Effect of Antibody Dose on the Imaging and Biodistribution of Indium-111 9.2.27 Anti-Melanoma Monoclonal Antibody

Jorge A. Carrasquillo, Paul G. Abrams, Robert W. Schroff, James C. Reynolds, Clive S. Woodhouse, Alton C. Morgan, Andrew M. Keenan, Kenneth A. Foon, Patricia Perentesis, Sharon Marshall, Mitchell Horowitz, Janusz Szymendera, Josie Englert, Robert K. Oldham, and Steven M. Larson

Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bethesda; and Biological Therapy Branch, Biologic Response Modifiers Program, National Cancer Institute, Frederick, Maryland

Eleven patients with metastatic melanoma underwent serial gamma camera imaging and biodistribution measurements after i.v. injection of escalating doses of [^{111}In]9.2.27, an anti-melanoma murine monoclonal antibody. Patients received a fixed dose of 1 mg of [^{111}In] 9.2.27, with no additional 9.2.27 (five patients), or co-infused with 49 mg (five patients) or 99 mg (one patient) of unlabeled, unconjugated 9.2.27. Higher doses resulted in prolonged blood-pool retention, less uptake in spleen and bone marrow, and appeared to have a positive effect in improving tumor imaging. A dose of 1 mg of 9.2.27 permitted detection of tumors in two of five patients and two of ten lesions, while with ≥50 mg, tumors were detected in all patients and in 24 of 32 lesions. Human gamma globulin injected prior to administration of [^{111}In]9.2.27 failed to block the prominent liver, spleen, and bone marrow uptake. No toxicity was observed. These results indicate the feasibility of imaging metastatic melanoma with [^{111}In]9.2.27 and suggest that antibody dose may be a critical determinant of biodistribution and tumor uptake.

J Nucl Med 29: 39–47, 1988

Many studies have used radiolabeled anti-tumor antibodies (*1–18*) in efforts to improve the staging of tumors and the detection of recurrent disease. The initial work used polyclonal antibodies (*1,2,6,11,12*). More recently, with the advent of monoclonal antibodies (MoAb) (*19*) with their well-defined specificity and uniformity between lots and abundance, a shift toward using these reagents has occurred (*5,7–9,13,20–22*).

A common problem in most of these studies has been a limited sensitivity for tumor detection that has required use of image-enhancement techniques. These image-enhancement techniques (*1,23*) have proven cumbersome, prone to artifact production, and suboptimal (*5,24,25*). In addition, most of the labeling has been performed with isotopes of iodine that are subject to de-iodination in vivo and may be less suitable for imaging than indium-111 (^{111}In) labeling with the bifunctional chelate technology (*17,25–27*). Several studies have reported dose-dependent effects on biodistribution and improved tumor targeting (*3,20,21,28*) with higher antibody doses than those commonly used (*1,2, 4–7*). This study was designed to evaluate the effect of monoclonal antibody dose on biodistribution of [^{111}In] 9.2.27, an anti-melanoma MoAb, in patients with metastatic melanoma.

MATERIAL AND METHODS

Monoclonal Antibody

The antibody 9.2.27 is a murine MoAb (Ig2a, k) that recognizes a high molecular weight (250 kD chondroitin sulfate/proteoglycan) cell surface antigen present on most (90%) human melanomas (*29,30*). Extensive testing by immunoperoxidase has shown that it is relatively selective for tumor cells; there is some cross reactivity with blood vessels endothelium, basal cells, and sweat glands of skin, collecting duct of kidney, and benign nevi (*31*). The antibody was purified from hybrid-

Received Oct. 17, 1986; revision accepted Aug. 10, 1987.

For reprints contact: Jorge A. Carrasquillo, MD, Bldg. 10 Room 1C401, Nuclear Medicine Dept., National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

oma ascites by Na_2SO_4 precipitation as previously described (*32*).

The ^{111}In labeling was performed by a modification of the bifunctional chelating method of Krejcarek (*33*). The antibody was received in a kit* contained 1 mg of diethylenetriaminepentaacetic acid (DTPA) conjugated 9.2.27 in 1% human serum albumin. Labeling was performed by incubating ~5 mCi of ^{111}In with the MoAb in a citrate buffer. Excess DTPA was added to scavenge any free ionic ^{111}In as the final step prior to injection.

Quality Control

The incorporation of ^{111}In onto the MoAb was determined by instant thin layer chromatography (ITLC) in silica gel with 5% ammonium acetate in methanol: water (1:1). To determine colloid formation, chromatography with 0.9% NaCl in Whatman No. 1 paper was performed (*34*). (Pyrogen and sterility testing was performed by Hybritech, Inc., La Jolla, CA).

The immunoreactivity was determined by a cell-binding assay. Graded doses of live, antigen-bearing cells (up to 8×10^6) were incubated with 5 ng of [^{111}In]9.2.27 at 4°C for 1 hr. The cells were then separated by centrifugation in phosphate-buffered saline (PBS), 1% bovine serum albumin. The cell pellet was counted in a gamma counter and the immunoreactivity was expressed as the percentage of radioactivity on cells compared with the total added. To obtain the immunoreactivity of the protein bound ^{111}In (corrected immunoreactivity) the immunoreactivity value was divided by the ^{111}In protein bound fraction.

Indium-111 9.2.27 plasma levels were determined by gamma counting of circulating ^{111}In. In order to determine that the circulating ^{111}In was in the MoAb fraction, an enzyme-linked immunoassay (ELISA) was used to trace the antibody independently of the radiolabel. Microtiter plates were coated with 100 ng per well of goat antimouse [IgG + IgM (H+L)]][†] and then blocked with 5% normal chicken serum. They were incubated with serial dilutions of patients' serum or 9.2.27 standard, washed, incubated with alkaline phosphatase-conjugated goat anti-mouse IgG[†] and developed using p-nitrophenyl phosphate[†] The optical density was determined at 405 nm using a micro ELISA reader.[‡] For each assay, a standard curve of absorbance versus concentration of 9.2.27 was generated and serum values were determined by linear regression and interpolation.

