Radioimaging of Melanoma Using $^{99m}$Tc-labeled Fab Fragment Reactive with a High Molecular Weight Melanoma Antigen

Lamki M. Lamki, Alexander A. Zukowski, L. Joy Shanken, Sewa S. Legha, Robert S. Benjamin, Carl E. Plager, Darrell F. Salk, Robert W. Schroff, and James L. Murray

Deportments of Nuclear Medicine [L. M. L.]. Clinical Immunology and Biological Therapy [A. A. Z., L. J. S., J. L. M.] and Medical Oncology [S. S. L., R. S. B., C. E. P.], The University of Texas M. D. Anderson Cancer Center, Houston. Texas 77030, and NeoRx Corporation, Seattle, Washington [D. F. S., R. W. S.]

Abstract

Twenty patients with metastatic malignant melanoma were studied with $^{99m}$Tc-labeled monoclonal antibody (MoAb) Fab fragment (NR-MI-05) reactive with a high molecular weight (M, 240,000) melanoma associated antigen. Patients received 40 mg unlabeled irrelevant MoAb (NR-2AD-IgG) and 7.5 mg unlabeled NR-MI-05 (whole IgG) prior to infusion of 10 mg $^{99m}$Tc-labeled (10-25 mCi) NR-MI-05 Fab. Unlabeled MoAb were given to block nonspecific and specific binding sites. Gamma camera scans and single photon emission computed tomography were performed at 8 and 24 h postadministration. Of 172 preexisting lesions, 136 were imaged for a sensitivity of detection of 79%. Imaging was site and size dependent with the greatest sensitivity for liver lesions (100%) and the least for bowel (0%). Six sites (2 skin, 1 lung, 3 liver) were detected by single photon emission computed tomography that were missed on routine planar images. Forty-one additional unconfirmed sites were seen. Of these, 7 (17%) have been confirmed as tumor after a median follow-up of 6 months. False positive scans included scar tissue, areas of chronic inflammation, an infected femoral aneurysm, and septic emboli. Nonspecific uptake of radioactivity occurred in kidney, gallbladder, bowel, thyroid, and myocardium. Human anti-mouse antibodies were detected in up to 69% of patients. In summary, radioimaging with $^{99m}$Tc-NR-MI-05 is a sensitive test, especially for detecting liver lesions. It is safe, simple to administer, and convenient for the patient. Biodistribution and imaging sensitivity differ significantly from studies in which $^{111}$In-labeled anti-melanoma MoAb have been used.

Introduction

Diagnosis and treatment of metastatic malignant melanoma remain ongoing challenges to the medical oncologist. The incidence of cutaneous melanoma is currently on the increase with over 24,000 new cases diagnosed per year (1). Response to single or combination chemotherapy remains low and overall survival has not changed appreciably (2). Hence, the development of novel diagnostic as well as therapeutic approaches to this disease remains paramount.

Recent clinical trials have demonstrated the safety and feasibility of detecting melanoma using radioabeled MoAb. The majority of these studies have used either $^{131}$I (3-5) or $^{111}$In (6-13) as the radioisotope. Problems related to the use of $^{111}$In- or $^{131}$I-labeled MoAb, respectively, are unusual biodistribution and instability of the immunoconjugate. For example, dehalogenation of $^{131}$I-labeled MoAb (14) and a predilection for significant liver uptake of $^{111}$In-labeled MoAb (6) have often limited their utility as imaging agents.

The availability, favorable 6-h half-life, ideal $\gamma$ energy of 140 KeV, and lack of $\beta$-radiation make $^{99m}$Tc an attractive isotope for immunoscintigraphy trials. Adventitious binding of $^{99m}$Tc to protein has been largely circumvented through recent development of a stable, reproducible, relatively simple method of linking $^{99m}$Tc to MoAb via a bifunctional chelating agent (15). The use of monoclonal antibody fragments, such as Fab, rather than whole IgG, has been shown to greatly enhance image resolution, largely through more rapid clearance with subsequent decrease in background radioactivity and also greater specific localization of Fab compared to intact IgG in tumors (16). On the basis of these findings, we performed a pilot study to determine safety and sensitivity of $^{99m}$Tc-labeled anti-melanoma Fab fragment of MoAb, NR-MI-05, in detecting metastatic melanoma. The results of this trial are presented below.

