

Use of Gallium-67 Citrate and Indium-111 Monoclonal Antibodies in the Detection of Metastatic Melanoma

Metastatic melanoma is a cancer that may metastasize rapidly via the bloodstream and lymphatic system. Two nuclear medicine imaging procedures can provide invaluable information regarding the location and size of metastatic sites. Tomographic scanning with gallium-67 (^{67}Ga) citrate has shown promising results and monoclonal antibodies labeled with indium-111 (^{111}In) are currently in the clinical research phase.

MALIGNANT MELANOMA: THE DISEASE

Melanoma was considered a rare tumor in the past; however, there has been increased interest surrounding this disease. The frequency of melanoma has increased in the general population recently. Worldwide, mortality rates have almost doubled over the past 20 years, although mortality varies widely depending on ethnic group: Australia and New Zealand have the highest mortality rates, whereas Japan and Hong Kong have the lowest mortality (1). Physical characteristics also seem to play a role in the incidence of melanoma: patients with malignant melanoma are more likely to have light colored eyes, light complexions, and light hair color, and are also more susceptible to sunburn. The primary site in men is usually the trunk, whereas the primary site in women is usually the lower limbs. In addition, genetic inheritance may be a factor in melanoma. Studies have shown families with unusually high incidences of malignant melanoma throughout the generations.

Exposure to the sun and repeated injury to nevi (dark moles) may be etiologic factors of malignant melanoma. The average person has approximately 21 nevi. Moles that are frequently irritated or injured should be surgically removed (1).

Most melanomas arise from the melanocytes of the basal layer of the epidermis (Fig. 1). Malignant melanoma evolves as a result of various types of premalignant lesions. The first type is known as Hutchinson's melanotic freckle (an accumulation of melanin). Hutchinson's melanotic freckle was first described in 1892 as senile freckle. It has been estimated that only 5% of Hutchinson's melanotic freckles actually become malignant. The latent period between the appearance of Hutch-

inson's freckle and malignancy can last anywhere from 5 to 40 years. Invasive melanoma may begin with a preinvasive phase, such as dysplastic nevus (a precursor of melanoma that frequently occurs on the back, it has an irregular border and can consist of a variety of colors) or a nonmalignant precursor, or it may infiltrate the underlying tissue immediately (1,2).

There are three different histologic types of malignant melanoma: 1) lentigo maligna melanoma, 2) superficial melanoma, and 3) nodular melanoma.

Melanoma begins frequently as an enlargement of a preexisting mole. The mole may change by becoming darker, or it may become raised and ulcerated. Slight bleeding is yet another warning sign. Pigmented lesions that show any of these signs should be removed immediately (3), since malignant melanoma metastases are common.

The survival rates have improved over the years because of early diagnosis and better surgical treatment; however, many patients still die as a result of melanoma (2). Presently, surgery offers the greatest hope for cure. The excision must be wide and deep. The 5-year survival rate for Stage I (localized invasive melanoma) is 50%–80%. When regional metastases are present, the 5-year survival rate is only 15%–40%.

Regional chemotherapy has been widely used in malignant melanoma. The chemotherapeutic agent is delivered continuously via a drip method into the artery that supplies blood to the affected limb. This procedure may be selected for treatment of a limb with localized invasive melanoma. Regional chemotherapy can achieve excellent results in controlling regional metastases and the primary melanoma site. The best results are achieved when it is used in conjunction with surgical excision of the primary site along with regional lymph node excision. The 5-year survival rate for regional chemotherapy and surgery for patients with regional disease is 39%, with a 15-year survival rate of 35%.

Immunotherapy has shown occasional dramatic results, but these results have not been reproducible. The use of monoclonal antibodies labeled with a high energy isotope, such as iodine-131 (^{131}I), is still in the research phase. Immunotherapists are hoping that they will be treating cancer patients in the near future with this individualized therapy, which will be targeted to only the cancer cells, leaving the healthy cells to reproduce and take the place of the cancer cells.

