Indium-111-Monoclonal Antibodies in Radioimmunoscintigraphy

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This is the first article of a four-part update series on "nuclear medicine updates." Upon completion of this article, the reader will understand (1) the use of indium-labeled antibodies in the detection of cancer and other disease states; (2) nontumor radioimmunoscintigraphy with indium; and (3) quality control procedures for indium-labeled antibodies.

Thirty years ago, the basic science of immunology and the clinical science of nuclear medicine collaborated for the development of the radioimmunoassay technique. Although the use of radionuclides in in vitro assays is being replaced with non-radioactive detection methods, the basic discoveries are still in use and technical improvements continue.

Although initial attempts at imaging using radiolabeled antibodies were not successful (*1,2*), further immunologic improvements helped spur progress. A number of tumor-associated, as well as other equally important, antigens have been identified, purified and characterized (*3,4*). The development of the hybridoma technology for the production of single species of antibody molecules derived from one-cell monoclonal antibodies have made large constant supplies of highly specific antibodies available for study (*5,6*). Antibodies are produced from cell cultures instead of animals with concomitant improvements in purity, reproducibility and quantity. Monoclonal antibodies have been fragmented into smaller molecules which retain the ability to recognize and bind target antigens but improve the pharmacokinetics, nonspecific localizing properties, biodistribution and radiolabeling characteristics (*7,8,9*).

These advances along with equally important developments in camera technology, radionuclide production and availability, radiopharmaceutical chemistry and computer applications have led to the ability to locate cancer and other disease states at earlier stages of development when compared with other diagnostic tests and provide hope that therapeutic intervention with radiolabeled antibodies may someday become a reality (*10,11,12*). A number of excellent reviews of the use of radiolabeled monoclonal antibodies (MAbs) in diagnosis and therapy are available (*13,14,15,16,17,18,19,20, 21,22*).

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A major contribution provided by nuclear medicine scientists has been the development of radiolabeling techniques that has enabled the incorporation of medically useful radionuclides onto the protein structures according to selected need (*23,24*). Antibodies were first radiolabeled using radionuclides of iodine because of the availability of ^{125}I and ^{131}I along with the proven chemical methods of radioiodinating amino acid residues of proteins (*25,26*). The physical characteristics of ^{131}I and ^{125}I make them poor imaging radionuclides. Iodine-123 has physical properties which make it ideal for current instrumentation and the biological handling of antibodies and their fragments. However, the instability of the radioiodine label along with the expense and unavailability limited the use of ^{123}I in MAb imaging studies.

This report discusses the development and use of ^{111}In as a radiolabel for MAbs and their use in nuclear medicine imaging. The current importance of ^{111}In as the radiolabel for MAbs was illustrated at the recent 36th annual meeting of The Society of Nuclear Medicine where over half of the more than thirty presentations on radiolabeled antibodies in diagnosis utilized ^{111}In.

INDIUM-111 AS A RADIOLABEL FOR ANTIBODIES

Radioimmunoscintigraphy began with the use of radioiodinated antibodies because the chemistry for radiolabeling large protein molecules was well established. However, due to the limited availability of ^{123}I and its relatively short half-life, the majority of the early imaging studies were completed with ^{131}I. Along with improvements in the antibodies available for clinical investigations came the need for better radionuclides for diagnostic imaging. The availability of radiopharmaceuticals utilizing ^{111}In as the radionuclide for other diagnostic studies including abscess imaging, cisternography, bone marrow imaging and gastric emptying studies led to a desire to use ^{111}In for radioimmunoscintigraphy.

The 173 and 247 keV gamma photons had proven almost ideal for current instrumentation. The 67.9 hr half-life of the radionuclide allowed for localization times of 3–7 days prior to imaging.

Although the physical characteristics of ^{111}In were nearly ideal, the chemistry associated with radiolabeling proteins was an obstacle to overcome. Unlike radionuclides of iodine which have naturally occurring sites of incorporation on antibodies in the tyrosine and histidine amino acid residues, stable attachment of ^{111}In as well as other radiometals to antibodies needed development.

The modification of proteins by covalently attaching metal-chelating groups led to a method for radiolabeling antibodies with radiometals (*27,28,29,30*). The use of a class of compounds referred to as "bifunctional chelating agents" which provide both a metal chelating portion as well as a reactive functional group capable of covalently attaching to proteins led to radioimmunoscintigraphy with radioactive metals such as ^{111}In, ^{99m}Tc, ^{67}Ga and others. In addition, the promise of radioimmunotherapy which is based on the use of a number of beta emitting radiometals also depends on the use of chelate-tagged antibodies.

In addition to improved nuclear properties, ^{111}In radiolabeling using metal-chelate attachment brought additional advantages to radioimmunoscintigraphy. As mentioned previously, similar metal-chelates could be used to radiolabel antibodies with a number of useful radioactive metals for both diagnosis and therapy as listed in Table 1. A method of attachment and radiometal labeling that leads to high quality images should also prove successful for the delivery of high therapeutic doses.

TABLE 1. Radionuclides for Labeling Antibodies Using Bifunctional Chelates

Radionuclide	Tp (in hr)	Emissions (energy)
Diagnosis		
Indium-111	67.9	gamma (173 keV) gamma (247 keV)
Technetium-99m	6.02	gamma (140 keV)
Gallium-67	78.3	gamma (93 keV) gamma (185 keV) gamma (300 keV)
Therapy		
Yttrium-90	64	gamma (1761 keV) beta (2279 keV)
Copper-67	61.9	gamma (184 keV) beta (577 keV)
Rhodium-105	35.3	gamma (319 keV) beta (565 keV)
Rhenium-186	88.9	gamma (137 keV) beta (1100 keV)
Rhenium-188	16.7	gamma (155 keV) beta (2116 keV)

During radioiodination of antibodies, the chemical modification and radiolabeling occur simultaneously and cannot be separated. Radiometal labeling of antibodies is easily separated into two steps. The first involves the chemical modification of the protein by covalent attachment of the chelating agent. The second step is the addition of the radiometal in a chemical form capable of transferring to the chelate as shown in Figure 1.

This allows the development of radiopharmaceutical kit formulations for radiolabeling antibodies. In a manner similar to commonly used ^{99m}Tc radiopharmaceutical kits, the chelated antibodies may be prepared in bulk, purified, tested and stored in a nonradioactive form. The final preparation involves the addition of ^{111}In prior to patient administration as shown in Figure 2. This allows for the storage of chelated-MAb kits on-site and higher specific activity of the final product when it is prepared close to administration time.

