Radioimmunoscintigraphy: A Clinical Perspective

Patricia J. Perentesis, Penney S. Yolles, and Jorge A. Carrasquillo

National Institutes of Health, Bethesda, Maryland

This is the second in a series of Continuing Education articles related to developing radiopharmaceuticals. After reading this article the reader should be able to: 1) discuss the clinical applications of monoclonal antibodies; 2) understand the principles of antibody technology; and 3) recognize the problems associated with this procedure.

The use of radiolabeled antibodies for tumor localization was first suggested by Pressman and Korngold in 1953 (*1*). The initial human imaging studies with polyclonal antibodies met with limited success (*2*). Although some investigators have reported excellent sensitivity (*3*) for tumor detection with polyclonal antibodies, the image quality has been marginal and the results have not been reproducible (*4–6*).

In 1975, Kohler and Milstein succeeded in creating an immortal cell (hybridoma) capable of producing an antibody of predetermined specificity in unlimited quantities (*7*). To produce the hybridoma, mice are immunized with tumor cells, i.e., antigens. The mouse immune system responds to these foreign antigens and antitumor antibodies are produced by their lymphocytes. The spleen is excised and minced in order to obtain a single cell suspension of lymphocytes. Lymphocytes can survive but cannot grow in cell culture. These lymphocytes are harvested and then mixed with mouse myeloma cells. The mouse myeloma cells, which are derived from a mouse cancer, are "immortal" and in addition have been selected for an enzyme deficiency (hypoxanthine phosphoribosyl transferase). When these enzyme-deficient cells are grown in special media (HAT media) the myeloma cannot produce the necessary nutrients to survive and will die out. A fusing agent is added to the mixture of lymphocytes and myeloma cells and three cell populations result: 1) the unfused lymphocytes, which cannot grow in culture; 2) the unfused myeloma cells, which cannot grow in HAT media; and 3) the fused cells, i.e., hybridoma that have complimentary characteristics from each cell and that can grow in culture producing large amounts of antibodies. The fusion product (hybridoma) of a single antibody forming cell and a tumor cell will have the ability to secrete a single species of antibody, and the immortality to enable it to proliferate continuously. This provides an unending supply of antibody with a single preselected specificity (Fig. 1).

Monoclonal antibody (MoAb) technology has led to the discovery of a host of tumor-associated antigens on human tumors and to the production of a large amount of well-characterized, immune-specific antibody. The ability to have a well-defined, reproducible antibody in large amounts with little variability between lots has rekindled the interest in the radioimmunoscintigraphy (RIS) technique (*8–13*).

Currently available nuclear medicine and radiographic procedures depend on nonspecific findings such as mass effect, ultrasound reflections, differences in density, paramagnetic properties, and metabolism. The RIS technique is a new field in nuclear medicine, which for the first time provides specific diagnostic reagents which have potential for the diagnosis, staging, and follow-up of tumors.

PRECLINICAL EVALUATION

The development of a MoAb for human use involves many time consuming and labor intensive steps. Extensive in vitro characterization of the antigen and antibody are usually performed, including the nature, location, and abundance of the

