahepatic metastatic disease at this site was confirmed at time of laparotomy. (Reprinted by permission of Reference 8.)

protein as foreign and mount a human antibody response. Therefore, within weeks following injection of the murine MoAb, there is a human antibody response known as human anti-murine antibody (HAMA). This HAMA response has a number of important clinical implications.

First, a HAMA response, in theory, makes an allergic response to a second MoAb injection more likely. However, this has not to date been reported with ^{111}In-CYT-103. Second, a HAMA response, in theory, makes a successful second MoAb imaging procedure less likely. Third, a HAMA response may interfere with murine protein-based serum assays. In particular, the frequently used tumor markers carcinoembryonic antigen (CEA) and cancer antigen 125 (CA-125), when performed using some standard laboratory procedures, may no longer give valid results. Special techniques such as heat inactivation can be used to obtain accurate CEA and CA-125 assay values in patients with a HAMA response (*21*). Other options include using only nonmurine kits for these assays. However, the laboratory personnel must be aware of the potential HAMA response to MoAb so that they can take appropriate corrective measures.

CONCLUSION

The biotechnology revolution, which created MoAb imaging, has passed from a purely research phase into the early clinical phase. Executing the new MoAb imaging protocols will place additional professional demands on nuclear medicine physicians and technologists. The first approved MoAb for imaging human tumors, ^{111}In-CYT-103, requires ^{111}In-chloride labeling, filtration, and buffering during preparation. Both planar and SPECT MoAb imaging protocols demand relatively long imaging times when compared to the times required for ^{99m}Tc-based products. In executing these clinical protocols, an organized approach, which emphasizes attention to detail such as marking surgical sites and thorough bowel preparation, is the secret to success.

The detailed anatomic display provided by radiologic techniques such as CT and MRI tends to mask the specificity limitations of such imaging procedures. The highly specific chemistry of imaging tumor-associated antigens has now been probed with MoAb technology. MoAbs antibodies have the potential to convert oncologic diagnosis from a radiographic study in anatomy to a nuclear medicine study in chemistry. While challenging, arduous, and sometimes frustrating, the MoAb revolution has already created unique opportunities for oncologic imaging of colorectal and ovarian carcinomas.

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REFERENCES

1. Bogard WC, Dean RT, Deo Y, et al. Practical considerations in the production, purification, and formulation of monoclonal antibodies for immunoscintigraphy and immunotherapy. *Semin Nucl Med* 1989;19:202–220.
2. Chatal JF, Peltier P, Bardies M, et al. Does immunoscintigraphy serve clinical needs effectively? Is there a future for radioimmunotherapy? *Eur J Nucl Med* 1992;19:205–213.
3. Serafini AN, Vargas-Cuba R, Benedetto P, et al. Clinical experience in utilizing radiolabeled monoclonal antibodies. *Antibod Immunoconj Radiopharmaceuticals* 1991;4:77–83.
4. Serafini AN, Garty I, Jabir AM, et al. Monoclonal antibody imaging, clinical and technical perspectives. *Antibod Immunoconj Radiopharmaceuticals* 1989;2:225–234.
5. Britton KE, Granowska M, and Mather SJ. Radiolabeled monoclonal

antibodies in oncology I. Technical aspects. *Nucl Med Comm* 1991;12: 65–76.
6. Granowska M and Britton KE. Radiolabeled monoclonal antibodies in oncology II. Clinical applications in diagnosis. *Nucl Med Comm* 1991;12: 83–98.
7. Britton KE, Mather SJ, and Granowska M. Radiolabeled monoclonal antibodies in oncology III. Radioimmunotherapy. *Nucl Med Comm* 1991; 12:333–347.
8. Collier BD, Trembath L, Liu Y, et al. A practical approach to planar and SPECT imaging of In-111-CYT-103. In: Maguire RT, Van Nostrand D, eds. *Diagnosis of colorectal and ovarian carcinoma. Application of immunoscintigraphic technology.* New York: Marcel Dekker; 1992:191–210.
9. Serafini AN. From monoclonal antibodies to peptides and molecular recognition units: an overview. *J Nucl Med* 1993;34:533–536.
10. Collier BD and Foley WD. Current imaging strategies for colorectal cancer. *J Nucl Med* 1993;34:537–540.
11. Krag DN. Clinical utility of immunoscintigraphy in managing ovarian cancer. *J Nucl Med* 1993;34:545–548.
12. Texter JH and Neal CE. Current applications of immunoscintigraphy in prostate cancer. *J Nucl Med* 1993;34:549–553.
13. Unger M. New drug hastens 2 cancers' diagnosis. *New York Newsday*, January 12, 1993.
14. Marchione M. New cancer test may aid detection, area researchers say. *The Milwaukee Journal*, April 21, 1993.
15. Waldmann TA. Monoclonal antibodies in diagnosis and therapy. *Science* 1991;252:1657–1662.
16. Brown BA, Comeau RD, Jones PL, et al. Pharmacokinetics of the monoclonal antibody B72.3 and its fragments labeled with either ^{125} or ^{111}In. *Cancer Res* 1987;47:1149–1154.
17. Bhargava KK and Acharya SA. Labeling of monoclonal antibodies with radionuclides. *Semin Nucl Med* 1989;19:187–201.
18. Thor A, Ohuchi N, Szpak CA, et al. Distribution of onco-fetal antigen tumor-associated glycoprotein-72 defined by monoclonal antibody B72.3. *Cancer Res* 1986;46:3118–3124.
19. Collier BD, Abdel-Nabi H, Doerr RJ, et al. Immunoscintigraphy performed with In-111-CYT-103 in the management of colorectal cancer: comparison with CT. *Radiology* 1992;185:179–186.
20. Doerr RJ, Abdel-Nabi H, Krag D, et al. Radiolabeled antibody imaging in the management of colorectal cancer. Results of a multicenter clinical study. *Ann Surg* 1991;214:118–124.
21. Hansen HJ, LaFontain G, Newman ES, et al. Solving the problem of antibody interference in commercial "sandwich"-type immunoassays of carcinoembryonic antigen. *Clin Chem* 1989;35:146–151.