To define the translocation of ^{111}In from MoAb to autologous transferrin, serial assays of ^{111}In bound to transferrin were performed. Ten milliliters of rabbit anti-human transferrin[§] was coupled to 30 ml of Reacti-Gel (6x) beads[¶] following the manufacturer's protein-coupling procedures. Active groups were then blocked with 1.0*M* ethanolamine at pH 9.0 for 3 hr. The beads were then stored in PBS, 1% bovine serum albumin. Patient serum was obtained at different times and 10 μl of serum was incubated with 80 μl of Reacti-Gel beads (a sufficient amount to bind all the transferrin in 10 μl of serum, data not shown) for 1 hr and separated by centrifugation with two washings of PBS, 1% BSA. The pellet and supernatants were counted in a gamma counter; the percentage of the total counts on the pellet represented the percent of ^{111}In on transferrin.

To determine if there was pre-existing human anti-mouse antibodies (HAMA), 1 μl of patient baseline serum was incubated with 0.5 to 1 ng (10,000 cpm) of [^{125}I]B72.3 monoclonal antibody, a murine IgG1, for 18 hr at 4°C. Ten milligrams of formalin-fixed staphylococcus A cells[**] were added and, following a 15-min incubation (4°C), the bound counts were separated by centrifugation (3,000 rpm × 5 min). The percent binding for each patient's serum was calculated as bound counts/total counts × 100 and compared with the mean for a control group that had never been exposed to mouse antibodies. A serum was considered positive for HAMA if the percentage binding was at least 3 s.d. greater than the mean of the control group.

Tumor Preparation

Four patients had immunohistologic assessment of subcutaneous or lymph node metastases prior to MoAb infusion and six patients had biopsies within 7 days of MoAb injection. Frozen sections of their tumors were obtained for immunohistochemical analysis. An indirect, two stage immunoperoxidase procedure was used as previously described (*35*). Briefly, nonspecific binding of proteins was blocked by incubating cryostat sections with 100 μl of 4% rabbit serum in PBS containing 5% chicken serum (PCS). The slides were washed with PBS and then either incubated for 30 min with 100 μl of monoclonal antibody 9.2.27 culture supernatant followed by 100 μl of horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin[††] diluted 1/50 in PCS containing 4% pooled human serum for in vitro assessment of antigen status or stained directly with horseradish peroxidase-conjugated rabbit anti-mouse for in vivo delivery of MoAb to tumor. Sections were washed in PBS and incubated in 0.5 mg ml^{-1} diaminobenzidine hydrochloride[‡‡] containing 0.03% hydrogen peroxidase, washed in PBS, counterstained in Mayer's haemalum, dehydrated in graded alcohols, and mounted with Permount.[§§] Controls consisted of monoclonal immunoglobulins derived from RPC5 and P3-X63 (P3) plasmacytoma lines. Saturation in vivo (endogenous binding) with 9.2.27 was assessed by comparing the staining of sections with and without the additional 9.2.27. Intensity of staining was assessed as negative (−) or positive (+ to +++). The proportion of melanoma cells staining was approximated and expressed as a percentage in 5% increments.

Patients

Eleven patients with histologically confirmed metastatic melanoma were studied (Table 1). The mean age was 50 yr (range 35–73 yr); seven were male and four female. Staging studies included chest x-ray, bone scan, abdominal and brain computerized tomography, complete blood count, liver function test, and renal function test.

Dose escalation studies were performed at 1 mg (three patients), 50 mg (five patients) and 100 mg (one patient) by mixing and co-injecting 0, 49, or 99 mg, respectively, of carrier unconjugated 9.2.27 with ~1 mg of the [^{111}In]9.2.27. In an effort to block nonspecific liver, spleen, and bone marrow uptake, two additional patients received 7.5 g of human immunoglobulin (Gamma-immune) intravenously for 1 hr. followed immediately by 1 mg of [^{111}In]9.2.27. All MoAb were infused intravenously in 8 to 11 ml over a 2 hr constant infusion. Vital signs were monitored before starting and at the end of the infusion. Intravascular kinetics were determined by counting blood and plasma aliquots at 5 min, 30 min, 1 hr, 2 hr, 24 hr, 48 hr, 72 hr, 96 hr, and up to 7 days (when feasible).