Radiation therapy can be an effective palliative treatment

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TABLE 1. Stages of Malignant Melanoma

Stage I:	Local disease.
IA:	Primary lesion only.
IB:	Primary satellites within 5 cm of primary site.
IC:	Local recurrence within 5 cm of primary site.
ID:	Spread more than 5 cm of primary site but within primary lymphatic drainage area.
Stage II:	Nodal disease (regional draining nodes).
IIA:	Regional lymph nodes (clinically positive, histology not done).
IIB:	Regional lymph nodes (clinically negative, histology positive).
IIC:	Regional lymph nodes (clinically positive, histology positive).
Stage III:	Disseminated disease.
IIIA:	Remote cutaneous and/or subcutaneous melanoma.
IIIB:	Remote nodal involvement only.
IIIC:	Both IIIA and IIIB.
IIID:	Visceral spread.

TABLE 2. Radiation Doses for Gallium-67 Citrate

Organ	Dose	
	rad/mCi	mGy/GBq
Whole body	0.26	70.2
Liver	0.46	124.3
Marrow	0.58	156.7
Spleen	0.53	143.2
Upper large intestine	0.56	151.4
Lower large intestine	0.90	243.2
Gonads	0.26	70.2

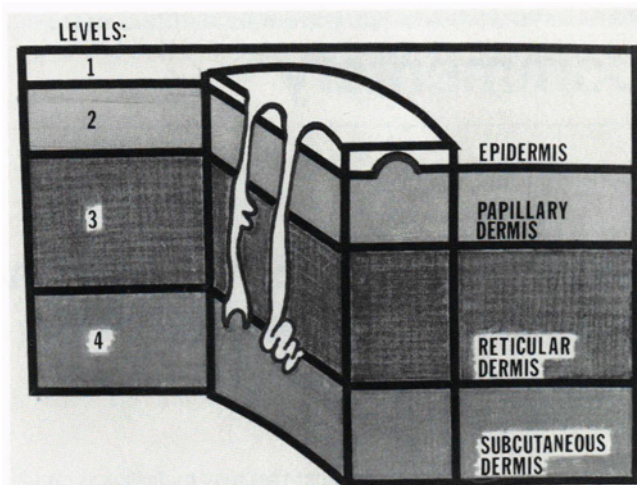
when used in high doses with long intervals between treatments. Radiotherapy has a palliative effect on metastatic melanoma of the skin, soft tissue, lymph nodes, bones, and brain.

Two classification schemes are used to describe and stage malignant melanoma. The Clark classification describes the level of skin involvement (Fig. 1), while the New York University Melanoma Classification Group (NYUMCG) scheme deals with staging the disease according to tumor size, lymph node involvement, and metastases (Table 1).

Treatment outcome and patient prognosis are very dependent on the disease stage, which in turn depends on the number of metastatic sites existing in the patient. The goal of the nuclear medicine diagnostic study in the patient with malignant melanoma is to determine the number of metastatic sites.

IMAGING OF MALIGNANT MELANOMA WITH GALLIUM-67 CITRATE

Gallium-67 citrate is a commonly used radiopharmaceutical with four principle gamma ray emissions: 93 keV(41%), 185 keV(23%), 300 keV(18%), and 394 keV(4%). The radiation

**FIG. 1.** Demonstration of Clark's classification of melanoma with the corresponding layers of skin.

doses to various organs for a diagnostic quantity are given in Table 2 (4).

Gallium-67 is not an optimal radionuclide for imaging with gamma camera systems. It has a complicated spectrum of gamma rays. Imaging with the low energy photons produces problems with scatter rejection or intrinsic resolution, whereas the high energy gamma rays are not detected efficiently by thin sodium iodide crystals. Collimators with thick septa are required to prevent septal penetration.

The exact mechanism of ^{67}Ga localization in tumors and abscesses is unknown. Experiments have provided evidence that ^{67}Ga has a strong affinity for iron-binding proteins, such as transferrin, lactoferrin, ferritin, and siderophores. After intravenous injection, the plasma clearance is slow; at 48 to 72 hours, approximately 5% is in the liver, 2% is in the kidneys, and 25% is in the bone and bone marrow (5). During the initial 24 hours the kidneys are the major route of excretion. Gallium-67 citrate normally localizes in the liver, spleen, bone marrow, skeletal system, kidneys, colon, adrenals, and lungs, as well as the nasopharynx, lacrimal glands, salivary glands, breasts, and genitals. Certain chemotherapeutic agents, such as vincristine, may suppress the accumulation of ^{67}Ga citrate in the liver (4).