Materials and Methods

Monoclonal Antibodies. NR-MI-05, a murine anti-melanoma MoAb of subclass IgG2b, was developed and purified for clinical use using standard hybridoma techniques by NeoRx Corporation, Seattle, WA. The MoAb recognizes a M, 250,000 high molecular weight proteoglycan antigen as described previously (17). Fab fragments were prepared using standard methods (18). A purified, irrelevant murine MoAb, NR-2AD (whole IgG2a), reactive with idiotypic determinants of surface IgG on a B-cell lymphoma was also supplied by NeoRx. NR-2AD does not cross-react with other tumors, including melanoma, or any normal tissues. Lyophilized MoAb were supplied in three different vials containing 20 mg/ml NR-MI-05 Fab (0.5 ml), 5 mg/ml NR-2AD (8 ml), and 5 mg/ml NR-MI-05 (1.5 ml).

Preparation of $^{99m}$Tc-MoAb Conjugate. An imaging kit was developed by NeoRx which uses the 2,3,5,6-tetrafluorophenyl active ester of sulfur protected 4,5-dithioacetamidophenolato, a diamide dimercaptide $N_2S_2$ complex (15). The $^{99m}$Tc-$N_2S_2$ complex was prepared by reconstituting stannous gluconate with 1 ml of sterile water and then mixing 0.75 ml of $^{99m}$Tc-pertechnetate (75-100 mCi) with this solution.

$^{99m}$Tc ligand ester was formed by pH adjustment of stannous gluconate solution using a glacial acetic acid-0.2 N hydrochloric acid solution and buffers. MoAb were added to the above reaction mixture and incubated for 20 min at room temperature, and the complex was purified using ion exchange columns and Millipore filters. Total radioactivity in the radioiodolated MoAb is measured using a dose calibrator. Labeling yield was typically 20 to 40% with a labeling efficiency of 90 to 95% as assessed by thin layer chromatography.

Patients and Study Design. Twenty patients with metastatic malignant melanoma were studied (16 with cutaneous and 4 with ocular melanoma). There were 17 males and 3 females with a median age of 41 years. All patients were ambulatory and had at least one measurable lesion as documented by physical examination and/or conventional investigations including plain radiography, ultrasound, or computerized tomographic scans. Five patients had no previous therapy, eight had received previous chemotherapy, two previous immunotherapy, and five both previous chemotherapy and immunotherapy. Patients had not received any treatment for a minimum of 2 to 3 days prior to the study. All participants gave oral and written consent to receive the radioconjugate in accordance with guidelines established by the Surveillance Committee at The University of Texas M. D. Anderson Cancer Center.

On the morning of study, patients received 40 mg of unlabeled MoAb NR-2AD i.v. in 30 ml of normal saline over 5 min. Twenty-five min later, 7.5 mg of NR-MI-05 (whole IgG) administered in similar fashion, followed 5 min later by 10 mg of NR-MI-05 Fab labeled with 10 to 25...
mCi $^{99m}$Tc. Irrelevant MoAb NR-2AD IgG and unlabeled NR-M1-05 IgG were given prior to radiolabeled MoAb Fab fragment because an earlier study demonstrated blocking of the reticuloendothelial system with enhanced uptake in tumor (18). Patients were observed for signs of toxicity from 1 to 2 h following MoAb administration. All patients received cathartics during this time to purge the bowel of nonspecific radioactivity.

All patients underwent nuclear scanning procedures 6 to 8 h following MoAb administration. These included total body digital scanning performed at 10 cm/min anterior and posterior simultaneously using a dual-head large field-of-view gamma camera (Toshiba Medical Systems, Tustin, CA) followed by multiple digital planar spot views of chest, abdomen, and pelvis (anterior and posterior); as well as skull and extremities when required. These were performed using GE Starcam gamma camera with a built-in Star computer (General Electric Medical Systems, Milwaukee, WI). Each image was acquired for 5 min at 512 x 512 matrix. In most patients, the spot views were repeated at 24 h but were acquired for 10 min each.

SPECT was performed for the chest and upper abdomen using the same GE Starcam camera at 64 x 64 matrix, 128 stops of 20 s each.

