Radioimmunoscintigraphy of Colon Cancer with Iodine-131-Labeled B72.3 Monoclonal Antibody

Jorge A. Carrasquillo, Paul Sugarbaker, David Colcher, James C. Reynolds, Jose Esteban, Gail Bryant, Andrew M. Keenan, Patricia Perentesis, Kunihiko Yokoyama, Diane E. Simpson, Patrizzia Ferroni, Raymond Farkas, Jeffrey Schlom, and Steven M. Larson

Department of Nuclear Medicine, Clinical Center; Department of Surgery, Laboratory of Tumor Immunology and Biology and the Department of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

The monoclonal antibody B72.3 is a murine IgG1 that is reactive with a wide range of carcinomas while demonstrating little or no reactivity to normal adult tissues. We have shown (27) quantitative analyses demonstrating selective targeting of [^{131}I]B72.3 IgG to metastatic colorectal cancer. We have also shown (*28*) that (a) B72.3 localization in metastases correlated with the percentage of tumor cells in the biopsy specimen; (b) B72.3 could localize in carcinomas of various degrees of differentiation with best localization in well-differentiated tumors and (c) [^{131}I]B72.3 could penetrate tumor masses, as determined by autoradiographic studies. We report here the various parameters effecting radioimmunoscintigraphy with [^{131}I]B72.3 IgG. Sixteen of 35 patients with colorectal carcinoma had positive scans (without blood-pool subtraction). High circulating TAG-72 antigen levels correlated with positive scans. No dose dependent differences were seen in biodistribution or tumor imaging. The plasma clearance and urinary excretion of [^{131}I]B72.3 and [^{125}I]BL-3 (nonspecific control) were not significantly different. No toxicity was noted. Approximately one-half of patients developed human anti-mouse immune response.

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Colon cancer has been one of the tumors most frequently imaged by the radioimmunoscintigraphy technique. Several studies have utilized anti-CEA antibodies of polyclonal (*3–13*) or monoclonal origin (*5, 14–16*) as well as antibodies directed at non-CEA antigens (*17–22*). Many of these antibodies have significant cross reactivity with normal circulating cells (*16,22*) or normal organs, limiting their clinical utility. Monoclonal antibody (MoAb) B72.3, a murine IgGl, was obtained by immunizing mice with membrane enriched fractions of a human breast carcinoma metastatic to the liver (*23*). The MoAb recognizes a high molecular weight glycoprotein (>10^6 D) with characteristics of a mucin, termed TAG (tumor associated glycoprotein)-72 (*24*). On the basis of immunopathologic findings, TAG-72 is expressed in ~85% of colon cancers, 70% of breast cancers, and 95% of ovarian cancers, while it shows minimal or no expression in normal adult tissue (*23,25–27*). The antigen is found on the cell surfaces and may also be secreted into the mucin pools of the tumor (*28*). We have previously reported (*29*) a detailed analyses of the quantitative distribution of [^{131}I]B72.3 in tumor and normal tissues of colorectal cancer patients. We have also reported (*30*) on the correlation of the quantitative distribution of [^{131}I]B72.3 IgG with tumor histology, tumor antigen level, and anatomic tumor location. We demonstrated (*29,30*) in 20 patients the selective localization of B72.3 in 99 of 142 tumors which had at least three times greater uptake per gram than normal tissue, while only 12 of 210 normal tissues had similar levels of uptake. We now report on various parameters effecting imaging in 35 patients receiving escalating doses of monoclonal antibody B72.3 IgG labeled with ^{131}I.

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For reprints contact: Jorge A. Carrasquillo, MD, National Institutes of Health, Nuclear Medicine Department, Building 10, Room 1C-401, ACRF, 9000 Rockville Pike, Bethesda, MD 20892.

MATERIAL AND METHODS

Antibody

B72.3 was purified from ascitic fluid by ammonium sulfate precipitation and ion exchange chromatography (*31*). The monoclonal antibody BL-3, an IgG1 directed against the idiotype of a B-cell lymphoma was used as a nonspecific control (*30*). Once purified, the IgG were filtered and all lots tested according to Food and Drug Administration "Points to consider in the manufacture of monoclonal antibody products for human use (*32*)." These included lack of mycoplasma, lack of adventitious viruses, sterility, lack of pyrogenicity, and general safety.