The use of bifunctional chelating agents has been shown to produce stable protein radiometal forms (*31*). In theory, as the radiolabeled antibody is metabolized, a chelated form of the radiometal which is excreted through the urinary system should be produced. This is in contrast to the in vivo handling of radioiodine from radioiodinated MAbs which results in high thyroid burdens unless steps are taken to block its uptake.

Just as each MAb and its fragments behave differently, changing the bifunctional chelate and method of radiolabeling can change the way an antibody is handled in vivo. The preparation of a chelated form of a MAb and radiolabeling with ^{111}In will generally increase the whole body retention

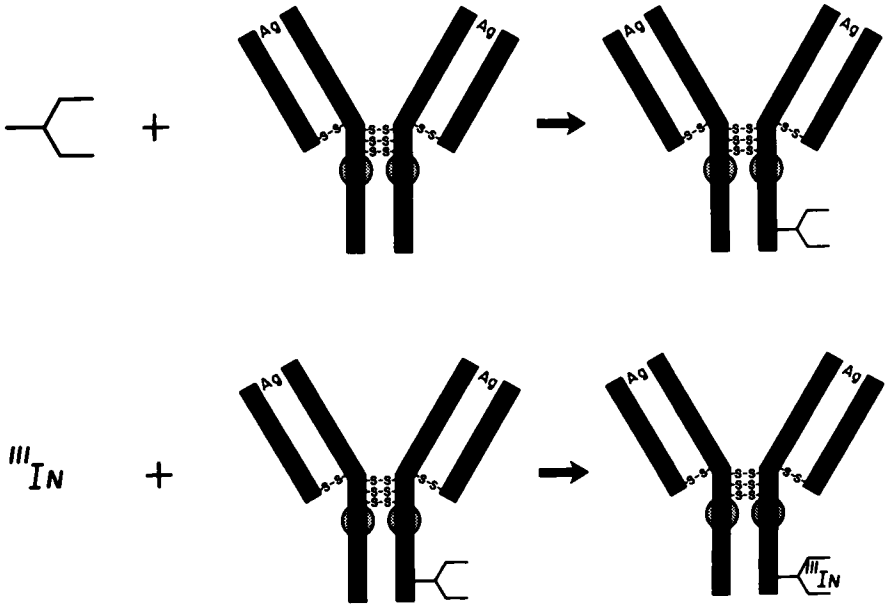

FIG. 1. General scheme for radiolabeling MAbs using bifunctional chelates showing the two step process. Step 1 involves the binding of the chelate moiety to the MAb. Step 2 involves the chelation of the radioactive ^{111}In.

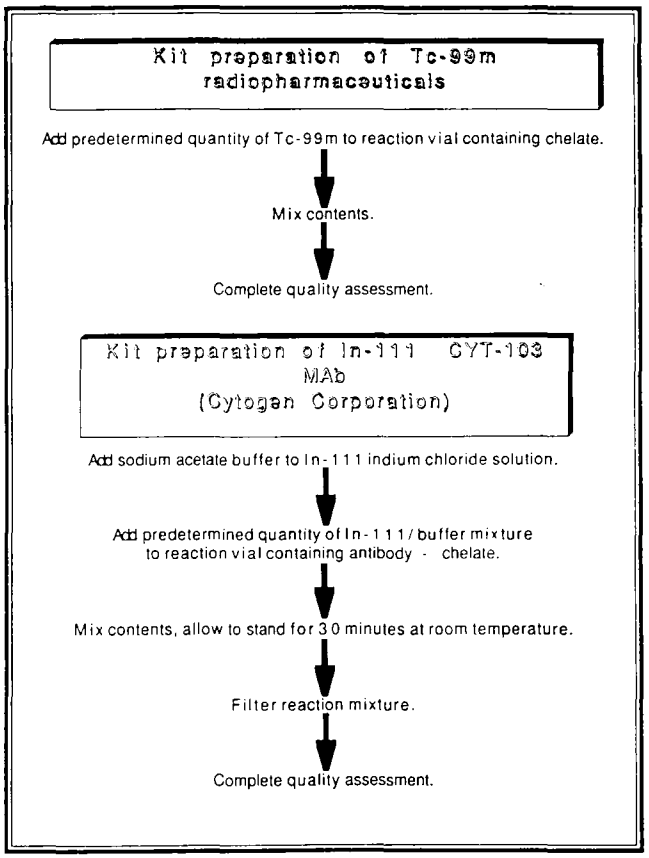

FIG. 2. Comparison of ^{99m}Tc radiopharmaceutical kit preparation with kit preparation of ^{111}In-labeled antibody.

time of radioactivity compared to its radioiodinated counterpart. In addition, prolonged elevated hepatic levels of radioactivity are seen with ^{111}In-labeled MAbs compared to the same MAbs that have been radioiodinated. Animal studies indicate the radioactivity in the liver is in the form of a low molecular weight substance (*32*). The high, prolonged liver uptake of ^{111}In remains the primary limitation of the clinical utilization of ^{111}In-MAbs for diagnostic imaging. Although the use of fragments of MAbs, "cold" antibody administration and higher dosages of cold MAbs given with radiolabeled MAb have been tried in an effort to improve the hepatic clearance, images of the upper abdomen remain suboptimal (*33*).

HISTORICAL OVERVIEW OF INDIUM-111 RADIOLABELING OF ANTIBODIES

Procedures for the radiolabeling of MAbs with ^{111}In and other radiometals grew out of the earlier work toward radiolabeling other large macromolecules such as albumin (*34,35*). An excellent chronology of the development of chelating agents for binding of radiometals to macromolecules can be found in a publication by Meares (*36*).

Unlike radioiodine, ^{111}In and other radiometals do not bind directly to any complex organic molecule. Because a strong, stable bond does not form directly between radiometals and MAbs, researchers tried bifunctional chelating agents such as DTPA and EDTA as a bridge for attachment of the radiometal to the protein (*37*). The polyaminocarboxylic acid chelates DTPA and EDTA appear to bind radiometals in a weak manner when compared to derivatives of the two (*38*). Sundberg et al. developed the first set of chelate-protein conjugates stable enough for in vivo investigations using derivatives of EDTA (*39*). Work with these compounds allowed basic requirements of protein-chelate compounds to be developed and provided standards for improvements. Derivation schemes investigated include the formation of an activated DTPA by preparing the mixed anhydride (*35,40,41,42*). Covalent attachment of the chelate derivative to the MAb is thought to occur on lysine residues (*43*). An improvement in the use of DTPA conjugates came about with the development of the cyclic anhydride derivative (*30,44,45,46,47*).

Although this early work led to stable ^{111}In MAb-conjugates, the coupling and radiolabeling procedures placed the ^{111}In on the MAb in an extensive random fashion much like that seen with radioiodination. Attachment of the chelate to the MAb molecule and use of this site for radiolabeling resulted in a stable product with minimal loss of radionuclide in vivo, but placing more than one chelate per MAb can interfere with antigen recognition and localization in the body.