Several proposals have been made to explain how ^{67}Ga localizes in tumors. There is the possibility that the increased vascular permeability of tumor vessels and their enlarged extracellular space may be responsible for the initial accumulation of ^{67}Ga (4). The rapid proliferation of tumor cells, as well as the decreased pH in tumor fluid are also factors that may influence the accumulation of ^{67}Ga . The lower pH of the tumor fluid causes the ^{67}Ga citrate to dissociate, which then allows the ^{67}Ga ions to bind to proteins within the tumor cell. It has also been suggested that ^{67}Ga binds to lactoferrin and leukocytes. Gallium-67 bound lactoferrin may be responsible for the localization of ^{67}Ga in inflammatory processes and abscesses (5). Another study suggests that ^{67}Ga accumulates in tumors via a tumor-cell associated transferrin receptor that binds ^{67}Ga -transferrin complexes to the tumor cell (4).

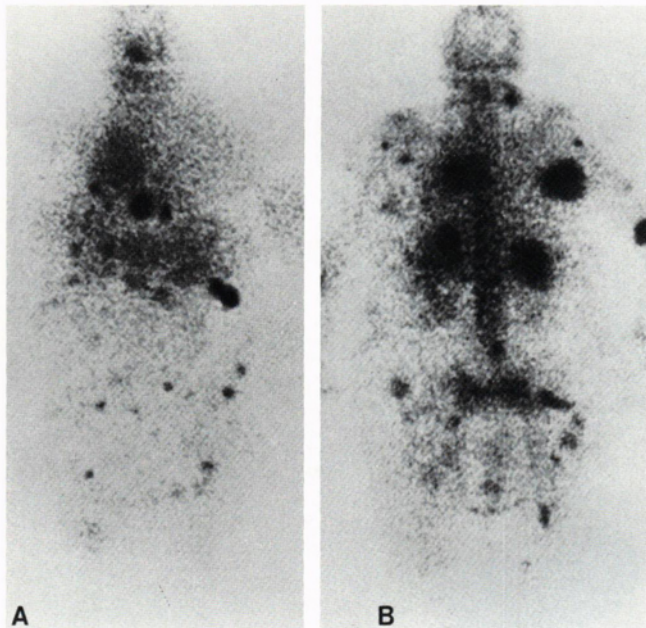


FIG. 2. Anterior (A) and posterior (B) projections of a whole body ^{67}Ga citrate image in a patient with multiple sites of melanoma.

The ^{67}Ga citrate is secreted by the intestinal mucosa and the appearance of radioactivity in the bowel often poses a problem for the diagnostician (5). There is controversy regarding the efficiency of routine bowel preparations (laxatives, purgatives, enemas) for ^{67}Ga citrate imaging.

Most melanomas concentrate enough ^{67}Ga to be imaged if the size of the tumor is at least 1–2 cm. A whole body Pho/Con* tomographic scan obtained 48 hours after injection of 10 mCi ^{67}Ga citrate is shown in figure 2. The use of selected views to complement the Pho/Con scan increases accuracy, especially for the head and the extremities. Gallium-67 may accumulate in recent biopsy and surgical sites, and should not be mistaken for tumor or metastases (6). The sensitivity of ^{67}Ga studies in the detection of melanoma was initially reported as approximately 50%, but recent studies have shown the sensitivity may be as high as 90% with the new techniques and advanced equipment in use today (4).

DETECTION OF MALIGNANT MELANOMA WITH INDIUM-111-LABELED MONOCLONAL ANTIBODIES

An antigen is a foreign substance that evokes an immunologic response. Antibodies for research are produced by injecting a small animal with antigen. The animal's immune system then produces antibodies that have unique molecular structures and bind to one or more receptor sites of the antigen. Polyclonal antibodies against the same antigen can have different molecular structures because they are made by unique lines of B-lymphocytes. Each lymphocyte line produces one specific antibody. Each antibody produced by the same line of B-lymphocytes will have the same sequence of amino acids, and they will all react in the same manner with only one antigenic