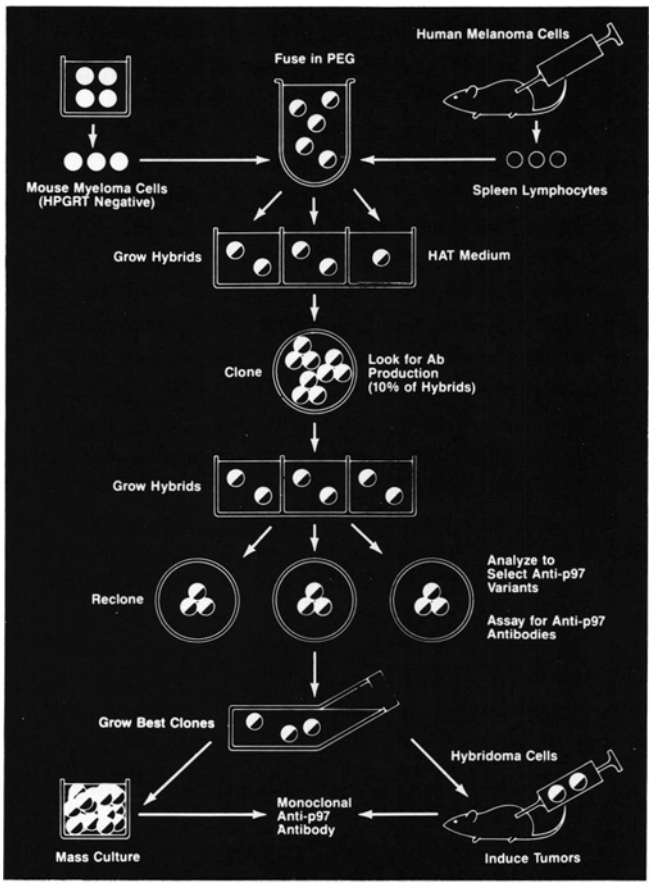

FIG. 1. Overview of MoAb production.

For reprints contact: Patricia Perentesis, Nuclear Medicine, National Institutes of Health, 9000 Wisconsin Ave., Bethesda, MD 20814-3810.

antigen, as well as binding characteristics of the antibody to tumor, reactivity to normal tissue, and antibody affinity. If the specificity and other characteristics look promising, then radiolabeling is performed. A variety of isotopes are being evaluated for imaging, including technetium-99m (^{99m}Tc), iodine-131 (^{131}I), iodine-123 (^{123}I), and indium-111 (^{111}In). In addition, several labeling methods are being investigated (*14–16*). Once the antibody is radiolabeled, it undergoes extensive testing to determine that it retains its ability to bind to the antigen, and animal studies are conducted to determine in vivo targeting. If these studies suggest the potential for clinical trials the labeled MoAb must then be approved for human use by the Food and Drug Administration prior to commencing clinical trials.

Before starting human trials the clinical protocols are reviewed and approved by both the Human Research Committee and the Radiation Safety Committee.

CLINICAL TRIALS

Following the above evaluation schema for development of MoAb for clinical use, investigators at the National Institutes of Health (NIH) have designed a diagnostic protocol for imaging colon carcinoma metastases with ^{131}I B72.3 MoAb. This protocol is representative of those used to test labeled monoclonal antibodies with clinical potential. B72.3 is an immunoglobulin G_1 (IgG_1) monocloncal antibody directed at a high molecular weight glycoprotein (mucin) present on 90% of colon cancers and has little cross reactivity with normal tissue (*17–19*).

The major goals for this clinical trial were to: 1) determine if any toxicity is associated with the administration of ^{131}I-labeled B72.3 at various doses and specific activities, 2) determine if radiolabeled B72.3 will selectively bind to carcinoma lesions versus normal tissue in patients with metastatic colorectal carcinoma, 3) optimize conditions for the use of B72.3 MoAb to detect occult carcinoma lesions via radioimmunoscintigraphy, and 4) potentially use high doses of ^{131}I-labeled B72.3 in clinical trials for therapy in carcinoma lesions for colorectal cancer.

B72.3 was labeled with ^{131}I (iodogen) with good retention of immunoreactivity. The antibody was administered as a 1-hr intravenous infusion. Patients were studied at a dose ranging from 0.27 to 20 mg and 1.1 to 10 mCi ^{131}I in order to assess the effect of B72.3 dose on biodistribution as well as the effect of improved counting statistics on the scintigrams. Fourteen of 27 patients had positive scans. Optimal images were obtained 3 days after infusion when background levels decreased. The scans showed no concentration in normal organs (Fig. 2). The specificity of targeting was documented by gamma well counting of surgical specimens obtained from patients coinfused with both ^{131}I and a nonspecific control antibody. The limited sensitivity for tumor detection is likely to be secondary to several factors, including those related to antibody delivery, antigen accessibility, tumor size, and dehalogenation.

Colon cancer will often metastasize to the peritoneal surface, and in a group of patients this may be the only site of recurrence. To determine if there was an advantage of direct delivery of B72.3 into the peritoneum, a study was performed administering ^{131}I intraperitoneally (IP) and ^{125}I intravenously (IV) to four patients. All patients subsequently underwent surgery. Surgical specimens counted in a gamma well counter showed preferential targeting of IP administered B72.3. In order to determine if this uptake was tumor specific five patients received IP coinfusions of ^{131}I B72.3 and ^{125}I BL3, a nonspecific MoAb. Gamma well counting of surgical specimens from these patients showed tumor-specific uptake with ratios of up to 100 to 1. Tumor uptake was seen in 9 of 12 scans and in all patients with pseudomyxoma peritoneii (7 of 7). In three patients the MoAb scan was the only evidence of metastatic disease. These preliminary studies suggest the potential of this route for therapy of IP carcinomatosis.