A more controlled random labeling of amino groups was obtained with the isothiocyanate derivative of DTPA, but any exposed amino group on the protein was a possible site of attachment (*48*). If a number of these sites, especially those that occur near the antigen binding site, are filled with bulky chelate-radiometal complexes, the biologic activity of the molecule, in the case of MAbs the immunoreactivity, may be reduced (*34,49,50*).

In an effort to increase the radiometal carrying capacity (specific activity) of the MAb and to preserve the ability to recognize and bind to specific antigen (immunoreactivity), methods were developed for covalently attaching many chelate moieties on the Fc portion of the MAb (*51,52,53*). As opposed to the random placement of chelate-radiometal complexes on the MAb, these methods of site-specific radiolabeling enables the placement of the radionuclide far removed from the "business" end of the MAb and preserves the antigen

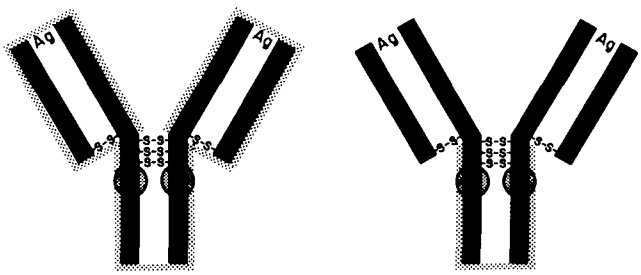

FIG. 3. Site-specific methods of radiolabeling places chelate groups only on the Fc (carbohydrate) portion of the MAb as indicated by dashed lines. Other methods place the chelate groups in a random fashion over the entire molecule.

binding capacity as shown in Figure 3. Attachment of up to nine chelate groups can be directed to the oligosaccharide structures which occur only on the carbohydrate chain of the MAb.

The goals of radiolabeling methods involving the use of ^{111}In or any other radionuclide are identical. They include the production of an in vitro and in vivo stable bond with the MAb or fragment, at a site on the protein molecule that does not interfere with immunoreactivity with a high specific activity to deliver high levels of radioactivity to the target site. If the radiolabeled MAb behaves in a similar manner to the native MAb, the radiolabeling procedure has been successful.

Although immunoglobulins have similar chemical and biological properties, each specific antibody may react slightly different under conditions during radiolabeling (*54,55*). The same is true of fragments of each MAb if in fact the MAb can be fragmented.

The number of chelates and ^{111}In atoms that can be placed on an antibody before the molecule begins to lose immunoreactivity must be carefully determined stoichiometrically. Improved chemical modifications and carefully controlled radiolabeling and purification procedures have led to higher affinity and avidity of radiolabeled MAbs. As each new method is attempted, careful in vitro and in vivo quality assessment procedures are completed to be certain minimum standards for radiochemical purity, pharmacokinetics and immunoreactivity are maintained. Some of the chelate derivatives used for radiolabeling MAbs with ^{111}In are listed in Table 2.

TABLE 2. Bifunctional Chelates for Radiolabeling Proteins with Indium-111

Bifunctional chelate	Reference
1-(p-benzenediazonium)-EDTA	27
Mixed anhydride of DTPA	35
Cyclic dianhydride of DTPA	30
1-(p-isothiocyanatobenzyl)-DTPA	48
glycyl-tyrosyl-lysine (GYK)-DTPA	51
N-hydroxysuccinimide DTPA pentaester	46

QUALITY ASSESSMENT OF INDIUM-111-LABELED ANTIBODIES

Currently, most antibodies under clinical investigation are monoclonal antibodies (MAbs) obtained from hybridomas of murine (mouse) origin. Even before these MAbs are available for radiolabeling, they must meet a number of production and testing guidelines established by the Food and Drug Administration's Office of Biologics (*56*). Included in this requirement is documentation of the MAb's origin (hybridoma cell line), production procedure (tissue culture or mouse ascites manufacture) and contamination testing (quantification of various murine viruses, murine DNA). These tests along with determination of the physicochemical properties of the MAb are completed by the manufacturer or laboratory involved in the scale-up production of the antibody (*9*). If the MAb passes these initial tests, it is available for human studies under the investigational new drug (IND) process. In the clinical setting, a series of quality assessment determinations are completed on the radiolabeled product to be certain a safe preparation in the correct radiochemical form capable of binding to the target antigen will be given.

As in the case of other radiopharmaceuticals, when the drug is developed in the institutional setting, it must be tested prior to human use to be certain of the radiochemical purity, sterility, freedom from pyrogens and immunoreactivity as listed in Table 3.

The investigator must be certain the chelate conjugation to the MAb and the radiolabeling procedure has not destroyed the ability of the MAb to recognize and bind to the target antigen. If the radiolabeled MAb preparation is obtained from a manufacturer's kit formulation, the sterility and endotoxin levels as well as immunoreactivity have been established during developmental testing. In this situation, a quick test to determine radiochemical purity after on-site radiolabeling is necessary to be certain shipping and storage of the kit components and/or radionuclide has not changed the ability of the chelated MAb to bind ^{111}In.

Radiochemical purity is a measure of the quantity of radioactivity in the preparation in the desired chemical form either as whole IgG or antibody fragments. Radiochemical impurities in the form of free ^{111}In, ^{111}In chelate not protein bound and/or ^{111}In protein aggregates can result from improper shipping or storage conditions, decreased binding of ^{111}In to chelated MAb or failure of final purification steps. Depending on the chemical form of these impurities, they may remain in the circulation for extended periods of time or may localize in an organ or tissue where a primary or metastatic lesion becomes difficult to detect. Indium-111-labeled antibody aggregates would localize in the liver and increase the already high background in that organ. Indium-111 chelate would cause increased kidney localization which is excreted. Indium-111 in the free, unbound form quickly binds to circulating proteins, primarily transferrin, which has a long biological half-life (*57,58,59*). Figure 4 shows the biodistribution of an

TABLE 3. Quality Assessment Procedures for Radiolabeled Antibodies

Biological tests	Sterility Limulus Amoebocyte Lysate (LAL) Immunoreactivity
Physicochemical tests	Instant Thin Layer Chromatography (ITLC) Trichloroacetic Acid Precipitation (TCA) High Performance Liquid Chromatography (HPLC)