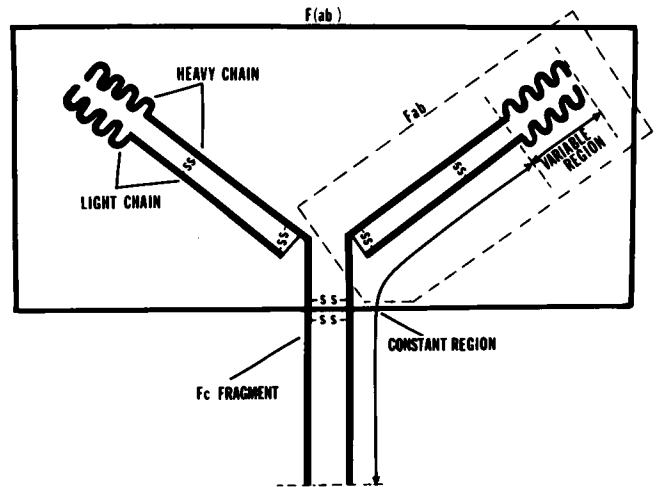


FIG. 3. Representation of the various regions and fragments of an IgG molecule.

determinant on the antigen (7).

It is impossible to obtain an antibody made by a specific B-lymphocyte unless the B-lymphocyte is separated from all other B-lymphocytes. This is known as cloning. Unfortunately, normal lymphocytes die when they are cloned. A way to produce large amounts of monoclonal antibodies by fusing antibody-producing lymphocytes from mice with mouse myeloma cells has been devised. The new hybridoma cells are able to survive cloning. These cells go on to produce their unique monoclonal antibodies (7).

The hybridomas function like cancer cells when implanted into the peritoneum of a mouse. The resulting tumor secretes the monoclonal antibody into the ascites of the mouse. This is one method used to harvest monoclonal antibodies. From both the B-cells and the mouse myeloma cells the hybridoma inherits the ability to proliferate continuously, making exact copies (clones) of themselves forever. The resulting antibodies are monoclonal: they all have the same affinity (attractive force between an antigen and an antibody). They are also of the same immunoglobulin class (IgG, IgM, etc.), the same molecular weight, and the same amino acid sequence (7).

Monoclonal antibodies' biodistribution and clearance rate vary according to the molecular weight along with other factors. Further production steps may be required to tailor the antibody to the objectives of a given in vivo study. Monoclonal antibodies are usually of the IgG class of plasma globulins. Immunoglobulin G antibodies have a molecular weight of about 150,000 daltons. They consist of two heavy chains and two light chains connected by disulfide bonds (Fig. 3). Part of the heavy chain is identical to the light chain. Immunoglobulin G molecules can be digested by enzymes to yield smaller molecular fragments that retain their immunoreactivity, but display different biodistributions in vivo. In some situations these fragments, when labeled with a radionuclide, have been found to produce images of better quality, and sooner after injection, than the whole IgG (7).

The enzyme papain can be used to cleave part of the heavy chain, leaving a Fab fragment that consists of the light chain

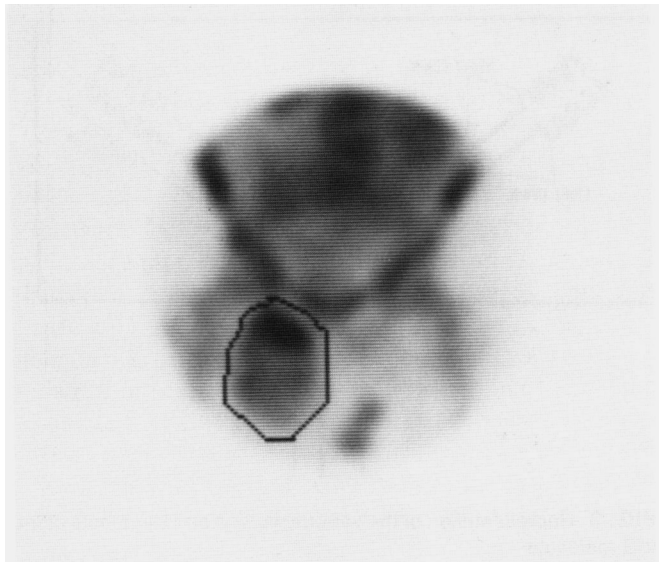


FIG. 4. Sixty-one-year-old man with metastatic melanoma. The primary site was on his right calf; as a result of the primary melanoma, his right leg was amputated above the knee. The enormous metastatic lesion seen in the region of interest above was detected 72 hours after injection of ^{111}In -DTPA monoclonal antibody specific to melanoma.