The NIH group has evaluated patients with cutaneous T-cell lymphoma (CTCL) (mycosis fungoides of Sezary syndrome) using T101 MoAb. Evaluation of extracutaneous disease in CTCL has been particularly difficult due to problems in histologic interpretation of tissue biopsy specimens and a reluctance to perform invasive procedures in these indolent lymphomas. T101 is a murine MoAb IgG_{2a}, directed against a cell surface pan T-cell antigen present in high concentrations in CTCL (*20*). This antibody* was labeled with ^{111}In via the

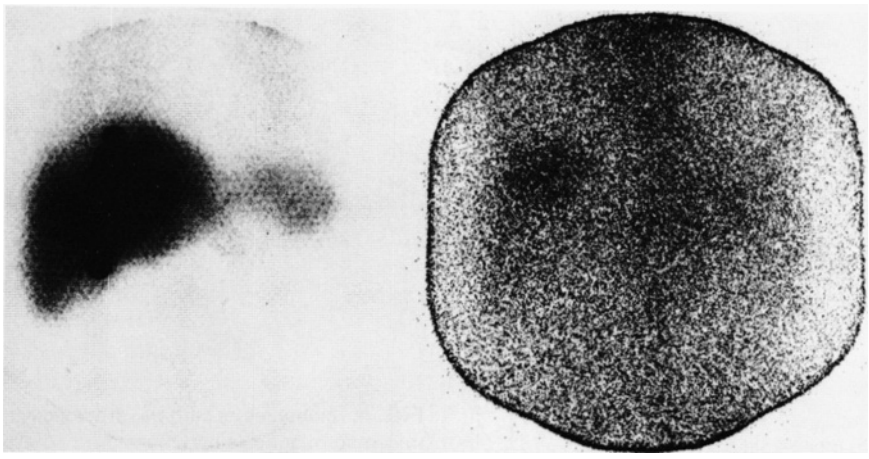

FIG. 2. Anterior abdominal views of a ^{99m}Tc sulfur colloid scan (left) and a MoAb scan using ^{131}I B72.3 (right). The tumor appears as a "cold" area in the sulfur colloid image and as a "hot" area in the MoAb image.

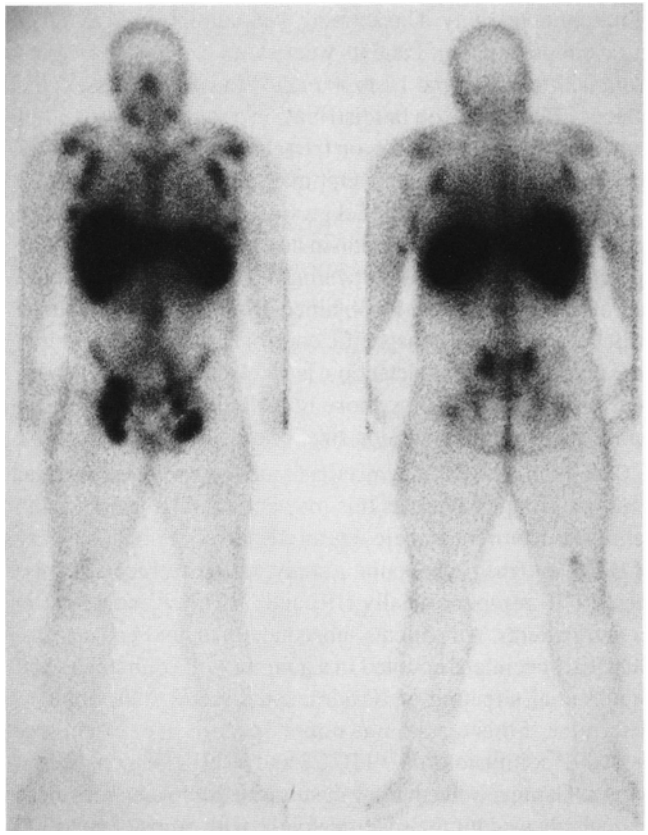

FIG. 3. Anterior and posterior whole body images at 48 hr with 5 mCi ^{111}In T101, 10 mg.

mixed anhydride bifunctional chelating method.