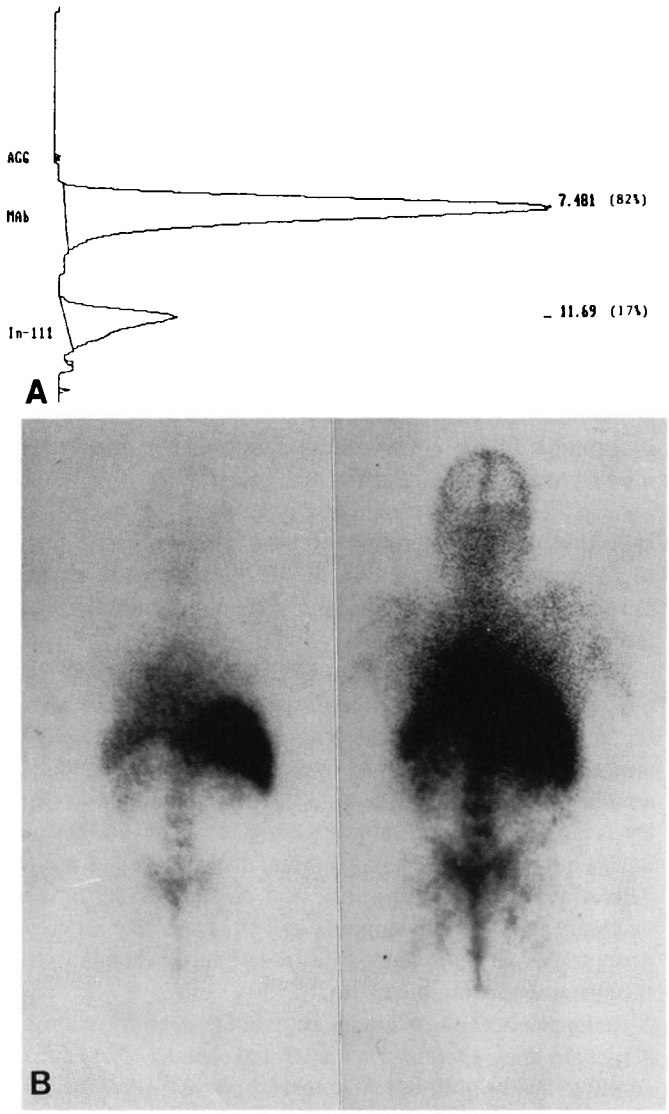

FIG. 4. HPLC radiochromatogram of ^{111}In-MAb (A) indicates 17% of the radioactivity in the preparation is not protein bound. This preparation results in high skeletal and kidney levels of radioactivity as seen in the posterior view (B) 3 days postinjection.

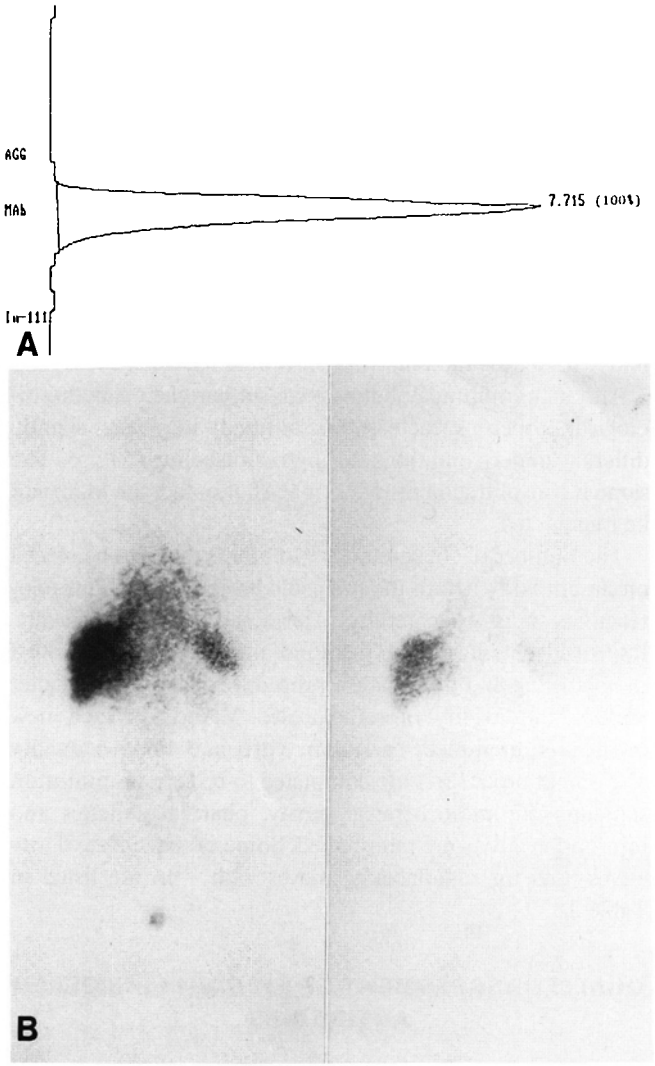

FIG. 5. HPLC radiochromatogram of ^{111}In-MAb showing only one peak of radioactivity occuring at the IgG retention time. This preparation results in minimal skeletal or kidney radioactivity as seen in the posterior view (B) 3 days postinjection.

^{111}In-MAb with elevated levels of free, unbound ^{111}In. Figure 5 illustrates the biodistribution of the same ^{111}In-MAb with little unbound ^{111}In.

A number of techniques common to nuclear medicine are used to quickly quantitate the levels of protein bound and free ^{111}In in radiolabeled antibody preparations. Various investigators as well as manufacturers of ^{111}In antibody labeling kits recommend different chromatography media as well as solvents to separate between the possible radiochemical forms.

Instant thin layer chromatography (ITLC), thin layer chromatography (TLC) and paper chromatography have been used. Because the ^{111}In protein structures are relatively large molecules, most solvent systems move "free", unbound ^{111}In away from the protein which remains at the origin. Percentage of the total activity on the strip remaining with the protein is determined by cutting and nuclear counting of the strip or radiochromatogram scanning as seen in Figure 6. Results are typically in the 90–95% range with acceptable values established for each radionuclide-antibody combination and the ultimate utilization.

The use of trichloroacetic acid (TCA) to precipitate proteins out of solution has been utilized in nuclear medicine for a number of years to quantitate protein bound radionuclide. In order to determine the percentage of ^{111}In bound to MAbs, duplicate aliquots of the preparation are placed in a series of test tubes and 0.5ml of a 20% solution of TCA added. The tubes are assayed for total radioactivity then centrifuged to pellet the radiolabeled protein. The supernate is removed carefully and the tubes counted again. Percentage of ^{111}In bound to protein is determined from the two counts as illustrated in Table 4.