TABLE 1

Patient	Age (yr)	Sex	Staging	Size	Scan	Biopsy	Prior Treatment	Gamma-immune	[111In]9.2.27 mCi	Mg
1	39	M	Axillary node	10 × 10 cm	+	Y	Brain radiation	N	4.7	0.92
			Liver	4 × 4 cm	−					
			Spleen	3.1 × 5 cm	−					
			Kidney	1.2 × 1.2 cm	−					
2	65	M	Chest	2.4 × 2.4 cm	−	Y	Lung surgery	N	5.0	0.75
			Subcutaneous	0.5 × 0.5 cm	−					
3	31	F	Rectal	4 × 4 cm	+	N	Surgery, local chemotherapy and hyperthermia	N	5.0	0.93
			Lung	2 × 2 cm	−		Tamoxifen			
4	63	F	Hilar	2.3 × 1.7 cm	−	Y	Surgery of subcutaneous lesion and Interferon	Y 7.5 g	3.97	0.90
			Subcutaneous	1.5 × 1.5 cm	−					
5	51	M	Subcutaneous	5 × 3 cm	−	N	Surgery Interferon	Y 7.5g	4.03	0.92
6	52	F	Subcutaneous (15 lesions)	1.5 × 1.5 cm up to 9.5 × 8 cm	+	Y	Surgery Poly-ICLC	N	4.7	50
7	73	M	Lymph node	6.5 × 5 cm	+	Y	Surgery	N	5	50
			Lymph node	7.5 × 8 cm	+					
			Liver	3 × 5 cm	−					
8	42	M	Subcutaneous (two lesions)	<1 cm	−	Y	Surgery of axilla	N	5	50
			Lung	3 × 3 cm	+		Vaccinia oncolysate			
			Bone		+					
9	49	M	Lung	2.5 × 2.5 cm	−	N	Interferon and chemotherapy	N	4.01	50
			Cervical node	<1 cm	+					
			Abdominal mass	7 × 7 cm	+					
			Right pararectal	4.5 × 4.5 cm	−					
10	63	F	Humerus		+	Y	Humerus radiation	N	5	50
			Subcutaneous	2 × 2 cm	−					
			Inguinal	<1 cm	−					
			Subcutaneous	1 × 1 cm	−					
11	35	M	Subcutaneous	2 × 3 cm	+	Y	Surgical dissection	N	4.95	100
			Axillary node	2 × 3 cm	+		Vaccinia oncolysate			

Indium-111 radioactivity in the patients' samples and the injected standard was determined by gamma counting. The percent of the injected dose circulating in the plasma was calculated from the patients' estimated plasma and blood volume (*36*). From the same blood and serum samples we determined if there was binding to circulating cells by calculating what proportion of the counts in the blood aliquots were due to radioactivity in the plasma. Pre-infusion and 96-hr to 1-wk postinfusion complete blood cell counts, serum creatinine, and liver function tests were obtained.

Twenty-four-hr urine collections were performed daily up to 96 hr. Whole-body retention of radiolabel was measured with a 2 × 2 in. diameter sodium iodide crystal placed at 7.1 m with the patient in a fixed geometry, taking the immediate postinfusion value as 100%.

Scintillation camera images were recorded within 2 hr postinfusion, at 24 hr, 48 hr, 72 hr, and at 6 or 7 days. Images were recorded with a GE 535 gamma camera with a medium-energy collimator. Separate 20% windows over the 173-keV and 247-keV gamma-ray peaks of ^{111}In were used to record anterior and posterior whole-body scans and spot views (5 min). The typical spot images had 500,000 to 1,000,000 counts, while the whole-body images had 1.5 to 3.5 × 10^6 counts (96 and 2 hr, respectively). No blood pool or organ subtraction was performed. In addition to analog recording of images on film, digital acquisition on a Hewlett-Packard Scintigraphic Data Analyzer[¶] was performed. To calculate serial changes in radiolabel distribution, spot images were analyzed with manually drawn regions of interest (ROIs) over the entire anterior and posterior liver and spleen. Small regions of interest were also performed over a portion of the bone marrow and tumor. Values were expressed as counts per 5-min per organ and in the case of the bone marrow and tumor the values were expressed as counts per 5 min per pixel. All counts were corrected for isotope decay.

RESULTS

All patients studied had evaluable metastatic disease. There were no major differences between the low dose (1 mg) and the high dose groups (>50 mg) in terms of disease stage, tumor size, or antigen expression, with the exception of Patient 6 who had a large number (15 lesions) of subcutaneous nodules.

Tumor uptake was seen in two of five patients receiving 1 mg [^{111}In]9.2.27 (Fig. 1), and two of ten lesions

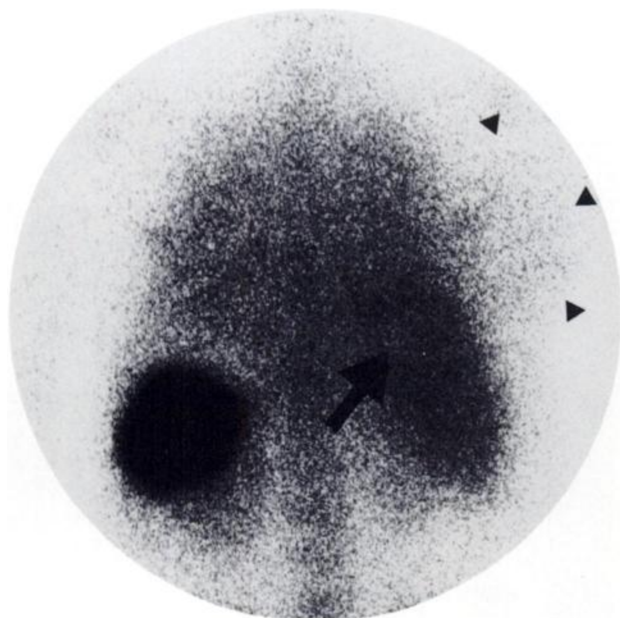

FIGURE 1
Posterior view of the chest obtained 48 hr after i.v. administration of [^{111}In]9.2.27 showing faint uptake of tracer in a right axillary metastasis (arrowhead) and a relatively photon deficient region in the liver corresponding to a metastatic lesion (arrow). There is prominent uptake in the spleen, liver, and bone marrow.

were identified. At least one tumor site was detected in all six patients receiving ≥50 mg of 9.2.27 (Fig. 2), with 24 of 32 lesions identified. When all patients were considered, the differences in tumor detection at the two dose levels were significant (chi-square, $p = 0.006$); when Patient 6, with 15 subcutaneous lesions, was eliminated from the analysis, nine of 17 lesions were positive and the difference was not significant (chi-square, $p = 0.26$). In addition, tumor to nontumor contrast was more prominent at ≥50 mg 9.2.27 than at 1 mg (Fig. 1 versus Fig. 2). Lesions not visualized at the 50-mg doses included one liver lesion, one lung lesion, five subcutaneous metastases and one lymph node metastasis (Table 1). No false-positive scans were obtained.