Patients with Sezary syndrome or mycosis fungoides were imaged with ^{111}In T101. In all patients ^{111}In T101 concentrated in pathologically or clinically involved sites, including several previously unsuspected sites. Thirty-eight of 39 clinically or pathologically involved sites were detected on images as well as 44 of 136 clinically negative sites. Focal uptake was seen in skin tumors and infilitrated erythroderma but not in skin plaques. In addition to skin and lymph node, all patients had prominent uptake in spleen, liver, and bone marrow (Fig. 3). Although the liver and spleen uptake is multifactorial in origin, it has been seen with other ^{111}In antibodies, making it difficult to evaluate these sites for tumor involvement. In contrast to targeting for solid tumors, the mechanism for localization appears to be related to binding to T cells, which can then carry the radioactivity to involved sites (*21*). The concentration of ^{111}In T101 in biopsied lymph nodes has been 10 to 100 times higher than that usually reported for this technique. This higher concentration together with the biodistribution data suggest that yttrium-90 (^{90}Y) T101 may be useful in treating CTCL and plans for a phase 1 therapeutic trial are in progress.

Larson et al. (*26*) have used a new diagnostic and therapeutic strategy using MoAbs and their fragments for diagnosis and treatment of malignant melanoma targeting two melanoma associated antigens (Fig. 4). Melanoma is an aggressive tumor, and once it metastasizes, no therapy is effective. The antigen p97 is a glycoprotein of molecular weight 97,000 on the cell surface (*22*). It is expressed at very low concentrations in normal adult tissues and in high concentration in more than 90% of melanomas. The second antigen that has been targeted is

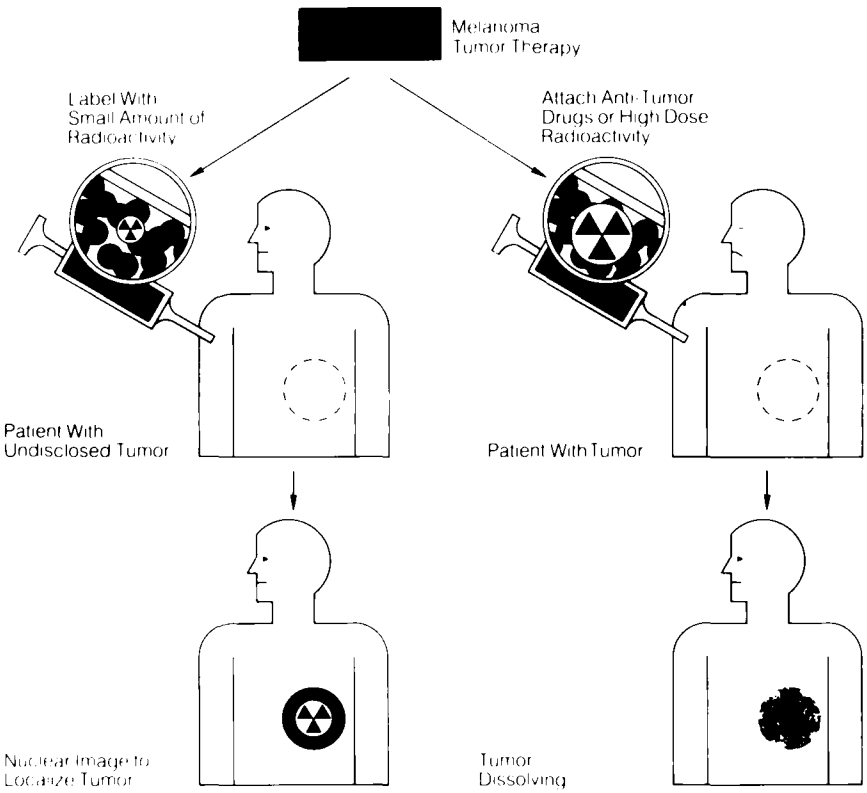

FIG. 4. Antibodies as carriers of radioactive tracers for diagnosis and therapy.

a proteoglycan on the cell surface, with a molecular weight of 250,000 and 400,000 (high molecular weight antigen) (*23*).