The procedures mentioned above are quick, easy and common to most nuclear medicine departments. However, they only provide data on the level of protein-bound radionuclide and cannot separate between different forms of protein. High

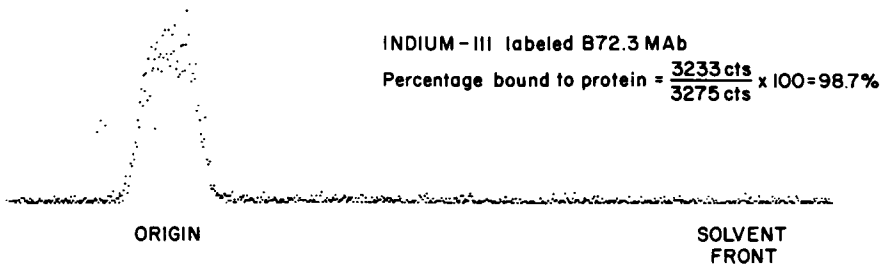

FIG. 6. Radiochromatogram scan of ITLC strip used for determination of "free," unbound ^{111}In. After spotting the strip with ^{111}In-labeled MAb, it was developed in 85% methanol solution, which would bring any nonprotein bound ^{111}In with the solvent front.

performance liquid chromatography (HPLC) is a column chromatography technique that provides high resolution of radiolabeled protein fragments, whole IgG and protein aggregates (*60*). If the ^{111}In MAb is a whole IgG, the presence of undesirable fragments and/or aggregates (dimers, trimers, etc.) would lead to altered biodistribution of the radiolabeled antibody with aggregates being removed by the reticuloendothelial system and fragments being excreted by the urinary system. Figure 7 illustrates the presence of radiolabeled protein aggregates in an ^{111}In labeled anti-CEA MAb.

Most HPLC systems used to evaluate radiochemical purity of radiolabeled MAbs utilize some form of size exclusion chromatography which separates molecules based on molecular size or weight. HPLC also quantitates non-protein bound forms of ^{111}In, as seen in Figure 7, although the expense and required expertise in the set-up, operation and trouble-shooting on the equipment precludes the general use in most nuclear medicine departments. HPLC is used initially to develop a procedure for radiolabeling a MAb that does not cause unwanted MAb fragmentation or aggregation and to make certain these undesirable protein forms are not present prior to radiolabeling. After confirmation of MAb radiolabeling techniques, the more convenient assays such as ITLC, TLC, TCA precipitation are used for a quick determination of adherence to established procedures.

Biological properties are studied to insure the radiolabeling, purification, dosage form preparation and storage allows a sterile, pyrogen-free and immunologically reactive product to be made. The U.S.P. Sterility Test and Limulus Test for the presence of endotoxins are required on investigational radiolabeled MAbs. These tests are completed on batches of products to prove production techniques maintain sterility and apyrogenicity. Since further biological testing is not done on preparations of radiolabeled MAbs obtained under a manufacturer's I.N.D., strict adherence to aseptic techniques is important when compounding the final product.

Immunoreactivity refers to the ability of an antibody to recognize and bind a specific antigen. Native MAbs will have unique immune characteristics of identifying an antigen and forming a stable Ab-Ag reaction. The MAbs undergo many changes in the amino acid configuration during chelate binding and labeling with ^{111}In. These chemical reactions along with changes in the environment in which the MAb is placed (freezing, thawing, heating, different solvents, column chromatography, etc.) can lead to structural changes in the molecule.

If these changes in the ^{111}In-labeled MAb occur at or near the antigen binding region, a decrease or complete loss in immunoreactivity may result. A measurement of immunoreactivity is made using a culture of live tumor cells known to express the target antigen, tumor cell extracts or pure antigen. Percentage binding to cells or an inert media coated with antigen is determined after an incubation and washing step employing the ^{111}In MAb (*61*).

TABLE 4. Results of Trichloroacetic Acid Precipitation Test

^{111}In-CEA MAb, Lot No. 1CEA101888, October 25, 1988			
Pre-sample	**CPM**	**Post-sample**	**CPM**
#1	106544	#1	97833
#2	104710	#2	96155
#3	108440	#3	97759
#4	101329	#4	91901
Background	158	Background	167

Assay results: #1 (91.8%); #2 (91.8%); #3 (90.1%); and #4 (90.7%).
Percent labeled = 91.1%.

INDIUM-111-LABELED ANTIBODIES: CLINICAL STUDIES

Research is currently underway in both industrial and academic settings on the utilization of radiolabeled MAbs to diagnose and treat a variety of diseases. The low specificity of currently available radiopharmaceuticals for diagnosis of can-

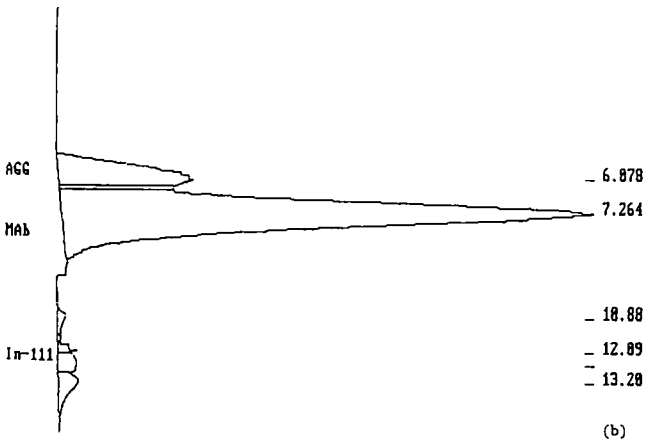

FIG. 7. HPLC radiochromatogram of ^{111}In-MAb showing the presence of protein aggregates as well as nonprotein bound, "free," ^{111}In.

cer makes the use of radiolabeled MAbs to a specific tumor-associated antigen an exciting possibility and in fact, most studies underway involve MAbs for tumor diagnosis and therapy. However, MAbs have been developed which bind to antigens present in non-cancerous disorders.

Because of early work completed with the carcinoembryonic antigen (CEA) which led to an extensive knowledge base and good supply of pure antigen and antibodies, the majority of early clinical studies with radiolabeled antibodies continued with anti-CEA formulations. This continued investigation of MAbs to CEA for radioimmunoscintigraphy also illustrated the importance of this antigen as a tumor marker.

The first reported clinical trial involving the use of a radiolabeled antibody for diagnosis of a neoplastic disease appeared in 1978 and used an ^{131}I-anti-CEA polyclonal antibody from immunized goats (*62*). Only three years later, the first use of a monoclonal antibody for imaging was reported utilizing CEA as the target antigen again (*63*). This study involved patients with pancreatic and colon carcinomas and utilized ^{131}I-labeled MAb for the localization of tumors in 50% of the patients studied.

Another first was reported in 1983 when an ^{111}In-polyclonal anti-CEA antibody was used to detect various CEA-producing carcinomas (*64*). One of the first clinical studies utilizing radiolabeled fragments of an antibody to image cancer was completed with an $F(ab')_2$ fragment of a MAb to CEA (*65*).

