Radioimmunodetection of Human Melanoma with Indium-111-Labeled Monoclonal Antibody

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The purpose of the study was threefold: (1) to evaluate the efficacy of an ^{111}In-labeled murine monoclonal antibody (ZME-018) directed against a heavy molecular weight melanoma associated glycoprotein in localizing metastatic disease; (2) to determine the effect of unlabeled antibody mass (2.5, 5, 10, 20, and 40 mg) on labeled antibody blood clearance, biodistribution and lesion detection; (3) to estimate radiation dosimetry. Twenty-five patients with previously documented disease received an intravenous infusion of 2.5 to 40 mg of monoclonal antibody with 1 mg of the antibody labeled with 5 mCi of ^{111}In. There were no acute reactions. Patients were scanned without computer enhancement or background subtraction techniques at 24 and 72 hr after injection. Imaging detected tumor in 14/18 (78%) patients with active disease, identified 34/44 (77%) of lesions >1 cm and changed or specifically directed patient management in 22% (4/18) patients with tumor. There was a prolongation in blood clearance associated with decreased liver and spleen activity following administration of 20 and 40 mg of antibody compared to the three lower antibody dose levels. Assuming a biodistribution similar to [^{111}In]ZME-018, the radiation dose delivered to normal tissues by [^{90}Y]ZME-018 would restrict its use as a routine vehicle for radioimmunotherapy; however, it may be possible to deliver substantial tumor doses in selected patients.

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Pressman and others (*1*) initially conceived of using radiolabeled antibodies to detect human cancer but problems associated with poorly defined tumor antigens and polyclonal antibodies delayed the development of the field. With the advent of hybridoma technology and the subsequent availability of monoclonal antibodies directed against a single human antigenic determinant, a source of reproducible and predictable antibody became available (*2*). Considerable progress has been made in characterizing a number of tumor antigens associated with malignant melanoma (*5–7*). In 1983, Larson et al. reported the use of iodine-131 (^{131}I) Fab fragments specific for a 97 kilodalton glycoprotein associated with human melanoma and using a blood background subtraction technique successfully detected 22 of 25 (88%) of lesions >1.5 cm (*8*). In spite of their promising results, the maximum concentration of radioiodine in the tumor tissue 72 hr after injection was only 0.01% of the injected dose per gram (*8*). Larson et al. subsequently conducted a study using an ^{131}I-labeled Fab fragment directed against a high molecular weight antigen of human melanoma (*9*). While the data were not definitive, these investigators had the impression that the [^{131}I]Fab fragment targeting the high molecular weight antigen appeared to be more selective than the [^{131}I]Fab preparation targeting the P97 antigen. Halpern et al. suggested that the low concentration of radioiodine in the tumor as reported by Larson (*8*) may have been due to loss of tracer ^{131}I from the antibody (*10–12*). In a further study of 21 patients with proven or suspected metastases this group also used an indium-111 (^{111}In) labeled monoclonal antibody targeting the 97 kD glycoprotein and detected 48 of 79 (61%) metastases >1.5 cm in diameter. Similar results were obtained by Murray et al. using the same ^{111}In-labeled

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antibody (*13*). The mass of administered antibody appeared to affect the blood clearance and tumor detection and both groups theorized that increasing the amounts of unlabeled antibody might saturate nonspecific binding sites associated with nontumor tissue and improve tumor uptake. The following is a report of our first 25 patients evaluated with an ^{111}In-labeled monoclonal antibody directed against a high molecular weight antigen. In our study, we varied the mass of antibody administered from 2.5 to 40 mg to determine the effect on blood clearance, biodistribution, dosimetry, and tumor uptake.

MATERIALS AND METHODS

The ZME 018 murine monoclonal antibody in this study is of the IgG_{2a} subclass. The antibody was made by immunizing BALB/c mice with melanoma cell line M21 and fusing their splenocytes with the mouse myeloma cell line Sp2/0-Ag-14 to form hybrids as previously described (*5*). Hybridomas were grown in ascites fluid in BALB/c mice and the antibody was purified from ascites fluid by sodium sulfate fractionation and DEAE chromatography. ZME 018 recognizes an antigenic determinant on a 240,000 molecular weight glycoprotein present on the cell surface of 80% of melanoma cell lines and fresh tumor samples (*5*). The antibodies were labeled with ^{111}In by modification of the bifunctional chelation technique described by Krejcarek and Tucker (*14*) and modified by Halpern et al. (*11*). Prior to use, 5 mCi of buffered carrier-free ^{111}In was mixed with 1.0 mg of the diethylenetriaminepentaacetic acid (DTPA) side chain conjugated monoclonal antibody and was permitted to react in a pH 4.0 citrate buffer at room temperature for 30 min. Indium-111 remaining free in the reaction vial was chelated with DTPA. There is a large excess of DTPA and within the limits of sensitivity, there is no free indium in the system. Labeling efficiency was determined by paper chromatography which separates [^{111}In]DTPA from the ^{111}In-labeled antibody.