In initial studies with whole immunoglobulin labeled with ^{131}I, it was demonstrated that there was antigen-specific uptake in vivo in human tumors and that approximately 88% of metastatic lesions greater than 1.5 cm were detected (*24*). The two major problems identified in this study were: 1) the prolonged circulation in the blood pool, which reduced target to nontarget ratios, and 2) the rapid development of human and anti-mouse antibodies. This occurred in most patients within 2 wk after injection of 1 mg of IgG. As a result of this study the use of Fab fragments was explored.

Fab fragments are smaller pieces of antibodies obtained by papain digestion of whole IgG. These fragments are univalent (have only one binding site) and retain their ability to bind to tumor. Because they are smaller and they lack the Fc fragment (the part of the antibody that is removed to form Fab), which is the most immunogenic portion, they are less immunogenic and may be given in repeated injections. In addition, they have faster localization in tumors and faster clearance from plasma and background, resulting in higher tumor-to-nontumor ratios. Utilizing Fab fragments we have reported improved imaging with better contrast than seen previously with IgG and without the need for blood pool subtraction (*25*) (Fig. 5). Nevertheless only 20 of 33 patients had positive scans (*26*). The faster clearance resulted in favorable dosimetry for a phase 1 therapy trial (*27*), which is currently in progress. Fab 96.5 has been labeled at high specific activity with up to 300 mCi (10 mg) of ^{131}I with good retention of immunoreactivity and tumor targeting. Therapy with high doses of ^{131}I MoAb differs in a number of ways from diagnostic studies: 1) the patient is dosed in a private room designated to minimize exposure to the other patients and staff on the unit; 2) technical considerations in handling the patient are similar to that of patients receiving ^{131}I therapy for thyroid carcinoma; 3) the patients are imaged after reaching a level of $\leq$ 30 mCi; 4) the patient's thyroid and bone marrow are monitored serially; 5) nursing staff is thoroughly oriented to radiation safety precautions so as to ensure good patient management.

TOXICITY

The large cumulative expertise with radiolabeled murine MoAb has shown remarkably few side effects. The injection of a murine protein can stimulate the production of human anti-mouse antibody (HAMA) response (*28*). While this is not associated with side effects, a repeat dose can cause an allergic reaction ranging from simple rash and fever to chills, dyspnea, and even anaphylaxis. In our experience with patients receiving second antibody doses after immune response the reactions have been relatively mild and easily treated.

A significant problem nevertheless is that the antibody clearance will be accelerated and tumor targeting may be precluded (*24*). These reactions are rare with first injections of MoAb but with repeated administration the risk increases. We have found the presence of HAMA in 18 of 39 patients injected IV with whole IgG, whereas none of the 10 patients receiving single dose of Fab had an immune response. Our strategy has been to perform skin tests prior to the second antibody infusion. With some antibodies that react with circulating cells, such as T101, anaphylactoid reactions may be seen when large doses of MoAb are infused quickly (*21*). Serum sickness has been rarely reported.