The continued development of MAbs that react with CEA led to a number of reports which showed improved detection of smaller and deep-seated lesions as a result of improved target to background ratios seen with ^{111}In radiolabeling (*66,67*). Various tumors including gastric, colorectal, squamous cell lung carcinoma, and medullary thyroid carcinoma produce CEA (*68*) and have been shown to image well with ^{111}In-anti-CEA antibodies. Improvements in the production of anti-CEA MAbs and ^{111}In radiolabeling techniques have led to a number of clinical investigations which have brought ^{111}In-labeled antibodies to CEA ready for Food and Drug Administration approval for radioimmunoscintigraphy (*69,70,71,72,73*). Figure 8 shows an ^{111}In-anti-CEA MAb image in a patient with recurrent colorectal cancer.

As the knowledge base of tumor immunology grew with the resultant identification of many tumor associated antigens, the possibility of using MAbs which bind these antigens for radioimmunoscintigraphy was investigated.

An effort to improve radioimmunoscintigraphy of gastrointestinal cancers led to the use of radiolabeled MAb 17-1A. This MAb recognizes an antigen localized on colon carcinoma tumor cell membrane but not shed into the general circulation. In comparison, CEA is a shed antigen and can be found circulating in the blood. Although this makes CEA an excellent tumor marker for serum assays, the formation of immune complexes in the blood leads to prolonged blood levels and hinders radioimmunoscintigraphy (*74*). A number of radioimmunoscintigraphy trials utilizing the 17-1A antibody either as whole IgG or its fragments provided 50%–60% detection rate for tumors (*75,76,77*). These studies involved radioiodine labeled 17-1A MAb.

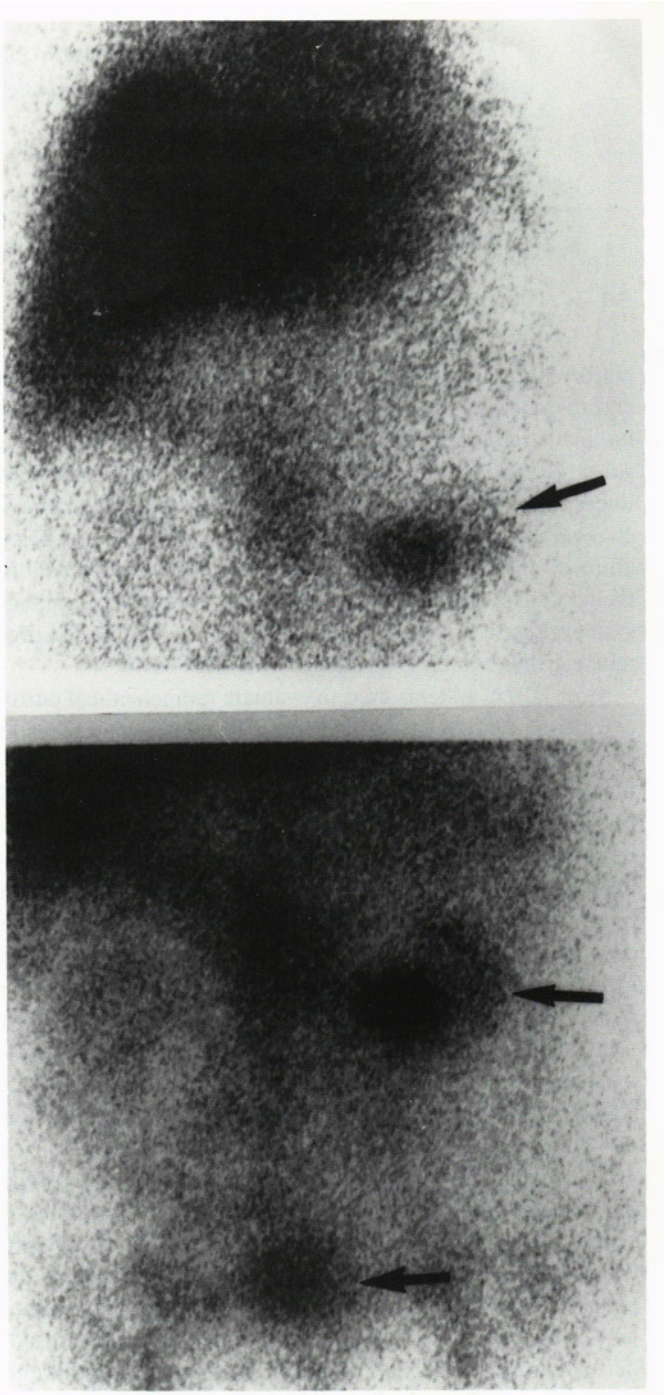

FIG. 8. Anterior views of the lower abdomen with liver and pelvis 3 days postinjection of ^{111}In-anti-CEA MAb. Arrows indicate sites of recurrence of colorectal cancer in the colon and vagina.

Improvement in tumor detection with ^{111}In-labeled 17-1A MAb was never obtained since no follow-up studies were conducted due to the MAb's less than impressive detection rate and the discovery of the antigen in normal tissue (*78*).

MAbs to the human milk fat globule were radiolabeled with ^{123}I and used to image ovarian and breast tumors (*79*). Antibodies to this same antigen radiolabeled with ^{111}In showed significant improvement in tumor detection (*80,81*).

The antibody OC 125 which recognizes the CA 125 antigen found on serous ovarian carcinomas (*82*) has been investigated for radioimmunodetection of gynecologic carcinomas (*83,84,85,86,87*). Although initial work utilized radionuclides of iodine for imaging, the results of these studies show tumor imaging of the pelvis region was superior with ^{111}In-labeled MAb. Approximately 70–80% of confirmed tumor sites were visualized using the OC 125 MAb labeled with ^{111}In. No mucous ovarian carcinomas expressed the CA 125 antigen while the CA 19-9 antigen was found in almost all of those tested. A suggestion was made to use the two antibodies in a complementary fashion as a radiolabeled antibody "cocktail" to further improve detection rates.

MAb B72.3 was produced using a breast carcinoma metastasis to the liver (*88*). It recognizes a high molecular weight tumor-associated glycoprotein antigen called TAG-72. This MAb has been studied extensively because of its reactivity with a wide variety of mucin-producing adenocarcinomas including colon, breast, ovary, pancreas and stomach, but an almost complete absence of reactivity with normal tissues (*89,90*). Early trials with radioiodinated B72.3 in patients with colorectal cancer provided evidence from surgical specimens of selective localization in primary and metastatic colorectal lesions (*91,92,93*). Metastatic colon cancer lesions were imaged with ^{131}I B72.3 in one-half of patients studied (*94*).