Immunoreactivity of this antibody was determined by binding to melanoma cells or purified membrane fractions containing the antigen. Immunoreactivity of both the chelated and labeled antibody and the unlabeled carrier are high, typically, in the range of 85%. The immunoreactivity is stable within the limits of sensitivity of the assay (plus or minus 5–10%) over a period of 2 yr. The immunoreactivities of the preparations used in the clinical trail did not vary from the specifications set by Hybritech in its IND.

Patient Studies

Patients were selected and studied according to a protocol approved by the University of Utah Institutional Review Board and the Food and Drug Administration. All 25 patients entering the protocol had previously documented malignant melanoma. At the time of study, 15 had known disease, five had suspected disease and five had no known active disease. There were 15 males and ten females with ages ranging from 24 to 84 yr. No patient was studied twice. All patients were admitted to the Clinical Research Center at the University of Utah. An admission history and physical examination were obtained and blood was drawn for a complete blood count, differential, platelet count, and serum chemistries; these measurements were repeated at 24 and 72 hr postinjection.

Blood was obtained just prior to antibody infusion and at 0.5, 1, 2, 4, 24, and 72 hr postinfusion. Urine was collected from 0–4 hr, 4–8 hr, 8–24 hr, and 24–48 hr after injection to check for ^{111}In activity; a urinalysis was performed preinfusion and on Days 1 and 3 postinfusion. A CBC was also obtained at 1, 24, and 72 hr postinfusion. A chemistry profile was obtained preinfusion and at 24 and 72 hr postinfusion.

All patients received ~5 mCi of ^{111}In-labeled to 1 mg of monoclonal antibody. Five patients received an additional 1.5 mg of unlabeled antibody such that total amount of antibody received by the first five patients was 2.5 mg; the second group of five patients received a total of 5 mg of antibody, the third group 10 mg of antibody, the fourth group 20 mg, and the fifth group 40 mg. The antibody preparation was diluted in 100 cc of saline and infused over 1 hr. Imaging was performed at 24 and 72 hr postinjection with earlier and later images occasionally obtained. All 24- and 72-hr images were recorded on the computer using a 128 by 128 matrix for subsequent data analysis.

Planar images were obtained by imaging the 173 and 247 keV photopeaks of ^{111}In using a large field of view gamma camera (Picker 415) with a 280 keV collimator (Nu Tech). One million count images were obtained of the chest, abdomen and pelvis; 500,000 count images were obtained of the femur and axilla and 250,000 count images were obtained of the legs and skull. All 24- and 72-hr images were recorded on floppy disks using a computer (Technicare 560). No computer enhancement of images was employed and no other radiopharmaceuticals were administered for background subtraction. In selected patients, single photon emission computed tomographic (SPECT) images were also obtained using a camera (General Electric 400 AT) interfaced to a computer (GE Star). Each SPECT image consisted of a 360° circular rotation of 64 views with a total SPECT imaging time of ~1.5 hr. Medium-energy collimators were used in all cases. Transaxial, coronal, and sagittal tomographic images were reconstructed using both nonlinear and linear filters. Confirmation of the lesions noted on imaging was obtained by clinical examination, ultrasound, computed tomography, chest x-ray, bone scan, surgery, biopsy, and clinical course.

Dosimetry estimates were based on estimates of organ uptake, urine excretion, and the plasma disappearance curves. Worst case dosimetry was estimated by assuming the maximum measured organ uptake occurred instantaneously and remained for a biologic half-life of 1,000 hr (effective half-life of 63 hr). The total-body dose was based on the assumption that 50% of the activity instantaneously distributed uniformly throughout the body; the remainder was assigned to specific organs (instantaneous uptake) or to blood. Activity in the gastrointestinal tract was assumed to be uniformly distributed with one-third in the upper small bowel, one-third in the lower small bowel, and one-third in the colon. Organ counts were converted to activity by counting a known amount of activity in a 250-ml cylindrical phantom in air and then immersed at a depth of 5.0 and 7.5 cm in a water bath. Dosimetry calculations were based on anterior views with the exception of the kidney and spleen. For these organs, we used posterior views. The mean kidney depth was estimated to be 5.5 cm and mean spleen depth was estimated to be 5.0 cm. Testes and superficial

tumor lesions were assumed to have no tissue attenuation. The pulmonary nodule was assumed to have a tissue attenuation of 5 cm. The ovaries were not visualized on the scan. Liver, heart, lungs/ribs, bowel, and femur were assumed to have a mean depth of 7.5 cm. Corrections for background activity were made and dosimetry calculations were made in accordance with the MIRD standards (*15,16*). Statistical analysis was performed using students t test for independent means.

RESULTS

Labeling Efficiency

Labeling efficiency determined by paper chromatography ranged from 85–97% with a mean of 95.6 ± 2.8% (s.d.).