All scans were initially reviewed in blinded fashion by one or more experienced nuclear medicine physicians, and findings were then correlated with lesions detected by physical examination and other radiographic techniques. Sensitivity was defined as the number of documented lesions associated with increased radioactivity relative to background divided by the number of lesions documented by other techniques. Areas of increased uptake at sites not previously known to harbor cancer were termed "uncorrelated lesions." Attempts were made to determine whether uncorrelated lesions were occult tumors through routine follow-up examinations.

Assay for HAMA. In 13 of the 20 patients studied, sera were drawn at 5, 8, and 16 weeks and analyzed for HAMA response using a previously published enzyme linked immunosorbent assay technique (19). Results were reported as normal human serum units, which are arbitrary units assigned to the enzyme linked immunosorbent assay results compared to a standard pool of more than 100 individual normal donors. Antiglobulin titers were considered elevated if they were greater than 2 SD above mean normal human serum units for the controls.

Results

Imaging Results. Optimal images were obtained at 7 h following infusion of $^{99m}$Tc-NR-M1-05 Fab; 24-h scans were generally inadequate due to low counts. An example of a typical 7-h total body scan is shown in Fig. 1A. There was normal localization of the labeled MoAb in myocardium (Fig. 1B), kidneys, gallbladder, and bone marrow. In two patients, we observed localization in the thyroid. There was no evidence that this normal distribution was that of free $^{99m}$Tc, but rather labeled Fab fragment of the antibody. There was very little uptake in the liver or spleen tissues, allowing metastases to be readily detected in these organs.

Table 1 summarizes the overall imaging sensitivity for previously known metastases in various organ sites from all 20 patients. Data are presented as the number of lesions detected by planar scans, planar and SPECT, and SPECT alone. One hundred thirty-six lesions were imaged compared to 172 previously known sites, giving a sensitivity of 79% (or false negative imaging rate of 21%). Imaging sensitivity was size and site dependent. The greatest sensitivity of detection occurred in liver and spleen with 100% of tumors appearing as areas of increased radioactivity relative to background. Only two of ten lesions less than 1 cm were imaged. In some cases, this finding accounted for the decreased sensitivity of detection in s.c. sites as well as brain and bowel, although several lesions >1 cm were also missed, chiefly in the lung. In 6 instances (2 s.c., 1 lung, 3 liver) lesions missed on routine planar scans were detected by
SPECT imaging. SPECT, however, could be performed only in one area of the body in each patient (Table 1), typically the chest and including the upper abdomen unless otherwise indicated.

There were also 41 areas of increased radioactivity observed which did not correlate with previously known areas of disease. Of these, 7, or 17% were eventually confirmed as being tumor by routine X-rays, scans, or physical examination after a median patient follow-up time of 6 months (3 cutaneous, 3 bone, and 1 lymph node metastases). Unfortunately, a large number of foci suspected to be "occult" s.c. or bone metastases could not be confirmed in two patients, due to early death (1 patient) or loss from follow-up (1 patient). If the additional 7 sites of disease are taken into account the overall sensitivity of the technique increases to 82%.

In nine instances increased foci of radioactivity were observed to localize nonspecifically in areas of old scar tissue, inflammation, or infection. An example of increased localization of conjugate in an area of infection is shown in Fig. 2. A 43-year-old male developed fever and septic emboli in his right foot and tibia shortly after receiving radiolabeled MoAb. Blood cultures were positive for staphylococcal organisms due to an infected femoral aneurysm of his right groin area where an intraarterial catheter had been placed. Punctate areas of increased radioactivity were seen in his foot (Fig. 2) and also in the groin area.

HAMA Response. No toxicity to the MoAb infusions was observed in any patient. Six of 13 patients (46%) tested for HAMA had elevated titers reactive with specific MoAb NR-MI-05, whereas 9 of 13 (69%) had elevated titers to the irrelevant MoAb NR-2AD, and 1 patient had HAMA reactive with polyclonal murine IgG (Table 2). These differences were statistically significant (P < 0.05, χ² test). Three of nine patients had HAMA which were monospecific (i.e., reacted with only NR-2AD), whereas six of the nine patients had HAMA which reacted with both NR-MI-05 and NR-2AD.