The ROIs analysis of organs at these two MoAb dose levels (Table 2) demonstrated significantly different biodistribution with more rapid clearance of radioactivity from the blood pool, and more prominent spleen (t-test, $p < 0.007$) and bone marrow uptake at the 1 mg level (Fig. 3) than at the ≥50 mg dose level. The liver activity was not significantly different (t-test, $p = 0.7$) at the two dose levels. Although some subcutaneous lesions were clearly visible by 2 hr postinfusion, lesions close to the surrounding blood pool required delayed imaging (Fig. 2). Optimal images were obtained after 72 hr when lesion accumulation of MoAb had leveled off and blood-pool background had cleared.

In an attempt to decrease the high "nonspecific" liver, spleen, and bone marrow uptake, we gave two patients 7.5 g of human gamma globulin immediately prior to infusion of 1 mg [^{111}In]9.2.27. Both plasma clearance and serial imaging showed no change in the biodistribution from that seen with 1 mg of [^{111}In]9.2.27.

The pharmacokinetics of clearance of [^{111}In]9.2.27 from plasma corroborated the visual impression from

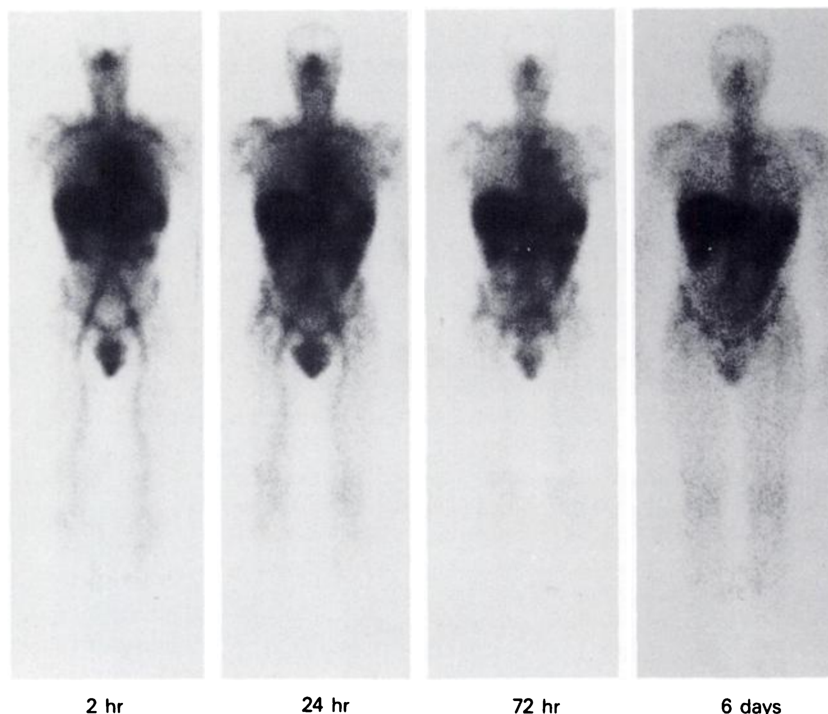

FIGURE 2
Serial whole-body images after i.v. infusion of 50 mg, 5 mCi of [^{111}In]9.2.27. Tumor localization in the left hilum is best seen at 6 days when blood pool has decreased. Prominent activity is seen in the liver and spleen, which showed no evidence of tumor on computerized tomography scan; activity in bowel is also seen.

TABLE 2
Region of Interest Data

Time of imaging	Liver* 1 mg	Liver* 50 mg	Spleen* 1 mg	Spleen* 50 mg	Bone marrow† 1 mg	Bone marrow† 50 mg
2 hr	336,566	421,348	398,876	85,432	144	90
1 day	590,058	515,750	396,889	85,087	236	86
2 day	629,562	555,280	364,114	83,480	237	114
6 day	596,440	765,148	241,273	103,936	182	113

* Geometric mean of whole organ counts per 5 min (three patients at each dose).
† Counts per pixel per 5 min over the L5 vertebral body.

the scans. The plasma clearance of the 1-mg dose was significantly faster than that of the ≥50 mg dose (Fig. 4) (t-test, $p < 0.01$). At 24 hr postinfusion of 1 mg, a mean of 14% (range 11–18%) of the injected dose was retained in the plasma. The plasma clearance of the two patients receiving pre-infusion of human gamma globulin was no different than that of the other three patients. The mean plasma retention at 24 hr for patients receiving ≥50 mg was 51% (range 45–60%). No significant binding to circulating blood cells was seen.

The mean total-body clearance of ^{111}In appeared to be more rapid for the 1 mg versus the 50 mg infusion.