The purified B72.3 was labeled with ^{131}I using the Iodogen method (*33*) [250 μg of iodogen and 500-1,500 μg of B72.3 IgG]. The MoAb was labeled at specific activities of 0.3 to 12.6 mCi per mg of antibody. Free iodine was separated from protein bound by gel filtration through a column packed with Pharmacia G-10 (Pharmacia, Upsala, Sweden). A mean of 98% of the ^{131}I was protein bound as determined by chromatography on Whatman No. 1 with 85% methanol. High performance liquid chromatography (HPLC) analysis of the percent protein bound iodine was performed using a TSK 2000 column, and TSK 3000 (Beckman Instruments Inc., San Ramon, CA) in tandem. The BL-3 was labeled with ^{125}I at specific activities of 2.44 ± 0.48 mCi per mg using a similar technique. A mean of 98% of the ^{125}I was protein bound. All products were shown to be sterile and passed quantitative endotoxin testing (limulus amebocyte lysate, Associates of Cape Cod, MA).

More than 80% of the [^{131}I]B72.3 remained immunoreactive based on sequential solid phase RIAs (*31*) with extracts of a carcinoma xenograft grown in athymic mice (LS-174T) which contains the TAG-72 antigen that reacts with B72.3. In addition [^{131}I]B72.3 has been tested in competition radioimmunoassays and competed identically to unlabeled B72.3 (data not shown). The [^{125}I]BL-3 was ~80% immunoreactive based on a solid phase RIA (*30*).

To evaluate the effect of protein mass on tumor localization, a limited dose escalation study was performed with doses of 0.16 mg to 20 mg of MoAb and 0.76 to 10 mCi. The MoAb was administered as a 1 hr intravenous infusion in <20 ml of phosphate buffered saline at a pH of 7.2. The 20-mg doses consisted of ~1 mg of ^{131}I-labeled B72.3 mixed with unlabeled, carrier, antibody to make a total of 20 mg and 10 mCi. As a control seven patients receiving a mean of 0.98 ± 0.13 mg of [^{131}I]B72.3 were co-infused with [^{125}I]BL-3 (0.97 ± 0.17 mg and 1.94 ± 0.1 mCi).

Patients

Thirty-five patients enrolled in a previously established NCI-Surgery Branch protocol for surgical resection of metastatic disease were evaluated. The protocol was approved by the Institutional Human Research Committee of the National Cancer Institute and all patients gave their informed consent. All patients had histologically documented colon cancer. Their ages ranged from 16 to 70 yr old, with a mean age of 51 yr. The group consisted of 22 men and 13 women. Baseline complete blood counts, liver function test, and renal function were obtained prior to radioimmunoscintigraphy and repeated 1 to 2 wk post-MoAb infusion. The initial workup included chest x-ray, abdominal computed tomography, full chest tomograms, and bone and liver spleen scans. Two patients had primary colon tumors (in one patient it was a second primary colon tumor) and the others had metastatic colon carcinoma. Thirty-four patients had previously undergone a resection of a primary colon cancer. Thirty-two patients underwent surgical exploration for "curative" or "debulking" resection of metastatic disease. Thirty patients had metastatic disease documented by conventional imaging techniques. Three patients had rising CEA with a negative radiographic workup and two patients had a tumor detected by colonoscopy. Two patients who did not undergo surgery had positive scans which were confirmed by radiographic workup.

All patients had determination of TAG-72 antigen status of their tumor by immunohistochemical studies with B72.3 antibody (*25*). The TAG-72 antigen is detected in the serum of approximately one-half of the patients with colorectal carcinoma cancer (*34*). In order to determine the effect of circulating antigen on biodistribution baseline serum samples prior to antibody injection were assayed for TAG-72 content using an immunoradiometric assay (*34*).

Imaging and Biodistribution

The patients were imaged within 2 hr of MoAb administration and daily until the day prior to surgery (range 4 to 15 days, mean 8 ± 2 days). A GE 535 gamma camera with a high-energy collimator was utilized. Utilizing the 364 keV gamma ray of ^{131}I and a 20% window, anterior and posterior whole-body images as well as multiple spot views (5 to 10 min) including chest, abdomen, and pelvis were obtained. In addition to analog images, digital images were recorded with a Hewlett Packard Scintigraphic data analyzer. Serial images were analyzed with manually drawn regions of interest over the major organs and tumor. Values were expressed as count per minute (cpm), corrected for isotope decay. The rate of isotope clearance from individual organs was compared to that from tumor.

