Continuing Education

Technetium-99m Imaging of Melanoma with Murine Monoclonal Antibodies

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This article on ^{99m}Tc monoclonal antibody and its specific usage in the detection of melanoma is the first in a five-part update series. Upon completion of this article, the reader will understand: (1) the benefits of ^{99m}Tc-labeled monoclonal antibodies; (2) the importance of careful labeling; and (3) the advantage of simultaneously imaging multiple organ systems.

Radioimaging of cancers using monoclonal antibodies linked to radionuclides is being pursued by a number of investigators. We report selected results of a multicenter clinical study of an imaging procedure using a ^{99m}Tc-labeled anti-melanoma antibody as part of the overall evaluation of suspected metastatic melanoma. Issues related to the labeling procedure and the use of these types of products in the nuclear medicine clinic are discussed.

Pressman, et al., demonstrated that iodine-131 (^{131}I) conjugated rabbit anti-rat kidney antibodies localized in rat kidney after intravenous (i.v.) injection (*1*). Since this first report of tissue-specific localization of radiolabeled antibody, numerous studies have been performed using polyclonal antibodies as carriers for the diagnosis and treatment of cancer (*2–8*). Monoclonal antibodies (*9*) offer advantages over polyclonal antibodies because they have greater specificity, purity, and wide availability, as well as lot-to-lot consistency. These factors have led to renewed interest in the use of radioconjugated antibodies for the detection and treatment of cancer.

Earlier studies employed ^{131}I, ^{111}In, or ^{123}I to radiolabel monoclonal antibodies (*10–22*). Although each of these radionuclides can be employed for tumor imaging using a monoclonal antibody, each has significant drawbacks. The gamma emission from ^{131}I is too high for optimal image resolution. In addition, its particulate beta emission and eight-day half-life limit the total dose that can be administered. Indium-111 may dissociate from the antibody and deposit in the reticuloendothelial system (RES), resulting in high radiolabel background due to slower clearance of the dissociated ^{111}In that was deposited in normal tissues. Of these radionuclides, only ^{123}I has a gamma ray energy ideal for imaging with conventional gamma cameras. However, as a 13-hr half-life cyclotron product, ^{123}I is both expensive and somewhat difficult to obtain.

Technetium-99m (^{99m}Tc), however, offers several advantages over iodine and indium as radioimmunodetectors. It has an ideal 140-keV gamma ray energy with a high photon flux that provides superior image resolution, and it is readily used in conventional nuclear medicine procedures. Its short physical half-life (6 hr) and lack of particulate radiation permits the administration of large doses (up to 30 mCi). Diagnostic images may be obtained in 7 to 8 hr. Fritzberg, et al. (*23*) have developed a diamide dithiolate chelating system that permits the stable labeling of antibodies with ^{99m}Tc.

There are now available monoclonal antibodies that react with a large variety of tumor-associated antigens with sufficient selectivity to be considered for clinical studies. Some or all of these antibodies may be useful in the diagnosis of metastatic disease when labeled with ^{99m}Tc. The slow disappearance of whole IgG antibodies from the blood however will require the use of more rapidly clearing F(ab')$_2$, Fab', or Fab fragments in order to achieve optimal tumor-to-background targeting with the imposed 6-hr half-life of ^{99m}Tc. This paper deals with some of the issues surrounding an imaging procedure using a ^{99m}Tc-labeled anti-melanoma monoclonal antibody (OncoTrac® Melanoma*) in patients with metastatic disease. Detailed reports of clincial investigations of this agent have been published elsewhere (*24,25*).

METHODOLOGY

Antibody Preparations

Three antibody preparations derived from murine hybridoma cells are used in the imaging procedure. Two nonradiolabeled antibody preparations are used to reduce the uptake of the radiolabeled antibody into normal tissue: a whole

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antibody preparation (NR-2AD) that does not cross-react with melanoma or any other normal human tissue and a nonradiolabeled whole antibody preparation (NR-ML-05) that binds specifically to the melanoma-associated 250KD glycoprotein/proteoglycan antigen (*26*).