Adverse Reactions

None of the patients had any reaction during the antibody infusion. Vital signs remained stable. The serum chemistries, CBC, and urinalysis at 24 and 72 hr postinjection were unchanged from the preinfusion values. One patient with unsuspected extensive disease developed fever, nausea, vomiting, and a skin rash beginning 4 days after a 10-mg antibody infusion. No infectious etiology was found and the patient recovered uneventfully after a short course of prednisone administration. The patient had no prior history of exposure to murine proteins and a subsequent blood sample showed no evidence of human anti-mouse antibodies. Although symptoms appeared earlier than usual and human anti-mouse antibodies were not documented, the patient's reaction best fits serum sickness (*17*).

Patient Results

The patient results are summarized in Table 1. The scan was prospectively abnormal in 14/18 (78% sensitivity) of patients with tumor and normal in 5/7 patients without tumor (71% specificity). The two false-positive scans represented uptake in the femur of a patient with a bone infarct or enchondroma and minimal uptake in the lung of one patient with pulmonary candidiasis. The bone lesion was considered to be diagnostic based on the radiograph and was not biopsied. Forty-five of 74 (61%) of presumed tumor sites were detected by imaging. Most of the 29 tumor sites not prospectively detected represented small pulmonary metastases or skin lesions; of these 29, 19 were <1 cm in diameter. The ten lesions larger than 1 cm included a 1.5-cm hepatic lesion and a 3-cm lower pelvis lesion; both tumor sites appeared to accumulate the ^{111}In antibody but the tumors were not prospectively detected as a result of the presence of normal activity in the liver and bowel and the scan was considered to be a false negative. A third lesion was a large unbiopsied pulmonary mass presumed to be melanoma in an 84-yr-old woman with recurrent melanoma on her right eyelid. Four undetected lesions occurred in a patient with multiple skin metastases, most of which were detected by imaging. The eighth and ninth undetected lesions represented brain metastases in a patient on high dose steroids who was undergoing radiation therapy. The final undetected lesion was a 1.5 × 1.5-cm skin metastasis; two other similar sized skin metastases in the same patient were positive. Scanning detected 61% (45/74) of all lesions and this figure increased to 77% (34/44) when only those lesions >1 cm were considered.

TABLE 1

Dosage (mg)	Patients with tumor	Abnormal scan	Number of lesions	Lesions detected	Lesions >1.0 cm	Lesions >1.0 cm detected
2.5	4	3	10	7	3	2
5.0	5	3*	9	5*	5	3*
10.0	3	3	32	23	25	21
20.0	3	3	17	6	5	4
40.0	3	2	6	4	6	4
Totals	18	14	74	45	44	34

*An additional patient had two lesions (liver, pelvis) which accumulated antibody but which were not prospectively detected. This is counted as false negative.

In three patients, the scan detected more extensive disease than was clinically suspected and in two of them, altered the planned therapy from surgery to chemotherapy. In a fourth patient, the scan detected a recurrent inguinal lesion in a patient with several previous inguinal dissections. The recurrent lesion was suspected by the patient who felt a "fullness" in the area but it was not clinically palpable. Supported by the scan results, surgery was performed and recurrent melanoma confirmed. A fifth patient was begun on chemotherapy after the scan confirmed multiple pulmonary metastases. In summary, the scan results changed or specifically directed therapy in 4/18 (22%) patient with tumor and supported the planned course of therapy in the remainder. Case histories are illustrated in Figures 1–4.

Blood Clearance and Urine Excretion

A blood sample was obtained immediately following antibody infusion. Plasma activity was assumed to represent 100% of the injected dose and residual activity was determined at 0.5, 1, 2, 4, 24, and 72 hr postinjection. Approximately 25% of the injected activity had left the blood by 4 hr postinjection and 50% by 24 hr (Fig. 5). There appeared to be no significant differences in the clearance rates at the 2.5, 5, and 10 mg dose levels. The 20 and 40 mg dose levels resulted in significantly slower plasma clearances at 4, 24, and 72 hr postinjection compared to each of the lower three dose levels ($p \leq 0.05$), but there was no significant difference in the plasma clearance between the 20 and 40 mg doses.