The images were interpreted as positive when focal areas of increased uptake were seen in the analog images not corresponding to sites of physiologic uptake (blood pool, bladder, and thyroid). Blood-pool or organ subtraction techniques were not applied to these images. As a "gold standard" scan results were correlated with a combination of surgical and radiographic findings.

Plasma clearance was determined by gamma counting plasma samples obtained at the end of infusion, 30 min, 1 hr, 2 hr, 24 hr, 48 hr, 72 hr, 96 hr and daily up to the time of surgery and multiplying the dose retained per milliliter of serum by the patients estimated plasma volume. Whole-body retention of ^{131}I was determined by probe counts with a 2 × 2 × 2 in. NaI crystal at 7 m from the patient taking the immediate postinfusion counts as 100%. In addition ^{131}I whole-body retention was calculated from the region of interest analyses of the anterior and posterior whole body (decay corrected geometric mean) taking as 100% the cpm in the immediate postinfusion scan. Serial 24-hr urine collections were obtained up to 72 hr postinfusion.

Human anti-mouse antibody (HAMA) determination (*35*) was performed on the baseline and post-antibody infusion serums. One microliter of the patients' baseline serum was incubated with 0.5–1 ng of [^{125}I]B72.3 monoclonal antibody

(10,000 cpm), for 20 hr at 4°C. Twenty milligrams of formalin-fixed staphylococcus A cells (BRL, Bethesda, MD.) were added, and following a 15-min incubation (4°C, the bound counts were separated by centrifugation (3,000 rpm for 5 min). The percent binding for each patient serum was calculated as bound counts/total counts ×100 and compared to the mean for a group of normal control serum from patients not previously exposed to MoAb. A serum was considered positive for HAMA if the percent binding was at least three standard deviations greater than the mean of the control group. Only patients with serum samples available at least 2 wk to 6 mo after MoAb infusion were evaluated for development of HAMA.

RESULTS

Sixteen of 35 patients had positive scans. No difference in tumor detection occurred at the different dose levels of B72.3 administered (Table 1). The anatomic distribution of lesions represented the usual metastatic sites of colon cancer. Table 2 indicates the sites of involvement and their imaging status. In all patients with clinical evidence of ascites the radiolabeled antibody concentrated in the peritoneal cavity (Fig. 1), while passive extravasation into a third space was likely a contributing factor, biopsy of the patient's tumor showed preferential tumor uptake (*29*). By scan, the smallest lesion detected was 2.5 cm in diameter with most detected lesions being 4 cm or larger.

A characteristic pattern of whole-body distribution was seen. The MoAb circulated in the blood pool with a mean terminal phase $T_{½}$ of 65 hr (weighed mean of all groups with a range of 32 to 106 hr that was not significantly different between doses ($p > 0.2$ to 0.9). No significant binding of the antibody to circulating blood cells was observed. The whole-body radioactivity cleared, with a mean $T_{½}$ of 82 hr, a range of 51% to 61% was retained at 72 hr. The whole-body retention calculated from the whole-body scans showed excellent correlation with the probe count measurements (Fig. 2). Gamma camera images showed that the antibody was predominantly in the blood pool with very little selective accumulation in normal organs (Fig. 3). However, in four patients with circulating antigen and evidence of immune complexes as determined by HPLC of serum, there was mildly increased splenic activity.

The radioactivity in the blood pool was a prominent fraction of that retained in the whole body (Table 3). The region of interest analyses showed a nearly constant ratio between the blood-pool activity and the liver and spleen activity (Table 4). Escalating doses of [^{131}I] B72.3 (up to 20 mg), had no effect on the plasma, whole-body clearance or biodistribution (Table 5). The plasma clearance (Table 6) and the urinary excretion (Table 7) of [^{125}I]BL-3 (nonspecific) MoAb was not significantly different from that of the co-infused [^{131}I]B72.3.