This MAb provides high tumor-to-nontumor ratios for various tumor types as seen by well counting of surgical specimens in these and other studies (*95,96,97,98*). The specificity and high degree of targeting observed with ^{131}I studies led to ^{111}In chelation labeling in an effort to improve imaging results (*99*). Major differences in pharmacokinetics and biodistribution of the ^{111}In chelated B72.3 compared with ^{131}I radioiodinated B72.3 were reported (*100*). The prolonged whole body retention of radioactivity, increased hepatic localization and bowel radioactivity have become the standard observations with ^{111}In MAbs compared with their radioiodinated counterparts (*101,102,103,104,105,106,107,108,109*). Although the localization of liver lesions was problematic in these investigations, the indications of higher levels of ^{111}In B72.3 in tumors as well as the superior physical characteristics of ^{111}In compared with ^{131}I encouraged further studies. Results of clinical trials of ^{111}In B72.3 for localization of colorectal carcinoma indicate a detection rate of 76% in surgically confirmed tumors while C.T. studies of the abdomen and pelvis had a detection rate of only 47% (*110,111*). A larger study confirmed this detection rate of 73% of presurgical cases with occult lesions seen in 17% of the patients (*112*). MAb B72.3 is also undergoing trials in ovarian and breast cancer detection (*113,114,115*). Figure 9 shows an image of ^{111}In B72.3 MAb in a patient with ovarian cancer. The limitation of this MAb may be only in the number of tumor cells that express high enough quantities of the TAG-72 antigen to facilitate localization (*116,117*).

Radiolabeled MAbs have been used extensively to image melanoma since several antigens expressed by melanoma cells have been identified. Two melanoma-associated antigens

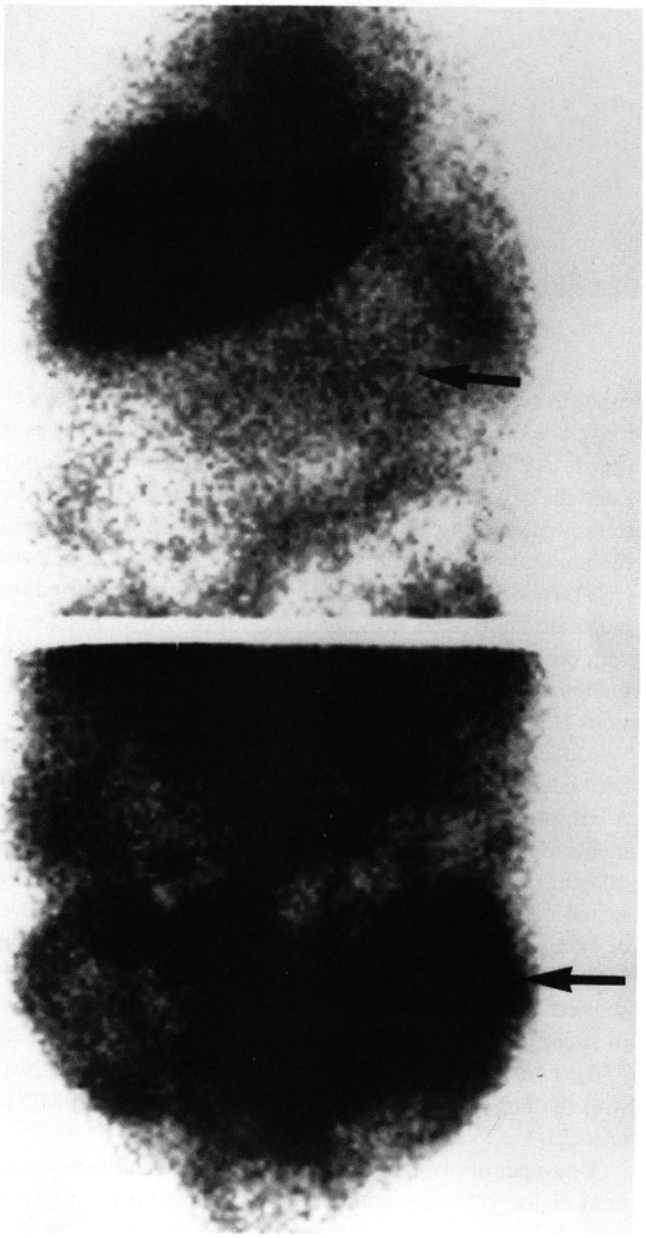

FIG. 9. Anterior views of the lower abdomen with liver and pelvis 3 days postinjection of ^{111}In B72.3 MAb. Arrows shows carcinoma of both ovaries with replacement of omentum of the upper abdomen with tumor.

that have been studied are the P97 antigen (*118*) and the high molecular weight melanoma-associated antigen or HMWMAA (*119*). Both antigens are expressed by most melanoma tumors and several MAbs to these antigens have been developed.

In early imaging studies using ^{131}I-labeled anti-p97 antibody (MAb 96.5), Larson et al. demonstrated localization in 88% of metastatic lesions although high liver uptake and prolonged blood retention of radioactivity reduced the lesion to normal tissue ratios (*120*). The same MAb was labeled using a chelated ^{111}In technique which resulted in improved contrast between tumor and normal tissue which made the images

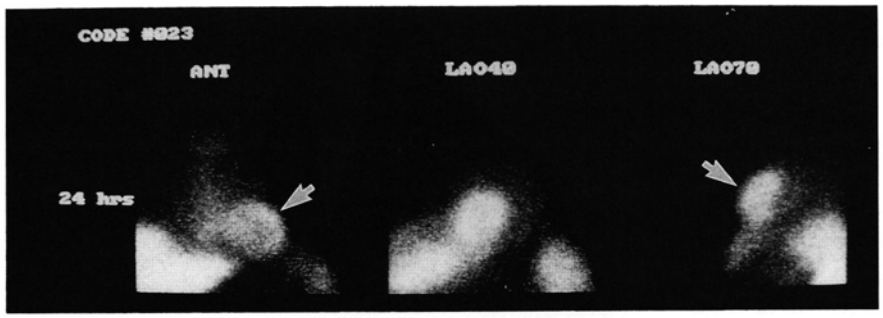

FIG. 10. Patient with a large antero-septal myocardial infarction 24 hr after infusion of ^{111}In-anti-myosin MAb. (Image courtesy of Todd Makler, MD, Albert Einstein Medical Center, Philadelphia, PA and Centocor, Malvern, PA)

easier to evaluate (*121*). This multicenter clinical study localized 81–83% of metastatic lesions at optimal dosage levels in this dosage ranging study. A continuation of this work indicated the best dosage of ^{111}In anti-p97 MAb to use for radioimmunoimaging were at 2 mg and greater (*122*). By increasing the amount of MAb infused, the uptake by liver is reduced (*123*). Participants in this Phase III clinical trial reported the major difficulty seen with the ^{111}In anti-p97 MAb images, as with other radiolabeled antibody preparations for diagnosing malignant disease, is the localization of micrometastatic lesions (*124,125*).