There appeared to be no difference in urinary excre-

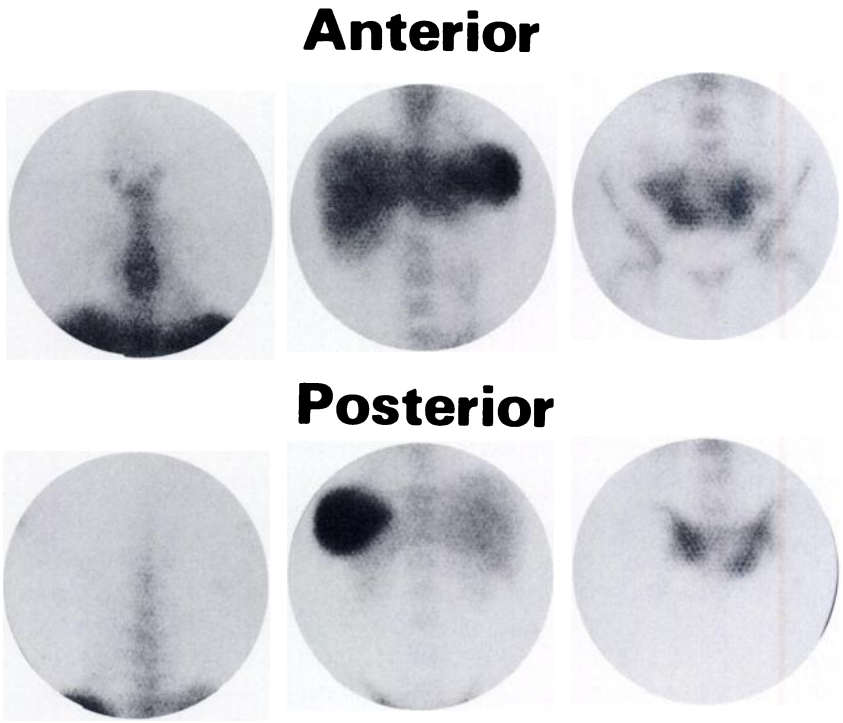

FIGURE 1
Patient 1: A 24-yr-old female who was well until December 1977 when a mole removed from her right calf proved to be malignant melanoma. In January 1983 she underwent a right groin dissection because of palpable adenopathy and one of eight nodes was positive for malignant melanoma. In December 1983 the patient noted several small subcutaneous nodules in the outer quadrant of her left breast which were surgically removed and found to be positive for melanoma. In June 1984 she again presented with three small subcutaneous nodules <1 cm in diameter in the outer quadrant of her left breast and she underwent a monoclonal antibody (5 mCi, 2.5 mg) scan prior to excisional biopsy. The 72-hr images following injection of 5 mCi of [^{111}In] ZME-018 (2.5 mg) reveal intense pelvic activity which persisted from 24 to 144 hr postinjection. Surgery confirmed unsuspected metastatic disease. The three small subcutaneous nodules (all <1 cm in diameter) in the outer quadrant of the left breast were not visualized by planar imaging.

tion of the ^{111}In at the five antibody dose levels (Table 2). Almost half of the total excreted activity appeared in the urine during the first 4 hr after antibody administration; most of this activity probably represents scavenged ^{111}In chelate that was never bound to antibody. Any free ^{111}In would be bound to transferrin and slowly eliminated (*18*).

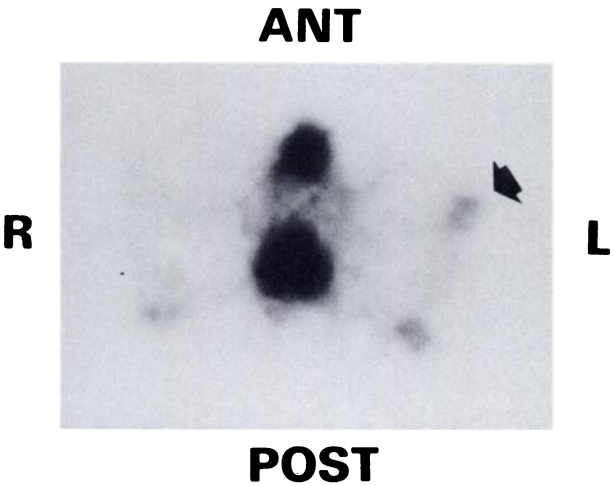

FIGURE 2
Patient 1: The largest of the three subcutaneous nodules was detected by SPECT imaging. Spine, sternum, and scapulae are also visualized.

Biodistribution at 24 and 72 hr

Within the limits of the technique, there appears to be little effect on the biodistribution of ^{111}In by increasing the dose from 2.5 to 10.0 mg (Tables 3 and 4). In all cases, liver activity increased from 24 to 72 hr. Heart activity as well as lung/rib activity decreased slightly, probably because of the drop in blood-pool activity. Administration of 20 and 40 mg of antibody produced a significant drop ($p \leq 0.01$) in liver and spleen activity and a concomitant increase in blood-pool activity. Activity in the remainder of the organs surveyed remained fairly constant. Of note was prominent activity in the bowel and testes.

Radiation Dosimetry

With the administration of 2.5 mg of MoAb, the spleen received the largest radiation dose with the liver, kidneys and testes receiving ~ four to five times the total-body dose. The dosimetry estimates for the various organs studied are presented in Table 5. Increasing the administered mass of antibody to 20 and 40 mg decreased liver, spleen, and kidney activity and consequently decreased the radiation dose to these organs. Total-body dose was estimated to be ~0.5 rad/mCi.