The optimum time for imaging was found to be about one week when the background activity had decreased and tumor to nontumor ratios were greatest (Fig. 3). Several liver tumor metastases presented as cold lesions early, which became of equal intensity to the liver and had more activity than the liver at the delayed time points (Fig. 4), indicating a slower access

TABLE 1
Scan Results by Dose

Dose*	0.28 mg	1.06 mg	4.18 mg	19.2 mg
Positive	6	7	1	2
Total	11	16	3	5

*Mean mg administered

TABLE 2
Scan Results by Site of Involvement

	Primary	Peritoneum	Liver	Lung	Bone	Retroperitoneum	Spleen
Positive	1	6	9	0	1	0	0
Total	2	15	17	6	1	5	1

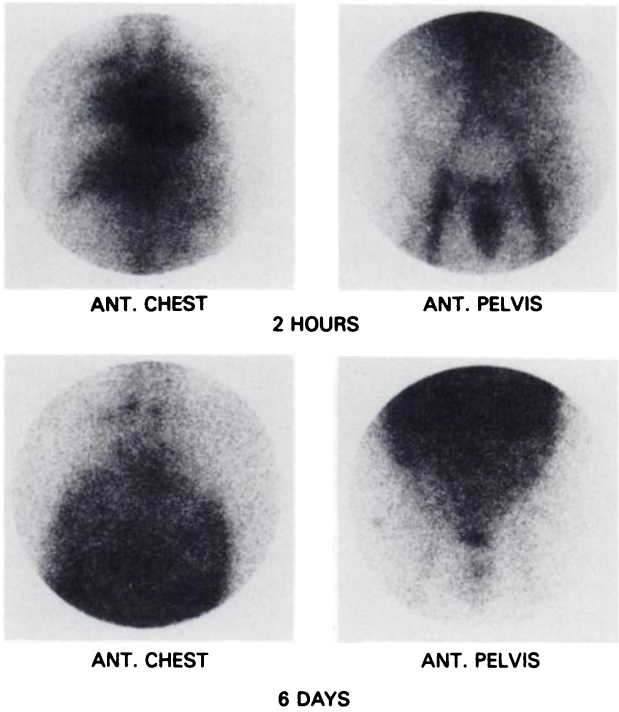

FIGURE 1
Anterior spot views of the chest and pelvis (10 mCi, 1.3 mg of [^{131}I]B72.3) from a patient with peritoneal carcinomatosis and ascites, demonstrating diffuse uptake in the peritoneal cavity at 6 days.

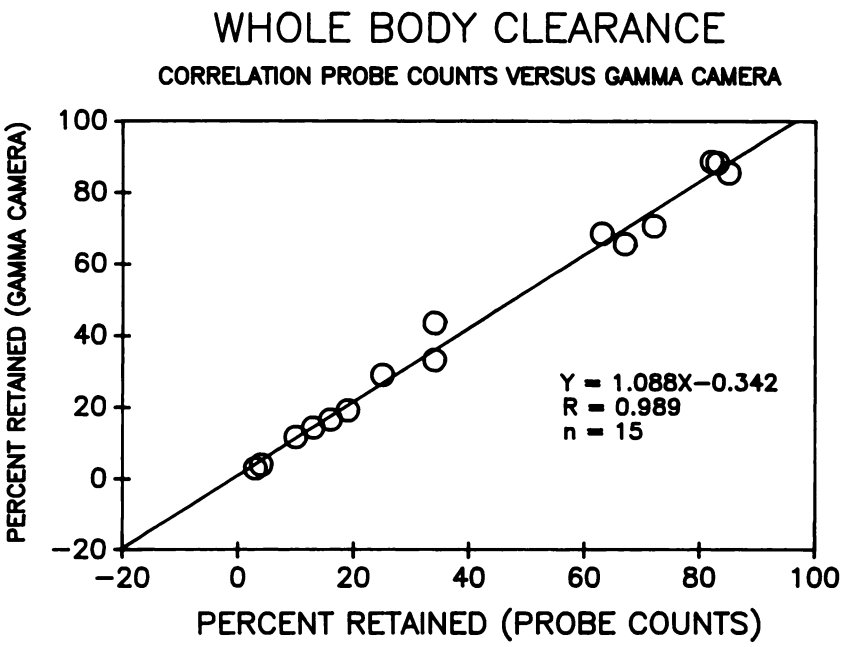

FIGURE 2
Determination of whole-body clearance of ^{131}I by probe counts shows excellent correlation to the whole-body clearance determined from the whole-body scans.

to tumor and more prolonged retention in liver metastases than in a normal liver.

There were several reasons for negative scans. The prolonged retention in the blood pool contributed to the high background. Areas of relative high activity such as the low pelvis near the urinary bladder were particularly difficult to interpret. In two patients a low concentration of antigen present in the tumor may have been responsible for poor imaging. No false-positive scans were observed.