The radiolabeled antibody preparation consists of a Fab fragment of NR-ML-05 that is radiolabeled with ^{99m}Tc just prior to administration. The imaging agent is provided as a cold kit that includes the antibodies and all the reagents needed to conjugate the imaging antibody with ^{99m}Tc, which is supplied by local radiopharmacies.

Technetium-99m Labeling

The first step involves the preparation of ^{99m}Tc-gluconate by reducing pertechnetate with stannous ion in the presence of a transfer agent (sodium gluconate). The ^{99m}Tc is then exchanged from ^{99m}Tc-gluconate into the ligand (diamidodithiol active ester) to form the ^{99m}Tc-ligand active ester. The antibody is conjugated with the ^{99m}Tc-ligand active ester to form the labeled antibody-ligand conjugate. Purification of the radiolabeled antibody is by ion exchange chromatography.

Technetium-99m is used in most conventional nuclear medicine imaging procedures, and it is therefore a very familar isotope to nuclear medicine technologists. However, the use of ^{99m}Tc to label antibodies is a recent development and presents new challenges in nuclear medicine. As biologicals, monoclonal antibodies must be handled in a different manner than the inorganic coumpounds commonly used for ^{99m}Tc imaging procedures such as bone imaging. Antibodies are subject to denaturation or damage of their capacity to bind to antigen during the radiolabeling procedure.

The OncoTrac® Melanoma labeling procedure is unique in that the labeling process consists of both a covalent chemical linking step and a metal chelation step. As indicated in Figure 1, the initial step of the labeling process is to chelate ^{99m}Tc to the ligand complex. Since this first step takes place prior to addition of the antibody, the chelation step can be carried out in rather harsh conditions (pH 2.8–3.1, 75°C) that would damage the antibody. The second step consists of a covalent linkage of the preformed chelate to the monoclonal antibody at basic pH. Proper pH adjustment of the preformed chelate is critical in order to ensure optimal conjugation of the antibody. The preformed chelate labeling process results in both a very gentle labeling procedure for the antibody and defined chemistry. Consequently, the immuno-reactivity of the radiolabeled antibody remains high. All of the ^{99m}Tc bound to the antibody is bound through the covalently attached ligand, yielding an extremely stable radioimmunoconjugate.

The labeling procedure can be performed by individuals with standard nuclear medicine technology background. However, the procedure does require a certain degree of training, as well as attention to detail.

Study Protocol

Eligibility for the study required that the patient be over eighteen years of age and that he or she have a histologically confirmed diagnosis of melanoma UICC Stage II or III. The

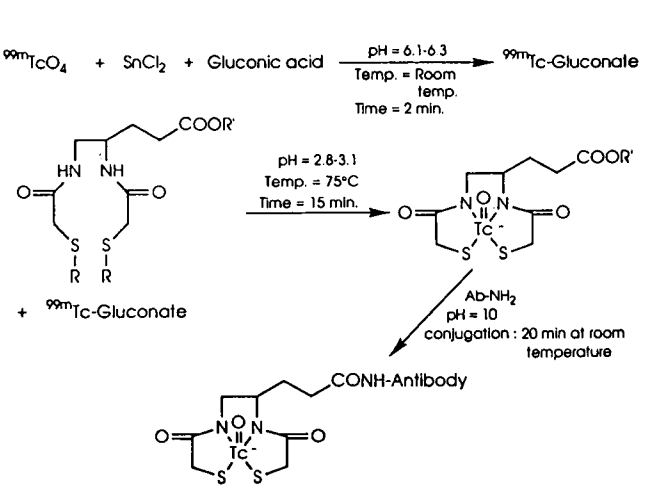

FIG. 1. Radiolabeling procedure consists of reducing pertechnetate ($^{99m}TcO_4^-$) using the stannous ion to ^{99m}Tc-gluconate, which then serves as a transfer agent for chelation of ^{99m}Tc into the N_2S_2 ligand. The chelation procedure is accompanied by loss of groups represented by an "R". The final process is the covalent linkage produced by the conjugation of an active ester (R') with the lysine amine of the antibody molecule.

patients must have had at least one evaluable lesion by physical examination or other diagnostic procedure.