Tumor uptake increased from 24 to 72 hr postinjection in almost 70% of the metastatic lesions; there was no change in activity from 24 to 72 hr in 19% and a decrease in 13%. In five sites (four superficial lesions and one pulmonary nodule) for which we have reason-

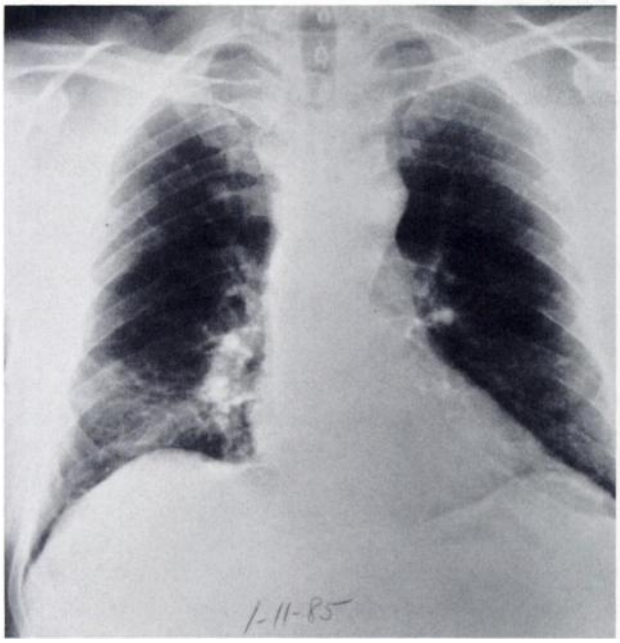

FIGURE 3
A chest radiograph revealed pulmonary nodules in an asymptomatic 60-yr-old male with recurrent melanoma resected from his back approximately a year earlier. The largest nodule is a 1 cm lesion to the right of the right heart border.

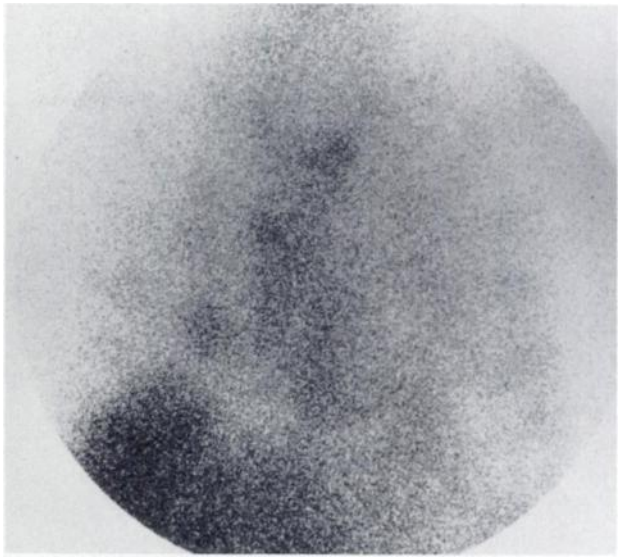

FIGURE 4
A 72-hr scan of the anterior chest following administration of 5.0 mCi (20 mg) of [^{111}In]ZME 018 shows marked uptake in the most prominent lesion noted on the chest radiograph (Fig. 3).

able estimates for tumor size and ^{111}In uptake, tumor activity was estimated to range from 0.002% of injected dose per gram to 0.67% (Table 6). The three intermediate values were 0.018, 0.052, and 0.014% of the injected dose/g, respectively.

DISCUSSION

The ^{111}In antibody targeting the high molecular weight antigen was clearly able to detect tumor in the majority of patients with metastatic malignant melanoma. Different amounts of antibody were used in this study because it has been theorized that increasing the