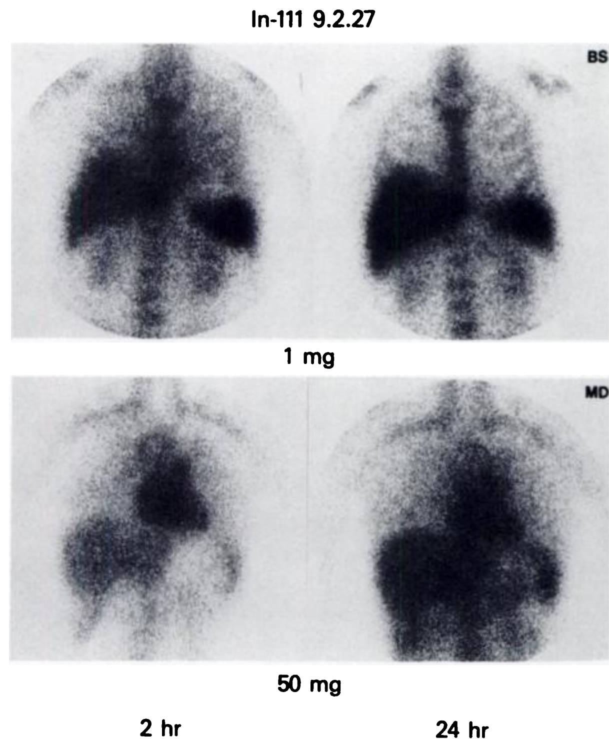

FIGURE 3
Anterior chest at 2 and 24 hr postinfusion of 1 mg, 5 mCi (upper panel) and 50 mg, 5 mCi (lower panel) of [^{111}In] 9.2.27. At the 50 mg dose level there is more prolonged blood-pool and less marrow and splenic uptake than at the 1 mg. No metastatic lesions are identified in either scan.

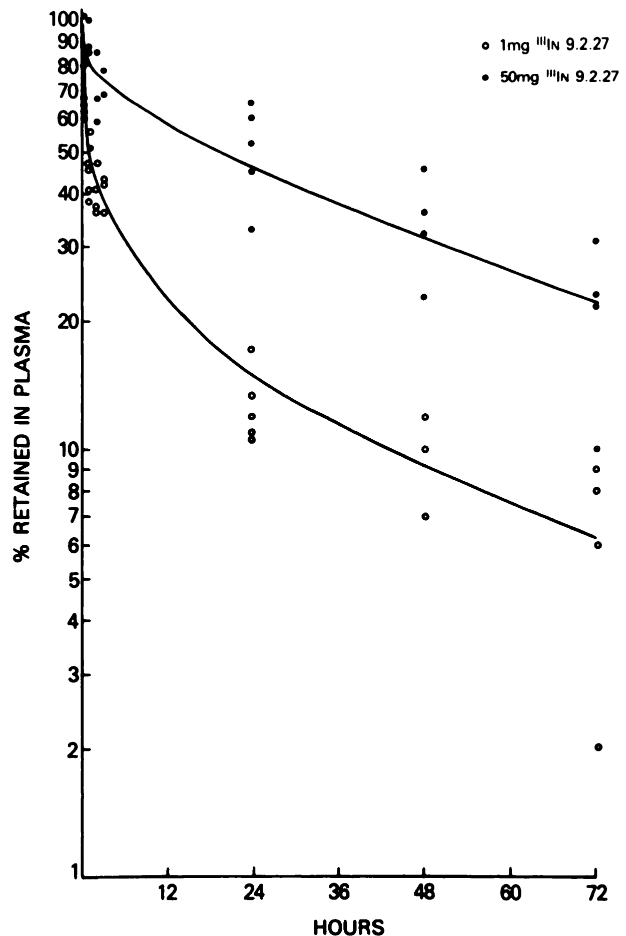

FIGURE 4
Plasma retention (see text) of [^{111}In]9.2.27 at 1 mg versus 50 mg of 9.2.27 MoAb. Individual data points are plotted.

This was due to the lower incorporation of ^{111}In during two labelings of 9.2.27 in the 1 mg group (70% and 74%) with consequent higher percentage of [^{111}In] DTPA excreted rapidly by the kidneys. When the total-body retention was corrected for the percent of total administered ^{111}In that was antibody bound, there was no statistically significant, difference between the two groups (t-test, $p > 0.1$). At both dose levels, more than 66% of the activity was retained by the whole body at 6 days. The urinary excretion in the first 24 hr ranged from 4% to 36%, and correlated well with the nonprotein bound ^{111}In ([^{111}In]DTPA). After the first 24 hr, the mean urine excretion of ^{111}In for all patients was 3% per day (range 1.8–3.8%) with no significant difference between both groups (t-test, $p > 0.1$). The chemical form of ^{111}In in the urine was not determined. There was no statistically significant difference between the urinary excretion measurements and whole-body retention probe counts (t-test, $p > 0.1$). The correlation coefficient between these measurements was excellent ($r > 0.9$), indicating that the kidneys were the predominant route of excretion of the isotope.

The MoAb levels determined by an ELISA assay for mouse IgG (antibody mass) correlated well with serum ^{111}In radioactivity counted in a well counter. For patients receiving 1 mg, the correlation coefficient was $r = 0.98$. For patients receiving 50 mg of 9.2.27, the correlation coefficient was $r = 0.95$. This indicated that the clearance of [^{111}In]9.2.27 was not significantly different from that of the unlabeled, unconjugated 9.2.27 (t-test, $p > 0.1$).

Histologic Correlation

Eight patients undergoing monoclonal antibody administration had biopsy of nodes or subcutaneous lesions that were positive for melanoma on histologic examination (Table 3). All of the biopsies tested showed that more than 75% of tumor cells expressed the 250 kD antigen when tested with 9.2.27. In Patients 4 and 8, subcutaneous nodules that were not detected on scan were biopsied and showed presence of the antigen. In four patients, no immunohistochemical analysis was available for antigen determination, three of these had at least one site positive on scan, while the fourth had no localization on scan. The concentration of ^{111}In in tumor biopsies ranged from 0.001 to 0.007% of the injected dose per gram. In biopsies of melanoma metastases from two patients who received 1 mg of 9.2.27, in vivo delivery of 9.2.27 could not be confirmed by immunohistology. In three of four patients receiving the ≥50 mg of 9.2.27 in vivo delivery by immunohistology confirmed the presence of the antibody at the tumor site. In Patient 8, although in vivo delivery of 9.2.27 was documented, the lesion (0.2 g) was not detectable on scan.