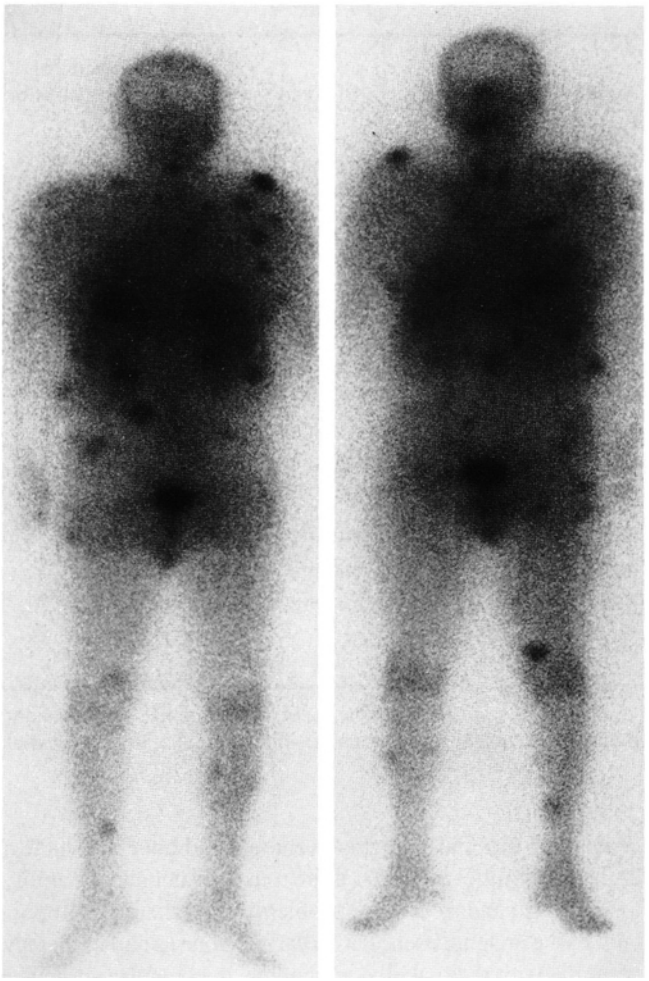

FIG. 5. Multiple subcutaneous lesions imaged 6 days after therapy dose of 100 mCi ^{131}I FAB 96.5, 10 mg.

SUMMARY

The use of MoAb for RIS is still in its early stages. Research efforts at developing clinically useful antibodies are in progress in many centers in the world. The current efforts of the NIH group are directed at finding ways to optimize the delivery, evaluating parameters such as (Table 1): 1) whole IgG vs. fragment, 2) choice of isotope, 3) choice of labeling method, 4) route (intravenous, subcutaneous, intraarterial, intraperitoneal, intralymphatic), 5) mouse MoAb vs. human MoAb, 6) planar imaging vs. SPECT.

When these tests do become a proven and approved part of nuclear medicine, many of these steps will be eliminated. The task of doing MoAb scans for the technologist in a clinical situation will still entail a wide range of responsibilities and expertise, for example: 1) the methods of infusion (i.e., IV vs. IP) have different degrees of difficulty and are more time

TABLE 1.

Antibody	Subclass	Disease	Isotope	Route of administration
Diagnostic antibodies				
9.2.27	IgG_1	melanoma	^{131}I, ^{111}In	IV, SQ
96.5	Fab	melanoma	^{131}I	IV, SQ
48.7	Fab	melanoma	^{131}I	IV, SQ
B72.3	IgG_1	colon cancer	^{131}I, ^{111}In	IV, IP, IA
	Fab	ovarian cancer	^{131}I, ^{111}In	IV
	Fab'(2)	breast cancer	^{131}I, ^{111}In	SQ
T101	IgG_{2a}	CTCL	^{131}I, ^{111}In	IV, SQ, IL
Antibombesin	IgG	lung cancer	^{111}In	IV, IB
LICO1688	HIgM	colon cancer	^{131}I	IV
LICO28A32	HIgM	colon cancer	^{131}I	IV
Therapeutic antibodies				
96.5	FAB	melanoma	^{131}I	IV
48.7	FAB	melanoma	^{131}I	IV
B72.3	IgG_1	colon cancer	^{131}I	IP
T101	IgG	CTCL	^{90}Y	IV

IV, intravenous; IP, intraperitoneal; IB, intrabronchial; HIgM, human monoclonal; SQ, subcutaneous; IL, intralymphatic; IA, intraarterial.

consuming than most other conventional radionuclide studies; 2) the possibilities of reactions are much greater and the ability to recognize and treat these problems immediately is important; 3) the imaging itself, particularly if done as part of therapy planning with radiolabeled antibodies, may require computer analysis with regions of interest and development of quantitative methods to calculate dosimetry.

NOTE

*Hybritech, Inc., La Jolla, CA.