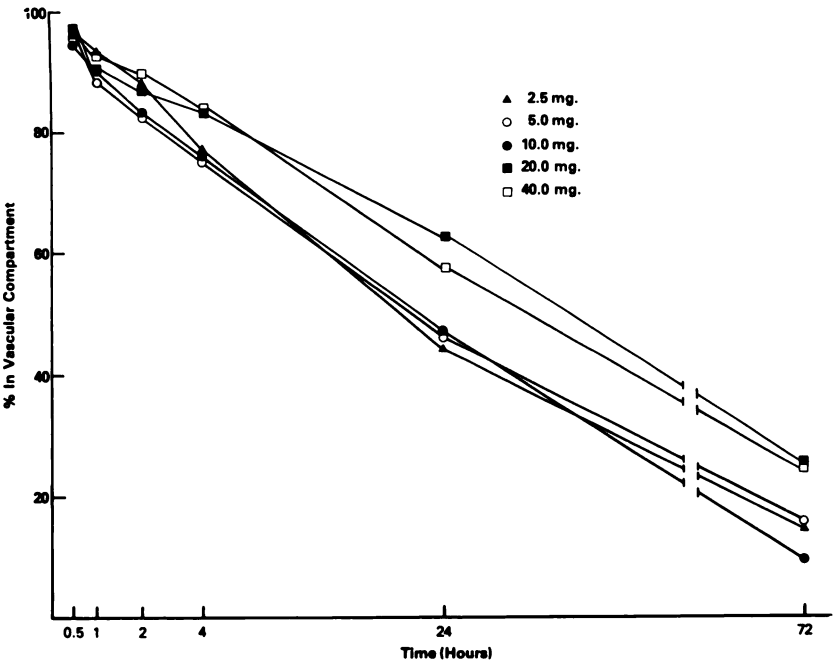

FIGURE 5
For the first 2 hr, there was no significant difference in the rate of clearance of ^{111}In activity from the vascular compartment for the five dose levels. Administration of 20 or 40 mg of ZME 018 delayed the plasma clearance at 4, 24 and 72 hr compared to the three lower dose levels ($p \leq 0.05$). For all points, the standard error ranged from 0.6 to 6.8 percentage point, with a mean standard error of 2.5 percentage points. This difference is further illustrated by comparing the 72-hr cardiac activity on Figure 1 (2.5 mg) and Figure 3 (20 mg).

TABLE 2
Urinary Excretion of ^{111}In (% Injected Dose) at Various Time Periods Postinjection*

Dose	0–4 hr	4–8 hr	8–24 hr	24–48 hr	0–48 hr
2.5 mg	6.7 ± 2.8	1.9 ± 2.2	1.8 ± 0.2	3.2 ± 1.2	13.6 ± 4.0
5.0 mg	6.8 ± 1.6	1.4 ± 0.5	1.4 ± 0.5	2.2 ± 0.4	10.9 ± 1.7
10.0 mg	5.9 ± 0.9	1.8 ± 1.1	1.6 ± 0.2	4.6 ± 1.2	13.9 ± 1.4
20.0 mg	7.9 ± 2.8	1.6 ± 0.7	1.4 ± 0.7	3.0 ± 1.6	13.8 ± 3.4
40.0 mg	6.1 ± 2.2	1.2 ± 0.4	1.4 ± 0.7	2.2 ± 0.5	11.0 ± 2.6

* Mean ± 1 s.d.

amounts of unlabeled antibody will saturate nonspecific binding sites associated with nontumor tissue (*11,12, 19*). Saturation of specific binding sites as a result of low but significant expression of tumor associated antigens on normal tissue may also occur but the possibility of saturation of nonspecific or low level specific binding sites may occur at the cost of diminished tumor uptake (*21*). Although more lesions >1 cm in diameter were detected at higher dose levels, the numbers are too small to support any definitive conclusions. In our study, 34 of 44 (77%) of lesions >1 were detected. These results are similar to the 73% and 60% overall lesion detection rate reported by Cornelius et al. and Murray et al. (*20,21*). Combining our results with those of Cornelius and Murray, 31% (9/29) lesions were detected with the 2.5 mg dose, 52% (13/25) were detected using 5.0 mg, 68% (52/77) were detected using 10 mg, 81% (94/112) were detected at 20 mg, and 76% (29/38) were detected at 40 mg. These combined results suggest a tendency toward a higher detection rate with greater amounts of cold antibody up to a dose of 20 mg.

Increasing the mass of antibody administered above 10 mg delayed the plasma clearance. These results are similar to those reported by Halpern (*12*) and Lamki (*22*) using different antibodies. Liver activity increased from 24 to 72 hr but ^{111}In activity in other tissues appeared to remain relatively constant over the time of the study. Following administration of [^{111}In]ZME-018 to rats, we have shown that the ^{111}In activity reaches the intestinal lumen primarily through the bowel wall rather than by hepatobiliary excretion (unpublished data). Bowel activity can interfere with imaging and did obscure a 3-cm lesion in one patient in our series.

The 72-hr images were clearly superior to the 24-hr image in diagnostic quality. Target to background ratios were enhanced, lesions were better identified and some lesions seen which were not apparent on the 24-hr study. Animal studies have suggested that little of the ^{111}In leaves the tumor during the first 72 hr following injection (*4*). Similar results were obtained in our study with tumor activity remaining constant or actually increasing from 24 to 72 hr in 89% of the lesions evaluated. Blood-pool activity was often present on the 72-hr scan and did diminish on later images. Due to the 68-hr half-life of ^{111}In, images obtained at 144 hr required substantially longer imaging times than the 24- or 72-hr image. Imaging at 72 hr is preferable to 24-hr imaging and images with higher target to background ratios can be obtained at 144 hr postinjection or beyond.