TABLE 3
Immunohistology

Patient	Interval between 9.2.27 infusion and biopsy*	9.2.27 in vitro† Intensity‡	9.2.27 in vitro† % positive§	9.2.27 in vivo¶ Intensity	9.2.27 in vivo¶ % positive
1	+6 days	+++	100	–	0
2	−18 days	++	90	PI**	PI
3	No biopsy			–	–
4	+1 day	++	95	–	0
5	No biopsy				
6	−8 days	+++	100	PI	PI
	+3 days	+++	100	–	0
7	+2 days	++	78	+	30
8	−10 mo	++	90	PI	PI
	+3 days	+++	100	+	25
9	No biopsy				
10	+2 days	Not available			
11	−1 mo	+++	95	PI	PI
	−3 days	+++	100	PI	PI
	+4 days	+++	100	++	35

* Date that biopsy was performed, + = time after antibody infusion, − = time before antibody infusion.
† In vitro antigen status of tumor (see text).
‡ Intensity = qualitative measure of antigen status (range 0 to +++).
§ Positive = number of melanoma cells expressing the HMW antigen.
¶ In vivo delivery of 9.2.22 antibody from the intravenous infusion (see text).
** PI = Preinjection biopsy, in vivo delivery is not applicable.

Quality Control

All 11 preparations were labeled with 4.0 to 5.0 mCi ^{111}In (mean 4.7 ± 0.4 mCi) (Table 4). The mean incorporation for the whole group of ^{111}In into the antibody was 90% with a mean of <9% [^{111}In]DTPA and <1% present as [^{111}In]colloid. When we corrected the immunoreactivity for the antibody-bound ^{111}In, eliminating from consideration the [^{111}In]DTPA in the preparation, we saw a mean immunoreactivity of 68%. Table 4 indicates the quality control results for the low and high dose of MoAb.

Three patients had serial plasma [^{111}In]transferrin determination. In the first 3 hr after the infusion, <2% to 3% of the injected radioactivity was on transferrin; at 24 hr a maximum of 3.2% to 4% of the injected dose was on circulating transferrin.

Toxicity

No side effects were encountered. Pre- and postinfusion, complete blood cell counts, liver function tests, and renal function tests were not significantly different ($p > 0.2$ to 0.9). Elevated human anti-mouse antibody levels were not present in any of the baseline samples.

DISCUSSION

The present study demonstrates dose-dependent pharmacokinetic alterations of [^{111}In]9.2.27 that are similar to those described by others (*3,20,21,28*). Our study with [^{111}In]9.2.27 indicates that, at low doses, there is rapid removal of large proportion of MoAb by liver, spleen, and bone marrow that renders it unavailable for tumor localization. The administration of

TABLE 4
Quality Control*

	1 mg	50 to 100 mg
^{111}In protein bound†	86 ± 13	95 ± 2
^{111}In colloid‡	1.6 ± 1.2	1.0 ± 1.2
Immunoreactivity§	58 ± 14	60 ± 14
Corrected-immunoreactivity¶	67 ± 11	62 ± 14

* Mean ± s.d.
† ITLC-SG.
‡ Whatman No. 1, HSA.
§ Cell-binding assay.
¶ $\text{Corrected immunoreactivity} = \frac{\text{immunoreactivity}}{\text{Fraction protein bound } ^{111}\text{In}}$.

higher doses of unlabeled MoAb caused a prolonged circulation of the [^{111}In]9.2.27 in plasma with less spleen and bone marrow uptake and with elevated plasma levels of MoAb and improved tumor localization. Although ROI data showed significantly less uptake in the spleen and marrow at the higher MoAb doses suggesting saturation, the concentration in the liver for both doses was not significantly different. Similar dose-dependent alterations have been reported by others (*20,21,28*), although this may not be the case for antibodies that do not have rapid binding to normal tissues (*37*).

This dose-dependent alteration in clearance indicates that there is a "sink" for 9.2.27 that can be saturated with higher doses (*38*). The mechanism of rapid removal from plasma with binding to liver, spleen, and bone marrow has not been completely elucidated. Quality control showed low levels of colloid in the preparation (<1%), that are not sufficient to account for the degree of liver or splenic uptake seen. Instability of [^{111}In]9.2.27 with transchelation of ^{111}In to other proteins in serum is unlikely to be the major cause of the observed distribution in liver, spleen, and bone marrow, since adding carrier antibody would not have increased the stability of the ^{111}In on the 9.2.27 or altered the distribution of any ^{111}In that separated from the MoAb. The comparison of clearance of ^{111}In radioactivity versus an ELISA determination of murine gamma globulin showed that [^{111}In]9.2.27 acted as a tracer to the carrier 9.2.27. In addition, ^{111}In translocation from MoAb to transferrin, <3% in the first 3 hr, was insufficient to explain the large amounts of radioactivity seen in liver, spleen, and bone marrow in the immediate postinfusion scan. Nonspecific Fc receptor binding cannot be excluded, although competition with [^{111}In]9.2.27 was not obtained by adding large amounts of human immunoglobulin, that is known to block Fc binding (*39*); it is possible that Fc binding of human IgG was less efficient than that of murine Fc. Future studies blocking with irrelevant, subclass-matched IgG2a or imaging with ^{111}In F(ab')$_2$ would be useful in better assessing Fc binding. Normal routes of catabolism of immunoglobulins may also contribute to the uptake seen in organs with high reticuloendothelial system (RES) resulting in prolonged retention of ^{111}In. In addition, cross reactivity with shared epitopes in liver, bone marrow, or splenic tissue cannot be excluded.