Discussion

The results of this trial suggest that the administration of ⁹⁹ᵐTc-labeled anti-melanoma MoAb (Fab fragment) is a convenient, safe, reproducible method of detecting metastatic melanoma. It offers additional advantages over traditional diagnostic techniques of being specific for melanoma and being able to image about 80% of tumors at a single time point during the same day of administration. It suffers from problems similar to those seen in other immunoscintigraphy trials: (a) a fairly high incidence of false negatives (21%); (b) inability to reliably detect tumors smaller than 1 cm which in many cases resulted in a decreased sensitivity of detection; and (c) the development of human antigramulbin against murine antibodies in 40 to 70% of patients. Additionally, (d) the N₂S₂ method of labeling the Fab with ⁹⁹ᵐTc is still fairly laborious taking 1.5 to 2 h to perform. The sensitivity of this technique appears as good as if not superior to the results from other immunoscintigraphy trials in melanoma. A sensitivity of tumor detection of 79% was comparable to that seen by Siccardi et al. (12) using ⁹⁹ᵐTc-labeled F(ab')₂ fragments of a similar MoAb, and Larson et al. (3) using ¹³¹I-labeled Fab fragments of 96.5, a MoAb which recognizes the separate M, 97,000 antigen, and 9.2.27, a MoAb recognizing M, 240,000 glycoprotein (4). It is slightly superior when compared to trials in which ¹¹¹In-labeled MoAbs have been used (i.e., 50 to 70% sensitivity), although in the majority, whole IgG rather than Fab fragments were studied (6–13). Undoubtedly, the combination of excellent physical properties of ⁹⁹ᵐTc and the use of the Fab fragment have contributed to the relatively better results observed by us.

Table 1 Imaging sensitivity of ⁹⁹ᵐTc-labeled MoAb Fab-NR-MI-05 for previously known melanoma lesions

<table>
<thead>
<tr>
<th>Lesion site</th>
<th>No. of known lesions</th>
<th>Planar only*</th>
<th>SPECT only</th>
<th>SPECT and planar</th>
<th>% of total lesions imaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>49</td>
<td>1</td>
<td>2</td>
<td>32</td>
<td>76</td>
</tr>
<tr>
<td>Lung</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Brain</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Lymph node</td>
<td>22</td>
<td>4</td>
<td>0</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>Bone</td>
<td>26</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>81</td>
</tr>
<tr>
<td>Liver</td>
<td>26</td>
<td>0</td>
<td>3</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Orbit</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Bowel</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>30</td>
<td>6</td>
<td>100</td>
<td>79</td>
</tr>
</tbody>
</table>

* Total number imaged was 136 lesions.

Table 2 HAMA response to the antibodies injected

<table>
<thead>
<tr>
<th>HAMA to</th>
<th>No. of patients positive* / no. tested (%)</th>
<th>Range of HAMA titers (normal human serum units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-MI-05</td>
<td>6/13 (46)</td>
<td>7.65–26.6 (normal &lt; 7)</td>
</tr>
<tr>
<td>NR-2AD</td>
<td>9/13 (69)</td>
<td>2.1–43.7 (normal &lt; 1.8)</td>
</tr>
<tr>
<td>Polyclonal murine immunoglobulin</td>
<td>1/13 (8)</td>
<td>7.13 (n&lt; 5.5)</td>
</tr>
</tbody>
</table>

* Positive HAMA response defined as greater than 2 SD above titers for normal control population.

Discussion

The results of this trial suggest that the administration of ⁹⁹ᵐTc-labeled anti-melanoma MoAb (Fab fragment) is a convenient, safe, reproducible method of detecting metastatic melanoma. It offers additional advantages over traditional diagnostic techniques of being specific for melanoma and being able to image about 80% of tumors at a single time point during the same day of administration. It suffers from problems similar to those seen in other immunoscintigraphy trials: (a) a fairly high incidence of false negatives (21%); (b) inability to reliably detect tumors smaller than 1 cm which in many cases resulted in a decreased sensitivity of detection; and (c) the development of human antigramulbin against murine antibodies in 40 to 70% of patients. Additionally, (d) the N₂S₂ method of labeling the Fab with ⁹⁹ᵐTc is still fairly laborious taking 1.5 to 2 h to perform. The sensitivity of this technique appears as good as if not superior to the results from other immunoscintigraphy trials in melanoma. A sensitivity of tumor detection of 79% was comparable to that seen by Siccardi et al. (12) using ⁹⁹ᵐTc-labeled F(ab')₂ fragments of a similar MoAb, and Larson et al. (3) using ¹³¹I-labeled Fab fragments of 96.5, a MoAb which recognizes the separate M, 97,000 antigen, and 9.2.27, a MoAb recognizing M, 240,000 glycoprotein (4). It is slightly superior when compared to trials in which ¹¹¹In-labeled MoAbs have been used (i.e., 50 to 70% sensitivity), although in the majority, whole IgG rather than Fab fragments were studied (6–13). Undoubtedly, the combination of excellent physical properties of ⁹⁹ᵐTc and the use of the Fab fragment have contributed to the relatively better results observed by us.