In many of the cases, failure to detect a lesion was probably related to poor contrast due to the fact that the size of the lesion was <1 cm in diameter. In five patients SPECT imaging detected small lesions in the chest or axilla which were not observed on planar imaging; there was one false-positive study with SPECT imaging. Lesions in the liver may be missed if they concentrate the monoclonal antibody to the same extent as the rest of the liver. This almost certainly hap-

TABLE 3
Biodistribution of ^{111}In Activity (% Injected Dose) in Humans at 24 hr After Injection of 2.5, 5, 10, 20, and 40 mg of ZME-018*

Dose	Liver	Spleen	Heart	Lungs/Ribs	Kidney	Bowel	Femur†	Testes
2.5 mg	9.6 ± 4.6	3.6 ± 2.0	1.2 ± 1.3	2.0 ± 1.2	2.0 ± 0.5	1.6 ± 1.0	0.3 ± 0.2	0.4 ± 0.1
5.0 mg	7.8 ± 1.5	3.8 ± 1.7	0.8 ± 0.3	2.1 ± 1.0	2.1 ± 0.5	1.9 ± 1.1	0.1 ± 0.01	0.3 ± 0.04
10.0 mg	9.1 ± 5.0	3.7 ± 2.6	1.0 ± 0.4	2.4 ± 0.8	2.1 ± 0.9	2.1 ± 2.1	0.1 ± 0.04	0.5 ± 0.2
20.0 mg	7.3 ± 2.5	1.9 ± 1.1	1.5 ± 0.9	2.7 ± 0.8	1.6 ± 0.8	2.5 ± 1.4	0.1 ± 0.03	0.4 ± 0.3
40.0 mg	5.7 ± 3.2	1.1 ± 0.3‡	1.9 ± 1.5	2.0 ± 0.5	0.9 ± 0.4‡	1.0 ± 0.1	0.1 ± 0.06	0.3 ± 0.04

* Mean ± 1 s.d.
† Activity reported for the femur is based on a region of interest encompassing the midshaft of the femur.
‡ $p \leq 0.01$ compared to 2.5 mg.

TABLE 4
Biodistribution of ^{111}In Activity (% Injected Dose) in Humans at 72 hr After Injection of 2.5, 5, 10, 20, and 40 mg of ZME-018*

Dose	Liver	Spleen	Heart	Lungs/ribs	Kidney	Bowel	Femur	Testes
2.5 mg	14.2 ± 5.6	4.5 ± 2.8	0.5 ± 0.2	1.5 ± 0.5	2.9 ± 1.4	2.1 ± 2.1	0.4 ± 0.4	0.5 ± 0.1
5.0 mg	10.0 ± 3.2	3.5 ± 1.5	0.3 ± 0.2	1.7 ± 1.0	1.8 ± 0.6	1.2 ± 0.6	0.1 ± 0.01	0.3 ± 0.04
10.0 mg	12.7 ± 4.3	3.2 ± 1.2	0.3 ± 0.2	1.7 ± 1.0	1.8 ± 0.3	2.1 ± 2.3	0.1 ± 0.04	0.5 ± 0.2
20.0 mg	9.5 ± 2.4†	2.1 ± 0.4	0.8 ± 0.4	2.1 ± 0.7	1.6 ± 0.8†	0.2 ± 0.7	0.2 ± 0.1	0.5 ± 0.2
40.0 mg	7.0 ± 3.8†	1.3 ± 0.5‡	1.0 ± 1.1	1.6 ± 0.6	0.7 ± 0.4‡	1.0 ± 0.5	0.1 ± 0.05	0.3 ± 0.01

* Mean ± 1 s.d.; Activity reported for the femur is based on a region of interest encompassing the midshaft of the femur.
† $p \leq 0.05$ compared to 2.5 mg.
‡ $p \leq 0.01$ compared to 2.5 mg.

pened in one of our patients but might have been avoided if a conventional technetium-99m sulfur colloid liver scan had been obtained in conjunction with the monoclonal antibody images. In a second patient, a liver lesion was noted on the antibody scan which showed a central cold defect with an enhanced rim of greater intensity than the liver, probably representing viable tumor surrounding a necrotic core. Significant uptake does occur in the spleen and the gastrointestinal tract and this activity can also obscure abdominal lesions.

In their study of Fab fragments directed against the high molecular weight antigen, Larson et al. reported that not all of the lesions were detected by imaging suggesting pronounced heterogeneity of antigen expression at different tumor sites in the same patient (*13*). We observed a similar finding in one of our patients with a large number of skin metastases. Significant differences in the expression of the high molecular weight antigen have been reported based on analyses of melanoma tissue samples removed from different patients and from different sites on the same patient (*23*).

TABLE 5
Radiation Dosimetry Estimates (rad/mCi) Resulting from the Administation of [^{111}In]ZME-018 with Varying Amounts of Unlabeled Antibody*

	2.5 mg	5.0 mg	10.0 mg	20.0 mg	40.0 mg
Blood	0.51	0.49	0.50	0.48	0.46
GI (SI)	0.75	0.71	0.74	0.75	0.59
GI (ULI wall)	0.84	0.78	0.83	0.85	0.62
GI (LLI wall)	0.79	0.75	0.79	0.84	0.60
Kidneys	2.48	1.94	1.98	1.60	1.07
Liver	2.13	1.62	1.94	1.55	1.23
Lungs and ribs	0.76	0.73	0.79	0.79	0.67
Muscle	0.48	0.46	0.47	0.45	0.43
Spleen	5.55	4.72	4.69	2.91	1.92
Testes	2.51	1.92	2.68	1.99	1.79
Total body	0.57	0.54	0.55	0.53	0.48

* The organ dosimetry is very likely overestimated because of our conservative assumption that the maximum organ uptake was instantaneous that the maximum and the biologic half-life was 1,000 hr. Estimates of marrow dosimetry are given in the discussion.