Serial ROI measurements showed that there was clearance from the blood pool and progressive accumulation in liver. Tumor uptake increased from 2 hr and peaked at 24 hr, remaining constant thereafter. In contrast, studies utilizing ^{131}I-labeled MoAb generally have shown gradual clearance from major organs and tumor (*3,17,25*), probably related to catabolism of the antibody with separation of the radioiodine from the antibody and excretion of the label. Optimum imaging time was approximately at 96 hr when blood background was low and tumor uptake had peaked. Unfortunately, high concentrations in liver, spleen, and bone marrow make it difficult to see lesions in or around these sites as shown by failure to detect lesions in two patients (one with a liver lesion and one with a subcutaneous lesion overlying the liver). In addition, concentration of ^{111}In seen in the bowel at the higher dose levels is also likely to interfere with tumor imaging. Although the mechanism of bowel uptake is unknown, similar findings have been observed with other ^{111}In-labeled antibodies (*20,21*).

The sensitivity of tumor detection was significantly improved at the higher mg doses, with 75% of all lesions visualized and positive studies in all six patients studied as compared to minimal lesion visualization in two of five patients and only two of ten lesions. Although higher doses of MoAb resulted in improved imaging in this small group of patients, the limited sensitivity continues to be a problem for diagnostic application of this MoAb. Further work to improve the targeting and decrease the nonspecific uptake will be necessary to optimize 9.2.27 delivery.

Tumor uptake of MoAb is likely to be determined by multiple factors. Although antigen presence is a prerequisite for specific imaging, it does not guarantee a positive scan. In two antigen-positive patients, the scans were completely negative, and two patients with localization in some sites of known disease had negative scans in other sites that were subsequently biopsied and shown to be antigen-positive. In these cases, size (<2.5 cm) was probably an important factor, although some patients had larger lesions that were also negative on scan (>4 cm). Other factors that were not evaluated in this study but which may be important parameters of tumor uptake include tumor blood flow and capillary permeability.

In summary, (a) large doses of MoAb can be administered safely; (b) dose of 9.2.27 is an important parameter of antibody biodistribution; (c) larger doses of 9.2.27, in this limited number of patients, resulted in an improvement of tumor imaging; (d) further work to elucidate the mechanism of nonspecific organ distribution will be necessary before routine clinical use can be considered.

NOTES

* Hybritech, Inc., La Jolla, CA.
† Kirkegaard and Perry Laboratories, Gaithersburg, MD.
‡ Dynatech, Alexandria, VA.
§ Pharmacia Fine Chemicals, Uppsala, NY.
¶ Pierce, Rockford, IL.
** BRL, Bethesda, MD.
†† Dako, Accurate Chemical, Westbury, NY.
‡‡ Sigma, St. Louis, MO.
§§ Fisher, Pittsburgh, PA.
¶¶ Hewlett-Packard.

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of Pat Maloney and Margarita Lora, who performed the quality control and assays; Marge Cawley, Doris Fejka, Susan Lange, and Penney Yolles, who imaged the patients; Warren Rumble and Louise Lerario, RN, who monitored the whole-body clearance; radiopharmacists Ray Farkas, Richard Fejka, and Mark Rotman, who helped in the formulation of the antibody; and Jan Paluch, who prepared this manuscript.