Patients were evaluated for their immune response to murine IgG before infusion of 0.28 mg to 20 mg of murine MoAb B72.3 and in some cases B72.3 and BL-3. While none of the patients had detectable pre-existing anti-mouse IgG 52% of the patients developed an immune response to the injected MoAb after the single administration of the MoAb (Table 8).

Fifty-six percent (19/34) of the patients tested had significant levels of the TAG-72 antigen in their serum. The antigen level found in the serum had no effect on the plasma or whole-body clearance. The antigen level found in the serum did have a significant correlation

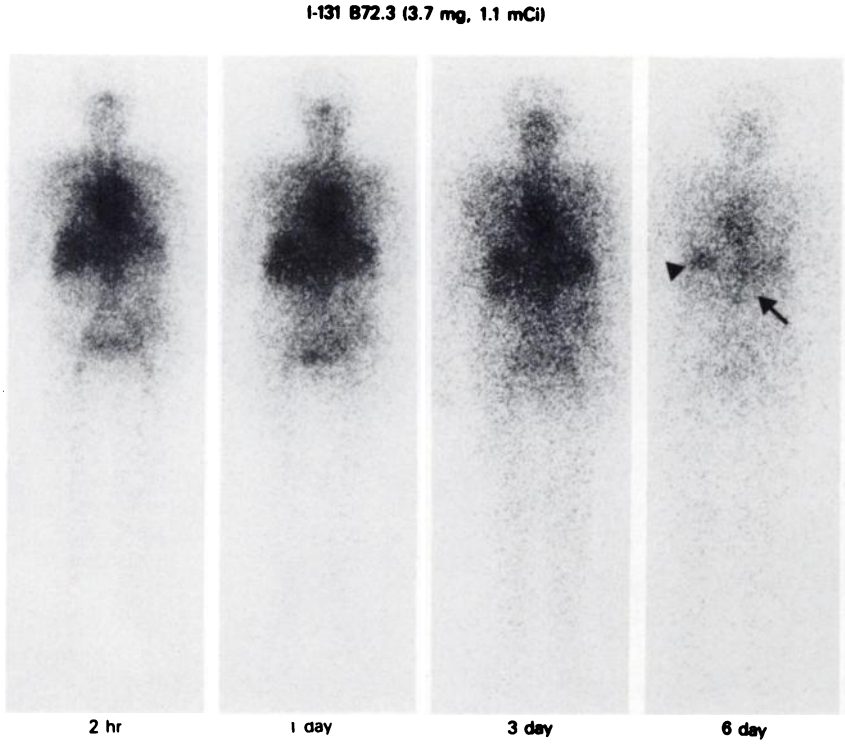

FIGURE 3
Serial anterior whole-body images from a patient with metastatic adenocarcinoma of the colon to the liver. The scans were obtained after i.v. injection of 1.1 mCi, 3.7 mg of [^{131}I] B72.3. There is a slow clearance of radioactivity from the blood pool and whole body. At 2 hr and at 1 day there is a normal appearing liver but at 3 days and 6 days, when the blood pool and liver background have decreased there is evidence of metastatic liver disease to the right lobe of the liver (arrowhead). A second metastases to the left lobe of the liver (arrow) is not clearly seen because of the high adjacent background in the heart and left upper quadrant.

TABLE 3
Fraction of the Radioactivity Retained in Whole Body that is in the Circulation*

B72.3 dose†	0.28 mg	1.06 mg	4.18 mg	19.2 mg
2 hr	0.95	0.92	0.88	0.82
24 hr	0.78	0.73	0.66	0.79
48 hr	0.67	0.68	0.58	0.64
72 hr	0.72	0.52	0.49	0.64

*Percent of the total injected dose in the plasma (gamma counts) divided by the percent of the injected dose retained in the whole body (probe counts).
† Mean milligrams injected.

TABLE 4
Ratio of Activity in Organ to Heart*

Days	Number of patients	Mean ± s.d.
Liver to heart		
0	12	0.692 ± 0.201
1	11	0.718 ± 0.200
2	9	0.709 ± 0.215
3	7	0.676 ± 0.246
6	6	0.797 ± 0.193
Spleen to heart		
0	12	0.557 ± 0.215
1	11	0.671 ± 0.317
2	9	0.628 ± 0.097
3	7	0.641 ± 0.170
6	6	0.962 ± 0.182

*From region of interest analyses.

with the ability to detect the lesions by gamma scanning. In 14 of 15 patients that had circulating levels of TAG-72 over 15 U/ml positive gamma scans were obtained, while only two of the 19 patients that had ≤15 U/ml had positive scans (Table 9).