REFERENCES

1. Pressman D, Korngold L. The in vivo localization of anti-Wagner osteogenic sarcoma antibodies. *Cancer* 1953;6:7619–7623.
2. Spar IL, Bale WF, Marrack D, et al. I-131 labeled antibodies to human fibrinogen. Diagnostic studies and therapeutic trials. *Cancer* 1967;20:865–870.
3. Goldenberg DM, DeLand FH, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med* 1978;298:1384–1386.
4. Mach JP, Carrel S, Forni M, et al. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. *N Engl J Med* 1980;303:5–10.
5. Ishii N, Nakata K, Muro T, et al. Radioimmunodetection of cancer using antibodies to alpha-fetoprotein and carcinoembryonic antigen. *Ann NY Acad Sci* 1983;417:270–276.
6. Sullivan DC, Silva JS, Cox CE, et al. Localization of I-131 labeled goat and primate anti-carcinoembryonic antigen (CEA) antibodies in patients with cancer. *Invest Radiol* 1982;17:350–355.
7. Kohler G, Milstein C. Continuous culture of fused cells secreting antibodies of predefined specificity. *Nature* 1975;256:495–497.
8. Mach JP, Buchegger F, Forni M, et al. Use of radiolabeled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. *Immunol Today* 1981;2:239–249.
9. Berche C, Mach JP, Lumbroso JD, et al. Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: First clinical results using radiolabled monoclonal antibodies against carcinoembryonic antigen. *Br Med J* 1982;285:1447–1451.
10. Chatal JF, Saccavini JC, Fumoleau P, et al. Immunoscintigraphy of colon carcinoma. *J Nucl Med* 1984;24:307–314.
11. Halpern SE, Dillman RO, Witztum KF, et al. Radioimmunodetection of melanoma utilizing ^{111}In-96.5 monoclonal antibody: A preliminary report. *Radiology* 1985;155:493–499.
12. Murray JL, Rosenblum MG, Sobol RE, et al. Radioimmunoimaging in malignant melanoma with ^{111}In-labeled monoclonal antibody 96.5. *Cancer Res* 1985;45:2376–2381.
13. Buraggi GL, Callegaro L, Mariani G, et al. Imaging with ^{131}I-labeled monoclonal antibodies to a high-molecular-weight melanoma-associated antigen in patients with melanoma: Efficacy of whole immunoglobulin and its $F(ab')_2$ fragments. *Cancer Res* 1985;45:3378–3387.
14. McConahey PJ, Dixon FJ. A method of trace iodination of proteins for immunologic studies. *Int Arc Aller* 1966;29:185–189.
15. Fraker PJ, Speck JC, Jr. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem Biophys Res Commun* 1978;80:849–857.
16. Krejcarek GE, Tucker KL. Covalent attachment of chelating groups to macromolecules. *Biochem Biophys Res Commun* 1977;77:581–585.
17. Stramignoni D, Bowen R, Atkinson BF, et al. Differential reactivity of monoclonal antibodies with human colon adenocarcinomas and adenomas. *Int J Cancer* 1983;31:543–552.
18. Thor A, Ohuchi N, Szpak CA, et al. Distribution of oncofetal antigen tumor-associated glycoprotein-72 defined by monoclonal antibody B72.3. *Cancer Res* 1986;46:3118–3124.
19. Colcher D, Keenan AM, Larson SM, et al. Prolonged binding of a radiolabeled monoclonal antibody (B72.3) used for in situ radioimmunodetection of human colon carcinoma xenografts. *Cancer Res* 1984;44:5744–5751.
20. Royston I, Majda JA, Baird SM, et al. Human T-cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen of T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. *J Immunol* 1980;125:725–731.
21. Carrasquillo JA, Bunn PA Jr, Keenan AM, et al. Radioimmunodetection of cutaneous T-cell lymphoma with ^{111}In-labeled T101 monoclonal antibody. *N Engl J Med* 1986;315:673–680.
22. Brown JP, Woodbury RG, Hart CE, et al. Quantitative analysis of melanoma associated antigen p97 in normal and meoplastic tissues. *Proc Natl Acad Sci USA* 1981;78:539–543.
23. Hellstrom I, Garrigues HJ, Cabasco L, et al. Studies of a high molecular weight human melanoma associated antigen. *J Immunol* 1983;130:1467–1472.
24. Larson SM, Brown JP, Wright PW, et al. Imaging of melanoma with ^{131}I-labeled monoclonal antibodies. *J Nucl Med* 1983;24:123–129.
25. Larson SM, Carrasquillo JA, McGuffin RW, et al. Use of ^{131}I-labeled murine Fab against a high molecular weight antigen of human melanoma: Preliminary experience. *Radiology* 1985;155:487–492.
26. Larson SM, Carrasquillo JA, Krohn KA, et al. Localization of I-131 labeled p97 specific FAB fragments in human melanoma as a basis for radiotherapy. *J Clin Invest* 1983;72:2101–2114.
27. Carrasquillo JA, Krohn KA, Beaumeir PL, et al. Diagnosis and therapy of solid tumors with radiolabled antibodies and immune fragments. *Cancer Treat Rep* 1984;68:317–318.
28. Reynolds JC. Carrasquillo JC, Larson SM. Human anti-murine response following administration of radiolabeled monoclonal antibodies (abstr). *J Nucl Med* 1985;26:P55.