The problem of liver uptake seen with ¹¹¹In-MoAb conjugates (6, 20) was nonexistent, which might conceivably be due to differences in chelation techniques, favorable characteristics of the ⁹⁹ᵐTc which, unlike ¹¹¹In, does not localize in the liver, and rapid in vivo clearance and metabolism of the Fab fragment (16). Rather than localize in liver, ⁹⁹ᵐTc-N₂S₂ had a propensity to target nonspecifically to bowel, gallbladder, kidneys, myocardium, and occasionally the thyroid gland. The ⁹⁹ᵐTc-N₂S₂ complex is excreted in bile and also by bowel lumen, and it is known that both F(ab')₂ and Fab fragments are metabolized and excreted by the kidney (12, 16). The mecha-
nism(s) underlying myocardial and thyroid uptake are unknown. The activity over the heart is a true myocardial uptake by 7 h when the scan is performed and not related to blood pool activity as evidenced by SPECT tomograms (Fig. 1B). There is no clear explanation for this myocardial activity. Preliminary studies by NeoRx failed to reveal reactivity of MoAb NR-M1-05 with myocardial muscle, although it does react with smooth muscles of the coronary arterioles. Also $^{99m}$Tc-labeled Fab-NR-M1-05 did not localize in the myocardium. Hence, the transient localization in myocardial interstitium is probably a peculiarity of the $^{99m}$Tc-[N$_2$S$_2$]-NR-M1-05 conjugate. Thyroid localization was observed only in two patients and it may have been a result of inflammatory or other thyroid disorder that we could not establish.

In six instances, SPECT was able to discern lesions that were not initially seen on planar films. In these instances where routine planar scans were negative, SPECT was useful in detecting questionable disease sites. An example is illustrated in Fig. 3. A 35-year-old white male presented to the medical oncologist with pulmonary nodule on chest X-ray (Fig. 3A). Following infusion of $^{99m}$Tc-NR-M1-05, planar scans of the chest failed to reveal an area of increased uptake (Fig. 3A). Coronal SPECT views of the chest, however, revealed a small area of increased uptake in the left upper lobe corresponding with the chest X-ray (Fig. 3C). The patient was taken to thoracotomy where a 1-cm metastatic nodule was removed. A phase III study using this antibody was able to confirm the usefulness of SPECT, particularly for detecting lung lesions (21). Larger prospective trials comparing SPECT to planar imaging are needed to determine the overall usefulness of this modality.

A 4% incidence of false positive scans was noted, chiefly involving areas of inflammation (such as arthritis), old surgical scars (including that of the primary melanoma), and infection. Whether these findings relate to increased blood flow and vascular permeability (22), shed and/or residual antigen, or occult disease in the affected areas remains unknown at present. Although very preliminary, the incidence of HAMA formation is comparable with an earlier phase I and II study of $^{99m}$Tc-labeled NR-M1-05 (18). The greater incidence of HAMA reactivity against the irrelevant MoAb NR-2AD is interesting and suggests that coadministration of irrelevant antibody may decrease HAMA against specific MoAb (18). In our study, 46% of patients had HAMA reactive with both NR-M1-05 and NR-2AD.

In summary, immunoscintigraphy of metastatic melanoma with $^{99m}$Tc-NR-M1-05 appears sensitive and is a relatively simple procedure to perform. Currently, it may be used as an adjunctive test along with conventional scanning procedures and appears capable of detecting occult disease up to 17% of the time in this patient group.

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*Unpublished observations.

$^5$ NeoRx, personal communication.


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