No toxic side effects were noted during any of the antibody injections. Vital signs and temperature remained unchanged up to the time of surgery. No changes from the baseline blood counts, liver function test or rental function test were observed.

DISCUSSION

Radioiodinated B72.3 targeting colon cancer xenografted in nude mice showed unusually high stability to de-iodination and has been associated with some of the highest percentage uptake reported for animal tumor models (*31*). Our previously published quantitative results (*29,30*) with B72.3 show among the highest tumor to nontumor ratios reported for radioimmunoscintigraphy (*4,21,29,36–39*) although, as in other studies the absolute concentration of radiolabeled antibodies has been much lower than in xenograft models (*5,21*).

The gamma camera images showed prolonged circulation in the blood pool with very little localization in normal organs beyond that expected from the normal blood pool. The clearance from the liver and spleen appeared to be proportional to the blood-pool clearance. The antibody had a gradual clearance from the plasma (~65 hr terminal phase $T_{½}$) and a longer whole-body retention ($T_{½}$ of ~82 hr). The whole-body retention by probe counts correlated well with the whole-

TABLE 5
Dose Effect of B72.3 on Whole-Body and Plasma Retention

Dose of B72.3	0.28 ± 0.09 mg (n = 11)	1.04 ± 0.2 mg (n = 16)	4.18 ± 0.71 mg (n = 3)	19.2 ± 0.8 mg (n = 5)
Whole-body retention*				
Time				
To†	100	100	100	100
1 day	79	78	83	80
2 days	61	60	69	66
3 days	51	58	61	50
Plasma retention*				
Time				
To†	95	92	88	82
1 hr	91	90	89	75
2 hr	78	80	80	75
1 day	62	57	55	63
2 days	41	41	40	42
3 days	37	30	30	32
6 days	9	12	15	13

*Mean percent of the injected dose retained in the whole body or plasma.
† To end of the 1 hr antibody infusion.

TABLE 6
Plasma Retention of Patients Co-Infused with [^{131}I]B72.3 and [^{125}I]B1-3*

	[^{131}I] B72.3	[^{125}I]B1-3
To†	82 ± 10	83 ± 24
0.5 hr	77 ± 19	81 ± 24
1 hr	80 ± 16	81 ± 21
24 hr	50 ± 15	48 ± 16
48 hr	38 ± 17	36 ± 16
72 hr	32 ± 7	30 ± 6
96 hr	13 ± 8	10 ± 6

* Mean ± s.d. of the percent of injected dose in the plasma.
† To end of 1 hr infusion.

TABLE 7
Urinary Excretion of Patients Co-Infused with [^{131}I]B72.3 and [^{125}I]BL-3*

Time	[^{131}I]B72.3	[^{125}I]BL-3
0–24 hr	18 ± 11	21 ± 11
24–48 hr	14 ± 3	11 ± 4
48–72 hr	9 ± 3	9 ± 4

* Mean ± s.d. of the percent of the injected dose excreted.

body scan data and comparison with urinary excretion indicated that the urine was the main route of excretion.

Although some studies utilizing anti-CEA antibodies (*3,14,15*) have reported sensitivities of 85% to 95% for tumor detection of colorectal carcinoma other studies targeting the same or other antigens have been less successful (*4,6,7,13*). Most of these studies depended on background subtraction techniques producing low count suboptimal images and subtraction artifacts (*5, 36,41*) resulting in image interpretation of questionable certainty. In our study in which no blood pool or organ subtraction was performed, 46% of the patients were found to have scans positive for recurrent metastatic diseases. Although a higher sensitivity utilizing blood-pool subtraction techniques may have been achieved, we believe that these methods are cumbersome, have potential for false-positive results (*13*), and do not address the main issue of low absolute concentrations in tumors. Therefore, we chose not to employ them at this time.

The limited sensitivity obtained thus far may be related to several factors. The prolonged blood pool created a high background contributing to poor tumor visualization. In two patients, the antigen expression was very low and insufficient for imaging. Many tumors were very small and hard to detect because of limited resolution of our gamma cameras for ^{131}I (*41*). In addition, several tumors appeared as photon deficient areas early after antibody infusion and were only visualized above surrounding background at the delayed times. This finding corresponds with hypovascularity and or poor capillary permeability at the tumor level and, in conjunction with low absolute amounts of antibody delivered to tumor, suggests that a barrier exists to the delivery of the radiolabeled MoAb.