Patients received an i.v. infusion of 10 mg of NR-ML-05 Fab fragment radiolabeled with 10 to 30 mCi ^{99m}Tc. This was preceded by an i.v. infusion, 30 min prior to the radiolabeled antibody, of 40 mg of unlabled NR-2AD. Five minutes prior to the administration of the radiolabeled dose, 7.5 mg of unlabeled whole NR-ML-05 was infused. A cathartic was administered at an appropriate time to clear bowel activity resulting from hepatobiliary excretion of radiolabel. Patients were imaged 6–10 hr after antibody infusion. The schedule of antibody administration is indicated in Table 1.

Methods of Gamma Camera Imaging

Each patient had a total-body survey performed by multiple anterior and posterior planar large-field-of-view images or by full-length anterior and posterior total body views. Left and right lateral views of the head were encouraged. In each case, selective spot views of areas of known disease or suspected occult lesions were obtained to augment lesion visualization or verify lesion position. Although not required, additional SPECT images were encouraged of lungs, liver, and other organs in order to improve the detection or positioning of a lesion.

Quality assurance testing was done on the day of imaging to confirm good field uniformity, resolution, linearity of the gamma camera using at least 2×10^6 counts per image. Tests were done using ^{99m}Tc sources. In all cases, a high-resolution, low-energy collimator was used. The initial imaging began with an anterior thorax view obtained for 10^6 total counts, which took ~ 5 min. All subsequent survey views were done for the same amount of time. Special spot views such as obliques and laterals used individual count acquisitions and

TABLE 1. Schedule of Antibody Imaging Study

−30 min	40 mg (in 20 mL)*	NR-2AD (whole): (Nonmelanoma antibody)
−5 min	7.5 mg	Anti-melanoma NR-ML-05 (whole): (Unlabeled anti-melanoma antibody)
0 min	5–10 mg†	Anti-melanoma NR-ML-05 (Fab): labeled with 10–30 mCi ^{99m}Tc
4–5 hr		Cathartic
7–8 hr		Imaging

* Diluted in normal saline and infused intravenously over 3–5 min.

† The amount of labeled antibody is based on mCi of activity, which varies depending on the radiochemical yield of the labeling procedure.

intensities in order to best elucidate the lesions. The energy window was 15% centered at 140 keV (132.5–147.5 keV) or on the full width at half maximum (FWHM) of the photopeak.

Occasionally, in fairly obese patients, subtle increased activity artifacts were noted in the skin folds of the axilla or breast. This was due to low angle forward scattering near skin folds with little attenuation at the skin surface. In the axilla, for example, this was confused with labeled lymph nodes and was checked by re-imaging with the arms extended overhead.

RESULTS

The antibody imaging procedure for metastatic melanoma can define and potentially extend definition of the disease. The multicenter clinical study of the OncoTrac® Melanoma agent consisted of 145 patients with a total of 622 clinically identifiable lesions (*25*). After antibody imaging, 3% were determined not to be melanoma and an additional 204 lesions were detected that were previously unsuspected. Forty-six percent of these previously unsuspected lesions were evaluated and by a second diagnostic modality, determined to be melanoma. Twelve false-positive lesions were imaged. The false-positive lesions resulted from localization to non-tumor tissues, including two benign thyroid nodules, a meningioma, two vascular lesions, four areas of inflammation, two lesions of uncertain etiology, and one area of artifact from radiolabel activity in the gut.

Overall detection rates were 87% for liver and bone lesions, 68% for subcutaneous lesions, and 77% for lymph node lesions. For unknown reasons, detection of pulmonary lesions was less, ~40%. The combination of results from physical examination, chest x-ray, and antibody imaging accurately staged 97% of the patients in the study. In comparison, "routine evaluation" of these patients based upon a physical examination, chest x-ray, and special diagnostic procedures (bone scan, computerized tomography, magnetic resonance imaging) performed because of specific symptoms or physical findings accurately staged 92% of patients (Table 2). The advantage of the antibody imaging procedure is that one procedure can be used to simultaneously image multiple organ systems throughout the body.