Other factors which might affect variation in tumor uptake include dose administered, modulation, shedding, multiple clones, cell cycle related events, tumor blood flow, and the extraction efficiency of the monoclonal antibody.

The dosimetry of [^{111}In]ZME-018 has been calculated based on estimates of organ uptake at 24 and 72 hr and the blood disappearance curves. These values are based on certain assumptions which need to be refined in further work. Actual tissue samples would be preferable but in most cases are not feasible and tissue samples, particularly tumor tissue samples, may not be representative of the entire lesion. We also assumed instantaneous organ uptake and no decrease in organ activity after 72 hr; these assumptions very likely resulted in an overestimate of the actual dosimetry. Additional data collected at 96 and 120 hr postinjection would certainly improve our estimates. Marrow doses are critically important but our data for estimating marrow dosimetry are limited. If we make a tenuous extrapolation from our data in Tables 3 and 4 to assume that 2.5% of the injected dose distributes in the red marrow, then the marrow dose ranges from 0.95 (2.5 mg) to 0.68 (40 mg) rad/mCi.

Murray et al. obtained biopsy samples from lesions detected by scans in six patients 3–14 days after injection (*21*). Percent injected dose/g for three lesions ranged from 2.8 to 8.5 × 10^{-5}. Based on imaging of skin lesions, we estimated the % dose/g to range from 0.2 to 5.2 × 10^{-2}; in one patient (Figs. 3 and 4), we estimated one pulmonary lesion to accumulated

TABLE 6
Tumor Activity (% Injected Dose/g)

Dose	Location	Diameter	24 hr	72 hr
20.0 mg	Lung	1 cm	0.42	0.67
20.0 mg	Mandible	1.5 cm	0.0002	0.018
20.0 mg	Groin	2.0 cm	0.053	0.052
20.0 mg	Skin	1.5 cm	0.014	0.014
40.0 mg	Axilla	2.0 cm	ND*	0.002

* Not detectable at 24 hr.

0.67%/g. Both studies evaluated a small number of samples. The samples obtained by Murray et al. may not have been representative of the whole lesion. We obtained a tumor sample from a three centimeter pulmonary lesion and found much more activity in the viable surface of the tumor than in the necrotic core. Murray's values may have been less if his samples included necrotic tumor. Our estimates of uptake in relatively small skin lesions which were probably not necrotic are similar to those reported by Larson (~0.01% of the injected dose/g) using an [^{131}I]Fab fragment which targets a 97 kD glycoprotein associated with human melanoma (*8*). Alternatively, we may have overestimated the tumor dose. In the future, it will be important to correlate tumor uptake determined by imaging with in vitro measurement of activity in the whole tumor nodule.

Both Halpern (personal communication) and Hnatowich (*24*) have shown that ^{111}In and ^{90}Y labeled to DTPA-coupled IgG have a very similar biodistribution. Based on these data, we assumed [^{90}Y]ZME-018 would have the same biodistribution as the ^{111}In chelate and estimated the dosimetry for ^{90}Y. Based on our estimates and assuming 0.01% of the injected dose per gram of tumor, 100 mCi of [^{90}Y]ZME-018 would deliver 1,830 rad to the tumor, 220 rad to the total body and 790 rad to the liver. These values are similar to those of Wessels et al. who calculated that 100 mCi of ^{90}Y-labeled antibody given to a patient with a 500-g tumor would deliver 1,700 rad to the tumor, 200 rad to the total body, and 200 rad to the liver (*25*). While the dosimetry data suggest that whole antibody will not be appropriate for routine therapy with ^{90}Y because of the high radiation to normal tissues needed to achieve tumor irradiation, selected patients may well be candidates for therapy due to high tumor uptake of the radiopharmaceutical. For example, one of our patients with a 1-cm pulmonary lesion accumulated an estimated 0.67% of the injected dose per gram at 72 hr. Assuming the activity remained in the tumor until decay, administration of 20 mCi of ^{90}Y-labeled antibody could deliver ~20,000 rad to the tumor.

In summary, significant ^{111}In tumor uptake occurred in a large majority of unselected patients with metastatic malignant melanoma. In 16% of patients studied (22% of patients with tumor) the scan results specifically directed or modified therapy. While uptake in the liver, spleen, and bowel pose problems in image interpretation, increasing the mass of administered antibody decreases the activity in the liver, spleen and kidney. Furthermore, the absence of acute toxic reactions or changes in the serum chemistries, CBC, and urinalysis in the first 72 hr after infusion, suggest that monoclonal antibody scanning could be done as an outpatient procedure. The dosimetry data suggest that use of the whole unmodified antibody labeled with ^{90}Y will not be appropriate for routine therapy but that therapy may be beneficial in selected patients with high tumor uptake.

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