connected to a portion of the heavy chain by disulfide bonds. The Fab fragment weighs about 50,000 daltons. Two Fab fragments are formed for each antibody. The two heavy chains below the cleavage are referred to as the Fc fragment. The enzyme pepsin can be used to cleave the Fc fragment below a disulfide bridge. This leaves both arms of the antibody, including the light chains and their counterpart heavy chains, connected by a disulfide bridge and a small part of the Fc fragment (Fig. 3) (7).

The Fab fragment contains the sequence of amino acids known as the variable area, where the true specificity for the antigen occurs. The major portion of the antibody is made of amino acids; however, the Fc fragment contains carbohydrates. The function and effects of the carbohydrates on radioimmunoimaging are presently unknown (7), but some researchers feel that the Fc fragment may interfere with monoclonal antibody imaging.

Monoclonal antibodies are already being used for inexpensive pregnancy tests, diagnosing venereal disease, and determining blood types. They are also used in various assays for determining different antigens that may be present in the serum of plasma, such as hepatitis B. Precise localization of diseased or damaged tissues within the body is a promising new application of monoclonal antibodies. These antibodies have the ability to bind to specific antigens *in vivo*. By coupling monoclonal antibodies with a radionuclide, diseased tissue might be pinpointed after injecting the patient with the labeled antibody and imaging with a scintillation camera. The greater the accumulation of radiolabeled antibodies at a cancer site, the greater the chances are that it will be detected, and someday treated (7). Figure 4 is an example of a metastatic lesion that has accumulated a significant amount of tracer. The region of interest

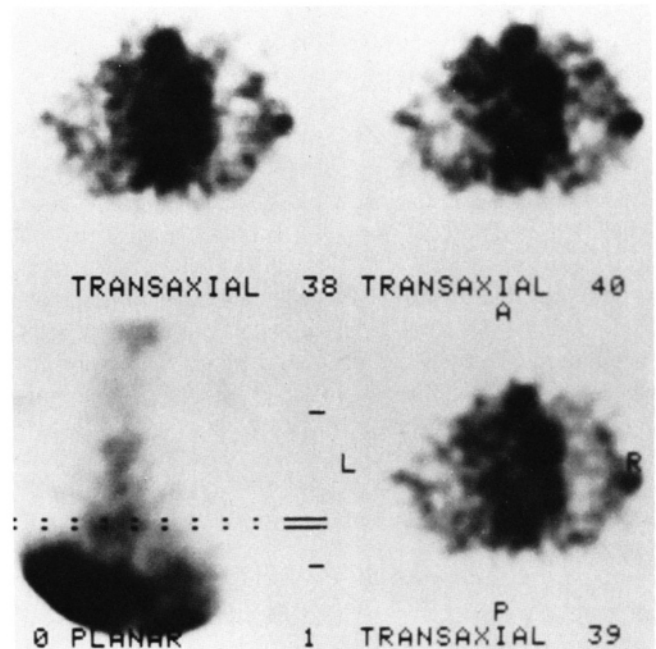


FIG. 5. SPECT generated ^{111}In -DTPA monoclonal antibody study. The horizontal lines on the planar view (bottom left) correspond with the transaxial slice 39; the remaining transaxial views correspond with the slices just above (38) and just below (40) the horizontal lines.

encircles a large metastatic lesion in the right thigh of a 61-year-old man. There are 138,000 counts in the region of interest. Unfortunately this was detected too late for this patient, but chances are that it won't be too late for many patients who undergo a ^{111}In -DTPA monoclonal antibody scan before their disease becomes as advanced as that illustrated.

$\text{F(ab}')^2$ seems to be the best choice for radioimmunodetection. It remains in the blood longer than the Fab fragments because its molecular weight is large enough to reduce its loss through the kidneys. Its divalency should keep the fragment on the antigen once it is attached, resulting in a higher extraction fraction by the tumor (7).