CASE REPORTS

The following cases were selected to provide examples of how the antibody imaging procedure can be of value in the management of patients with melanoma.

Case Number 1. A 62-yr-old male had an area of lymphadenopathy in the right neck that was defined by CT and proven by biopsy to be melanoma. After injection of the antibody imaging agent, SPECT images clearly demonstrated two individual lesions in this region and more extensive disease than was previously believed. After the extent of the cervical disease was recognized, surgical resection was canceled, and radiotherapy and chemotherapy were instituted. Antibody imaging thus affected patient management by accurately defining the extent of the soft tissue involvement. As illustrated in Figure 2, this case also illustrates how SPECT imaging provided greater detail than planar imaging.

Case Number 2. A 69-yr-old male had known metastases in the right femur and a palpable left axillary node. Both of these areas of metastasis were seen in the antibody images. In addition, the antibody images revealed unsuspected lesions in the liver, as well as in the left axillary and subclavian lymph nodes (Fig. 3). While the management of this patient did not change, the identification of previously unsuspected organ involvement is of value in the management of patients thought to have limited disease.

Case Number 3. This 43-yr-old male developed melanoma on the right side of his neck in January 1978. A superficial right parotidectomy and radical neck dissection was performed in February 1978. In August 1980, a CT examination of the head demonstrated a lesion in the left basal ganglion that was presumed to be melanoma. This lesion was subsequently irradiated. In May 1987, a bone scan demonstrated an abnormality in the area of the right 7th and 8th ribs and a right kidney mass was found on abdominal CT. The patient was referred for the antibody imaging procedure with a diagnosis of Stage III malignant melanoma. Because the right rib lesion and the right renal mass were not seen in the antibody images, the kidney mass was biopsied and proved to be renal cell carcinoma. The rib was presumed to be metastatic renal cell carcinoma or a fracture. In this case, the negative results of the monoclonal antibody imaging redirected the patient's diagnostic workup and led to the establishment of the correct diagnosis.

TABLE 2. Detection Rates of Diseased Organs or Diseased Organs with Metastases

Lesion site	OncoTrac® imaging alone	OncoTrac® plus chest x-ray and physical exam
Liver	91	96
Bone	95	96
Subcutaneous	79	100
Lymph Node	78	94
Lung	55	92

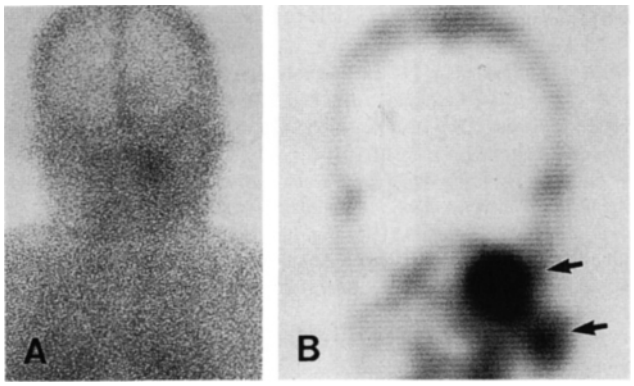

FIG. 2. A 62-yr-old male with one area of lymphadenopathy in the right neck defined by CT and proven by biopsy to be melanoma. Gamma camera view of the posterior neck (A) shows a definite but not well delineated area of increased activity on the right. (B) A coronal tomographic (SPECT) section through the same area clearly shows two individual areas of increased activity (arrows), suggesting more extensive disease than previously known. SPECT was very useful in demonstrating good detail in an area of low contrast due to overlying tissue activity. [Reprinted with permission from Salk D. and the Multicenter Study Group, *Semin Oncol* 1988; 15:608–618.]