Concurrently, studies were conducted with radiolabeled antibodies to the HMWMAA with similar results (*126,127*). A comparison of ^{123}I-, ^{99m}Tc-, and ^{111}In-labeled F(ab')$_2$ fragments of MAb 225.28S indicated similar effectiveness for imaging melanoma lesions regardless of the radionuclide used or the radiolabeling method (*128*). The anatomic site, level of antigen and size of lesion were mentioned as influencing the results of radioimmunoscintigraphy. A multi-center study showed ^{111}In anti-melanoma F(ab')$_2$ of 225.28S was inferior in lesion detection to the same antibody radiolabeled with ^{99m}Tc (*129*). Clinical investigations are currently underway with the Fab fragments of this MAb radiolabeled with ^{99m}Tc (*130,131*).

A number of investigators have demonstrated the potential clinical use of ^{111}In-radiolabeled antibodies in a variety of other less common cancers. Early studies indicate ^{111}In may be the preferred radionuclide in the radioimmunoimaging of nodal as well as cutaneous sites of lymphomas (*132,133,134*). Choriocarcinomas, rhabdomyosarcomas, breast cancer, hepatomas, lung and brain tumors have all been imaged with mixed results using antibodies labeled with radionuclides other than ^{111}In. Some malignancies such as renal and bladder cancer need further studies before acceptable results are obtained.

Nontumor Radioimmunoscintigraphy with Indium-111-MAbs

The development of radiolabeled MAbs for the diagnosis of noncancerous diseases has been as exciting as the work with malignant disorders. For example, the nuclear imaging of myocardial infarction with a radiolabeled MAb may soon be a common procedure, although over a decade of work has led to this accomplishment. Khaw et al. demonstrated cardiac myosin becomes accessible after myocardial injury due to increased capillary permeability and destruction of the injured cardiac muscle cell membrane (*135*). This phenomenon permits access of circulating anti-myosin antibodies to the myocardial protein which is normally found intracellularly in healthy cardiac muscle (*136*). Because of this cell damage, the radiolabeled circulating anti-myosin MAb binds to infarcted myocardium leading to areas of increased activity or "hot spots" (Fig. 10). Setting the trend for other antibody imaging development, the investigators systematically evaluated fragments of the polyclonal antibody radiolabeled with different radionuclides and finally developed anti-myosin MAbs in order to optimize the imaging procedure (*137,138,139,140*). It was determined that fragments of the anti-myosin MAb were needed in order to lower the radioactivity in the circulation quickly and obtain images early. In-111-anti-myosin fragments have established clinical usefulness through multicenter trials (*141,142,143,144*) evaluating myocarditis (*145*), cardiac transplant rejection (*146*) and drug-induced cardiotoxicity (*147*) as well as myocardial infarction.

The radioimmunoscintigraphy of blood clots appears promising with the development of anti-fibrin MAbs that do not react with fibrinogen (*148,149*). Specificity for fibrin is extremely important since fibrinogen circulates in large quantities and would quickly bind a cross-reacting antibody and impede imaging results (*150*). Preliminary results of clinical trials for imaging fresh and aged venous thrombi indicate ^{111}In anti-fibrin MAb is highly sensitive in detecting clots even in patients on heparin therapy (*151,152,153*). More recently, anti-fibrin MAb Fab' fragments have been radiolabeled with ^{99m}Tc and studied in animals with images of induced thrombi becoming positive within 1–4 hr after injection (*154*).

MAbs that strongly bind to leukocytes have been shown to be reliable in the detection of sites of abscess and inflammation (*155,156,157*). MAbs specific for other cell populations including lymphocytes and platelets have also been identified (*158,159*). The immunologic labeling of cells with ^{111}In will bring a fresh approach to the studies which currently are conducted using ^{111}In-oxine. The oxine indiscriminately radiolabels all blood cells as well as transfers the radionuclide to plasma proteins which necessitates a potentially cell damaging separation method prior to addition of radioactivity. MAbs which bind exclusively to surface receptors on the respective cells allows intravenous administration of the ^{111}In cell specific MAb with earlier imaging times following administration (*160*).

CONCLUSIONS

When considering the advances in the use of radiolabeled MAbs that have occurred in only nine years, it is easy to have great expectations from the collaboration that is ongoing between immunology and nuclear medicine. There is hope that radioimmunoscintigraphy will play a significant role in the diagnosis and therapy of a number of disease states. The diagnosis and treatment of cancer is an area where nuclear medicine has not met expectations.

Future advances such as the development of "second generation" antibodies which recognize and bind to a different epitope or site on an antigen resulting in a stronger, more stable bond, should lead to increased uptake at the antigen site and improved target/non-target ratios (*161,162*). The development of human chimeric MAbs will decrease the concern over the possibility of an allergic reaction in those patients who need repeat infusions.

Further development of radiolabeled MAbs for diagnosis and therapy depends on the ability to move the radiolabeling technology into the clinical setting without compromising the quality of the final product. A list of some of the biotechnology firms making progress toward this goal is found in Table 5. Continued improvement of methods for radiolabeling with ^{111}In as well as other radionuclides for diagnosis or therapy should be made. The use of ^{111}In as a radiolabel for MAbs solves the problem of in vivo dehalogenation known to occur with radioiodinated MAbs and leads to improved targeting and stability of the preparation. A greater sensitivity of detection when compared with ^{131}I-labeled MAbs results from higher concentration in tumor and improved physical characteristics. The high degree of liver uptake seen with ^{111}In MAbs continues to be a problem in those studies where the liver is a possible site of metastasis.

Further improvements to optimize the radiolabel through improved labeling methods, better selection of MAbs and/or their fragments, use of a mixture of MAbs that bind to different antigens on the same target cell and fine-tuning of imaging techniques should help bring radioimmunoscintigraphy to the clinical setting.

TABLE 5. Monoclonal Antibodies in Clinical Trials

Company	Target site/Antigen bound
Cytogen	Colorectal/TAG-72
	Ovarian/TAG-72
	Prostate/prostatic carcinoma antigen
Hybritech	Colorectal/CEA
	Breast
NeoRx	Melanoma/HMWMAA
	Colorectal/tumor associated glycoprotein
	Lung/tumor associated glycoprotein
Immunomedics	Colorectal/CEA
	Abscess/WBC antigen
Centocor	Myocardial infarct/myosin
	Colorectal/17-1A

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