Monoclonal antibodies have many advantages over polyclonals. First of all, monoclonals are very specific, homogeneous proteins that react with immunologic identity. Second, it is possible to isolate a monoclonal antibody that reacts with a specific tumor-associated antigen, or antigenic determinant. Third, monoclonal antibodies are useful for *in vitro* monitoring of antigen levels, as well as *in vivo* tumor imaging. And fourth, large amounts of a single antibody can be produced from one hybridoma. Once a hybridoma is created it theoretically never stops reproducing (7).

To determine where the monoclonal antibody has localized in the body, a gamma-emitting isotope must be labeled to the antibody. Indium-111 has been widely used as a label for monoclonal antibodies. Indium-111 is produced by irradiating a source of cadmium-111 (^{111}Cd) with 15 MeV protons in a cyclotron. The ^{111}In is separated from the ^{111}Cd target by the solvent extraction method (5). The ^{111}In is attached to the antibody by a bifunctional chelation method. A chelating agent, such as DTPA, undergoes a chemical reaction in which one of its

carboxyl groups reacts with one of the amino acids (lysine) on the monoclonal antibody. The remaining four carboxyl groups on the DTPA molecule are then free to chelate (bind) the ^{111}In (7).

Whole body clearance of ^{111}In monoclonal antibodies is slow ($T_{1/2} = 160$ hr). The major route of excretion is via the urine at a rate of 0.26% of the injected dose per hour. At 24 hours after administration, the activity in the liver, spleen, and kidneys accounts for about 30% of the injected dose, circulating serum contains about 20%, and 50% of the activity is distributed throughout the interstitial spaces. The slow whole body clearance may increase the total tumor-to-antibody exposure, which will improve the accumulation of ^{111}In monoclonal antibody in the tumor. Optimal imaging time is 48 to 72 hours after administration (8).

The maximum accumulation in the spleen and kidneys is at 20 hours after administration, followed by a slow clearance. The mean value at 20 hours is about 2% of the initial dose in the spleen and 5% per kidney (8).

The liver has the greatest organ accumulation (20% of the injected activity). Maximum liver accumulation is achieved almost immediately and thereafter remains relatively stable. The mean value in the liver is $20\% \pm 8\%$ of the original activity at 24 hours after administration (8).

The clinical use of labeled monoclonal antibodies is very promising, but a number of challenges must be met before they can be used to their greatest capabilities. The development of better isotopic labeling chemistries may provide better localization. Indium-111 and $^{99\text{m}}\text{Tc}$ are two radionuclides that provide optimal characteristics for imaging. Imaging equipment also needs improvement. Planar nuclear imaging is slowly being replaced by SPECT (single photon emission computed tomography) (7). SPECT has a 94% detection rate, as compared to the 50% detection rate using planar views and subtraction techniques (7). Once the tomographic scan is acquired, transaxial images are generated from a selected planar view to determine the exact location of the tumor within the body (Fig. 4). Figure 4 demonstrates three transaxial slices and the planar view of a patient with multiple metastases between the sternum and vertebrae.

Although few direct adverse effects have been observed from injecting humans with animal-derived monoclonal antibodies, the immunogenicity of mouse proteins remains a concern. The development of human monoclonal antibodies for in vivo use is also underway (7).

The ultimate challenge of monoclonal antibody development awaits researchers all over the world: to apply monoclonal technology to in vivo therapy. It may be possible to produce monoclonal antibodies capable of destroying diseased cells

by triggering natural immune responses. Since it has already been shown that drugs, toxins, radionuclides, and radiosensitizers can be coupled with monoclonal antibodies, someday it may be possible to use monoclonal antibodies to deliver therapeutic substances to specific tissues that are reactive with the antibody, without harming the healthy surrounding tissue (7).

In summary, imaging with radiolabeled monoclonal antibodies will play a very important role in the future of nuclear medicine. Monoclonal antibodies offer new hope, diagnostically and therapeutically, for melanoma patients. The use of ^{111}In monoclonal antibodies and single photon emission computed tomography allows the best resolution in radioimmunoscintigraphy. Gallium-67 is not specific, which can result in false-positive readings. The specificity of ^{111}In monoclonal antibodies results in accurate, reliable diagnoses. Radioimmunoscintigraphy needs improvement, but it is the best technique for detecting malignant melanoma today.

NOTE

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