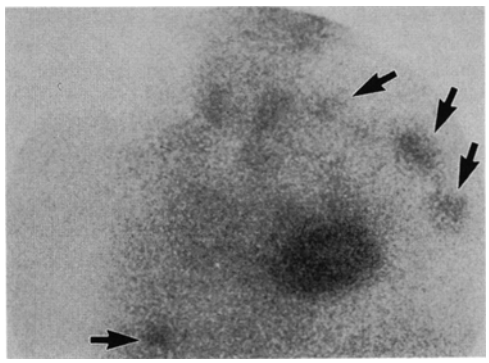

FIG. 3. A 69-yr-old male with known metastases in the right femur and a palpable left axillary lymph node, both of which were seen in antibody images. Anterior gamma camera view of the chest also shows multiple metastases to higher left axillary and subclavian lymph nodes and a single hepatic metastasis (arrows), none of which were seen on chest x-ray. Retention of activity in the myocardium is commonly seen and probably due to low levels of a cross-reactive antigen. [Reprinted with permission from Salk D. and the Multicenter Study Group, *Semin Oncol* 1988; 15:608–618.]

CONCLUSION

Tumor imaging with monoclonal antibodies is a technique of great promise and is expected to become a routine procedure in nuclear medicine. Due to the biologic nature of the antibody, special care must be taken in the radiolabeling and handling of the preparation to avoid loss of antibody immunoreactivity.

The potential role of antibody imaging in melanoma has been previously described (*25*). The imaging procedure for melanoma is safe and sensitive. As part of a basic evaluation including a physical exam and chest x-ray, antibody imaging identifies a large percentage of patients with metastatic disease. It provides rapid staging with a single test and extends the scope of detection by routine diagnostic imaging to subcutaneous tissues and lymph nodes. It can delineate the extent of regional lymph node involvement beyond that routinely detected by physical exam or chest x-rays, allowing better informed decisions about patient management. The high detection rates for lesions in the liver and bone allow for accurate evaluation of both these organs with a single procedure; at the same time, information is obtained about other regions and organs beyond those to which the physician's interest is initially directed by the patient's symptoms. These features are especially suited to the evaluation of melanoma, where the pattern of metastatic disease is variable. Accurate delineation of metastatic involvement or identification of distant metastasis may reduce the extent of surgery that is indicated, or may spare a patient unnecessary surgery. Other diagnostic tests can be directed to positive areas for confirmation, if necessary, or to suspicious negative areas for further clinical staging by a complementary modality. The majority of patients will require no further workup beyond the antibody images, with perhaps an occasional selected confirmatory test. Antibody imaging is useful as a second, complementary test when the cause of a patient's symptoms is not identified by another diagnostic modality that has already been performed, or when corroboration is desired for the antigenic/histologic nature of a lesion seen by another diagnostic modality.

In addition to its diagnostic value, antibody imaging has also shown utility in selection of patients for antibody-targeted therapy. At our institution, ^{99m}Tc-labeled monoclonal antibodies have been used to select suitable patients for entry into radioimmunotherapy studies using rhenium-186- (^{186}Re) labeled monoclonal antibodies. Rhenium-186 is a beta emitting radioisotope with therapeutic potential. Since ^{186}Re and ^{99m}Tc share many physical properties, the same ligand system described here for ^{99m}Tc has been used to label antibodies with ^{186}Re. In a current Phase I dose escalation study of patients with colon cancer, ^{99m}Tc-labeled antibody is used to evaluate the reactivity of a given patient's tumor with the antibody, and serial gamma camera image data are used to estimate the radiation exposure to the tumor and to various normal organs from the projected dose of ^{186}Re-labeled monoclonal antibody. The use of the ^{99m}Tc-labeled antibody in this manner provides for a rapid means of assessing eligibility for the therapeutic portion of the study as well as a means for selecting the appropriate therapy dose based upon normal organ radiation exposure.

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NOTE

*Oncotrac® Melanoma, NeoRx Corp., Seattle, WA.

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