REFERENCES

1. Goldenberg DM, DeLand FH, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med* 1978; 298:1384–1386.
2. Mach JP, Carrell S, Forni M, et al. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. *N Engl J Med* 1980; 303:5–10.
3. Larson SM, Carrasquillo JA, Krohn KA, et al. Localization of I-131 labeled p97 specific FAB fragments in human melanoma as a basis for radiotherapy. *J Clin Invest* 1983; 72:2101–2114.
4. Moldofsky PJ, Powe J, Mulhern CB, et al. Metastatic colon carcinoma detection with radiolabeled $F(ab')_2$ monoclonal antibody fragments. *Radiology* 1983; 149:549–555.
5. Mach JP, Buchegger F, Forni M, et al. Use of radiolabeled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. *Immunol Today* 1981; 2:239–247.
6. Fairweather DS, Bradwell AR, Dykes PW, et al. Improved tumour localisation using indium-111 labeled antibodies. *Br Med J* 1983; 287:167–170.
7. Farrands PA, Perkins AC, Pimm MV, et al. Radioimmunodetection of human colorectal cancers by an anti-tumor monoclonal antibody. *Lancet* 1982; 2:397–400.
8. Larson SM, Carrasquillo JA, McGuffin RW, et al. Use of I-131 labeled, murine Fab against a high molecular weight antigen of human melanoma: preliminary experience. *Radiology* 1985; 155:487–492.
9. Carrasquillo JA, Krohn KA, Beaumier P, et al. Diagnosis of and therapy for solid tumors with radiolabeled antibodies and immune fragments. *Cancer Treat Rep* 1984; 68:317–318.
10. Buraggi GL, Callegaro L, Mariani G, et al. Imaging with ^{131}I-labeled monoclonal antibodies to a high-molecular-weight melanoma-associated antigen in patients with melanoma: efficacy of whole immunoglobulin and its $F(ab')_2$ fragments. *Cancer Res* 1985; 45:3378–3387.
11. Goldenberg DM, Kim EE, DeLand FH, et al. Radioimmunodetection of cancer using radioactive antibodies to human chorionic gonadotropin. *Science* 1980; 208:1284–1286.
12. Goldenberg DM, Kim EE, DeLand F, et al. Clinical studies on the radioimmunodetection of tumors containing alpha-fetoprotein. *Cancer* 1980; 45:2500–2505.
13. Berche C, Mach JP, Lumbroso JD, et al. Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: first clinical results using radiolabeled monoclonal antibodies against carcino embryonic antigen. *Br Med J* 1982; 285:1447–1451.
14. Pateisky N, Philipp, Burchel J, et al. Radioimmunodetection in patients with suspected ovarian cancer. *J Nucl Med* 1985; 26:1369–1376.
15. Epenetos AA, Snook D, Hooker G, et al. Indium-111 labeled monoclonal antibody to placental alkaline phosphatase in the detection of neoplasms of testis, ovary, and cervix. *Lancet* 1985; 2(8451):350–353.
16. Davies JO, Davies ER, Howe K, et al: Radionuclide imaging of ovarian tumors with ^{123}I-labeled monoclonal antibody (NDOG2) directed against placental alkaline phosphatase. *Br J Obstet Gynaecol* 1985; 92:277–286.
17. Carrasquillo JA, Mulshine JL, Bunn BA, et al. Tumor imaging of In-111 T101 monoclonal antibody is superior to iodine-131 T101 in Cutaneous T-cell lymphoma. *J Nucl Med* 1987; 28:281–287.
18. Rainsbury RM, Westwood JH, Coombes RC, et al. Location of metastatic breast carcinoma by a monoclonal antibody chelate labeled with indium-111. *Lancet* 1983; 1:934–938.
19. Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495–497.
20. Halpern SE, Dillman RO, Witztum KF, et al. Radioimmunodetection of melanoma utilizing ^{111}In 96.5 monoclonal antibody: a preliminary report. *Radiology* 1985; 155:493–499.
21. Murray JL, Rosenblum MG, Sobol RE, et al. Radioimmunoimaging in malignant melanoma with ^{111}In-labeled monoclonal antibody 96.5. *Cancer Res* 1985; 45:2376–2381.
22. Epenetos AA, Shepherd J, Britton KE, et al. ^{123}I radioiodinated antibody imaging of occult ovarian cancer. *Cancer* 1985; 1:55:984–987.
23. DeLand FH, Kim EE, Simmons G. Imaging approach in radioimmunodetection. *Cancer Res* 1980; 40:3046–3049.
24. Bradwell AR, Fairweather DS, Dykes PW, et al. Limiting factors in the localization of tumours with radiolabeled antibodies. *Immunol Today* 1985; 6:1163–1170.
25. Sullivan DC, Silva JS, Cox CE, et al. Localization of I-131 labeled goat and primate anti-carcinoembryonic antigen (CEA) antibodies in patients with cancer. *Invest Radiol* 1982; 17:350–355.
26. Halpern SE, Hagan PL, Garver PR, et al. Stability, characterization, and kinetics of ^{111}In-labeled monoclonal antitumor antibodies in normal animals and nude mouse-human tumor models. *Cancer Res* 1983; 43:5347–5355.
27. Halpern SE, Stern P, Hagan P, et al. Labeling of monoclonal antibodies with indium-111: technique and advantages compared to radioiodine labeling. In: Buchiel SW, Rhodes BA, eds. Radioimmunoimaging and radioimmunotherapy. New York: Elsevier Publishing Co., 1983:197.
28. Carrasquillo JA, Bunn PA, Kennan AM, et al. Radioimmunodetection of cutaneous T-cell lymphoma with ^{111}In labeled T101 monoclonal antibody. *N Engl J Med* 1986; 315:673–680.
29. Morgan AC, Galloway DR, Reisfeld RA. Production and characterization of monoclonal antibody to a melanoma-specific glycoprotein. *Hybridoma* 1981; 1:27–36.
30. Bumol TF, Reisfeld RA. Unique glycoprotein-proteo-

glycan complex defined by monoclonal antibody on human melanoma cells. *Proc Natl Acad Sci USA* 1982; 79:1245.

31. Stuhlmiller GM, Borowitz MJ, Croker BP, et al. Multiple Assay Characterization of murine monoclonal antimelanoma antibodies. *Hybridoma* 1982; 1:447–460.
32. Oldham RK, Foon KA, Morgan AC, et al. Monoclonal antibody therapy of malignant melanoma: in vivo localization in cutaneous metastasis after intravenous administration. *J Clin Oncol* 1984; 11:1235–1244.
33. Krejcarek GE, Tucker KL. Covalent attachment of chelating groups to macromolecules. *Biochem Biophys Res Comm* 1977; 77:581–585.
34. Paik CH, Herman DE, Eckelman WC, et al. Synthesis, plasma clearance, and in vitro stability of protein containing a conjugated indium-111 chelate. *J Radioanal Chem* 1980; 57:553–564.
35. Gutter KC, Abhuluziaz BP, et al. Use of monoclonal antibodies for the histopathological diagnosis of human malignancy. *J Clin Pathol* 1982; 35:1253–1267.
36. International committee for standardization in hematology. Recommended methods for measurement of red cell and plasma volume. *J Nucl Med* 1980; 21:793–800.
37. Carrasquillo JA, Sugarbaker P, Colcher D, et al. Radioimmunoscintigraphy of colon cancer with ^{131}I labeled B72.3 monoclonal antibody. *J Nucl Med*: in press.
38. Eger RR, Covell DG, Carrasquillo JA, et al. Kinetic model for the biodistribution of an ^{111}In-labeled monoclonal antibody in humans. *Cancer Res* 1987; 47:3328–3336.
39. Imbach P, d''Apuzo V, Hirth RA, et al. High dose intravenous gammaglobulin for idiopathic thrombopenic purpura in childhood. *Lancet* 1981; 1:1228–1231.