Circulating TAG-72 antigen did not prevent tumor imaging but rather correlated with positive scans. Previous reports with radiolabeled anti-CEA antibodies have shown no interference of circulating CEA on tumor detection (*36*). This positive correlation between TAG-72 levels and scans suggest that patients with high levels would be good candidates for imaging.

The administered dose had no significant effect on tumor detection, or biodistribution over the 100-fold difference in dose range. In contrast other antibodies have significant dose dependent differences in clearance and biodistribution (*38,42,43*). The dose dependent differences in biodistribution seen with the latter antibodies appears to be related to rapid clearance of the administered MoAb into a saturable pool which appears to be predominantly in liver, spleen, and bone marrow. The nature of this binding is not completely understood and may be related to binding to antigen, Fc receptor binding, catabolism or other factors. The similarities in clearance between the [^{125}I]BL-3 and the [^{131}I]B72.3 suggested that the lack of dose dependent changes with [^{131}I]B72.3 are likely due to its lack of significant cross-reactivity with normal tissues.

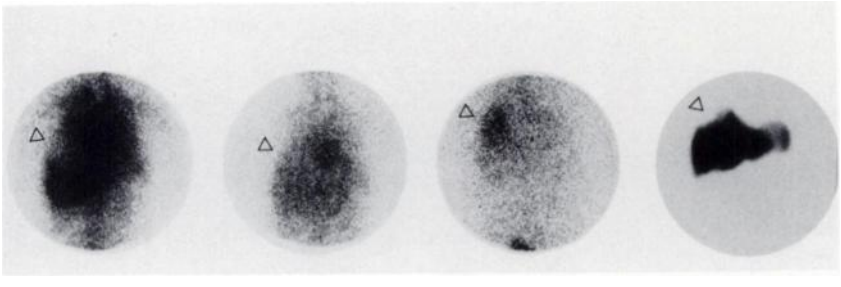

FIGURE 4
Serial anterior views of the chest (2 mCi, 0.34 mg of [^{131}I]B72.3) demonstrating a photopenic region in the liver corresponding to a large colon cancer metastases (arrowhead). At 1 day the lesion has equal activity to the normal liver while at 4 days the lesion has more uptake than the surrounding normal liver and background. A technetium-99m sulfur colloid scan (Tc-SC) shows a large metastases.

TABLE 8
Induction of Anti-Mouse Response in Patients Injected with [^{131}I]B72.3 IgG

	Positive	Borderline	Negative
Pre-injection	0/35	0/35	35/35
Postinjection	16/31	1/31	14/31

All infusions were well tolerated with no side effects. Our assay did not detect the presence of HAMA prior to the MoAb infusion although following the infusion 52% of the patients developed HAMA. The development of HAMA was not associated with any side effects.

The present results encourage further efforts and together with the biopsy data (*29,30*) indicate tumor specific targeting in ~70% of lesions. Although tumor targeting was observed, the limited sensitivity for tumor imaging indicates the need for strategies that optimize tumor uptake before we can use radioimmunoscintigraphy as a routine clinical tool. Future plans include: (a) investigating more favorable labeling methods and isotopes, such as the chelating techniques and ^{111}In (*43*); (b) using fragments, which with their smaller size should penetrate into tumors and provide quicker clearance from the blood pool (*38*); (c) using alternate routes of administration such as intraperitoneal (*45*) or lymphatic (*46*) or (d) finding methods to alter tumor capillary permeability and enhance local tumor delivery of the radiolabeled MoAb.

TABLE 9
Correlation of TAG-72 Serum Levels with Imaging of i.v. Administered [^{131}I]B72.3 IgG

TAG-71 U/ml	Scans
400.0	Pos
400.0	Pos
200.0	Pos
96.0	Pos
72.0	Pos
64.0	Pos
62.0	Pos
60.0	Pos
58.0	Pos
50.0	Neg
41.0	Pos
41.0	Pos
20.0	Pos
19.0	Pos
19.0	Pos
15.0	Neg
11.0	Neg
10.0	Neg
10.0	Neg
8.4	Pos
7.6	Neg
7.0	Neg
6.0	Neg
4.7	Neg
4.0	Neg
3.5	Neg
3.5	Neg
3.5	Neg
3.5	Pos
1.0	Neg
1.0	Neg
1.0	Neg
0.1	Neg
0.1	Neg

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