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# Safety and Role of Repeated Administrations of Indium-111-Labeled Anti-carcinoembryonic Antigen Monoclonal Antibody ZCE 025 in the Postoperative Follow-up of Colorectal Carcinoma Patients

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The safety and clinical utility of repeated administrations of  $^{111}\text{In}$ -ZCE 025 were evaluated in 25 patients who have undergone colorectal carcinoma resection. Fifteen patients were clinically and radiologically free of recurrences and asymptomatic while 10 had rising CEA and/or symptoms. We repeatedly imaged the patients following intravenous administrations of 40 mg ZCE 025, every 4 to 6 mo. Side effects occurred in 16% of patients who received two or more infusions. Sixteen lesions were detected by immunoscintigraphy in 11 patients who were free of disease by CT scans or other imaging modalities. Ten recurrences were surgically confirmed in seven patients. Radiographic and clinical follow-up confirmed the remaining 6 Mab-positive lesions. Elevated human anti-mouse antibody (HAMA) titers were detectable in the sera of 30% and 64% of patients following the 1st and 2nd Mab injection respectively, but did not interfere with successful immunoscintigraphy nor correlated with the occurrence of side effects. This study suggests that a negative Mab scan indicates that a patient will remain free of recurrence; conversely, a positive scan was associated with recurrences of disease.

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**F**ollowing curative resection of colorectal carcinoma, approximately 60% of patients will develop regional recurrences and/or distant metastases; the majority occurring within the first 2 yr after surgery (1). A rise in the serum CEA concentration in a patient after successful curative resection has been shown to portend tumor recurrence (2), and usually initiates a vigorous diagnostic

work-up to elicit the site of recurrence. The difficulty in differentiating local recurrent colorectal carcinoma from postoperative or post-radiation changes by computed tomography has been described by a number of authors (3-5).

Using a murine monoclonal antibody (Mab) against carcinoembryonic antigen (ZCE 025, Hybritech Incorporated, San Diego, CA) radiolabeled with  $^{111}\text{In}$ , we as well as others have demonstrated the feasibility of imaging primary colorectal carcinoma tumors as well as previously known sites of recurrences or metastases (6-10). In patients with rising serum CEA and negative radiologic and endoscopic work-up, we were able to identify tumor deposits in intraabdominal lymph nodes, local recurrences and metastases with  $^{111}\text{In}$ -ZCE 025 weeks to months before other x-ray modalities, including computerized tomography became positive (11,12), confirming similar findings by other investigators using this Mab (13-15) or different Mabs against colorectal cancers (16-18). Independently, Beatty et al. (15) and Patt et al. (13) have demonstrated benefits to their patients studied with  $^{111}\text{In}$ -anti-CEA Mab scanning, which allowed early resection of recurrent disease or initiation of definitive therapy.

Using  $^{111}\text{In}$ -labeled CYT-103 (monoclonal antibody B72.3 directed against TAG-72, a high molecular weight glycoprotein expressed on the majority of adenocarcinomas), we demonstrated beneficial patient management effects in approximately 20% of patients with primary and recurrent disease. In addition, antibody imaging detected occult disease in 12% of patients who subsequently underwent curative surgical resection (19).

In addition to endoscopy, laboratory tests and diagnostic x-ray modalities routinely used in the follow-up of colorectal carcinoma patients in the postoperative period, immunoscintigraphy is becoming a valuable tool for the diagnosis of recurrences and may be useful in conjunction

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with those tests. To fulfill that role, radiolabeled Mabs must be capable of re-administration without significant loss of safety and diagnostic efficacy as compared to the initial administration. Because the majority of Mabs used for radioimmunodetection clinically are derived from mouse tissues, the formation of anti-mouse antibodies by the patient might limit the repeated use of Mab and thus reduce their efficacy.

The objectives of this prospective trial were to evaluate the safety of repeated intravenous administration of murine monoclonal antibody ZCE 025 radiolabeled with  $^{111}\text{In}$ , and whether tumor recurrence and distant metastases could be detected by radioimmunoscintigraphy when anti-mouse antibodies are present in patients' sera. The role of multiple infusions of  $^{111}\text{In}$ -ZCE 025 in the follow-up of patients at high risk of developing recurrences also was evaluated.

## MATERIALS AND METHODS

ZCE 025 is a murine monoclonal antibody of the IgG<sub>1</sub> subclass directed against CEA with an affinity of  $6 \times 10^9$  mol/liter. The antibody is derived from the same clone as MAB 35 (20) and is produced and licensed by Hybritech, Incorporated, San Diego, CA.

In recently conducted multi-institutional clinical trials,  $^{111}\text{In}$ -ZCE 025 was shown to detect primary and recurrent colorectal carcinoma lesions with a sensitivity of 80%, and a positive predictive value (percent of positive scans confirmed histopathologically) of 94% (21,22). These studies also demonstrated that anti-mouse antibodies developed in approximately 40% of patients following single administration of  $^{111}\text{In}$ -ZCE 025 (23).

Antibody labeling and quality control is accomplished by the addition of 5.5 mCi  $^{111}\text{In}$ -citrate to the vial containing 2.0 mg ZCE 025, already conjugated with DTPA (diethylenetriaminepentaacetic acid) (24), followed by the addition of neutralizing buffer solution. Ninety percent or more of the radioactivity is bound to the antibody as demonstrated by instant thin-layer chromatography (Gelman Sciences, Ann Arbor, MI). The relative immunoreactive fraction of  $^{111}\text{In}$ -labeled ZCE 025 was assayed by a radioimmunoassay kit (RhoMed, Albuquerque, NM) (25). This test employs solid-phase antigen to colorectal tumors and is conducted under conditions of antigen excess (25). Using this assay kit, mean binding of  $^{111}\text{In}$ -ZCE 025 was  $59.7\% \pm 6.1\%$  and significantly exceeded the percent binding of positive control antigen (30%).

### Patients

Twenty-one male patients and four female patients participated in this prospective uncontrolled and non-randomized study which was conducted under a U.S. Food and Drug Administration Investigation New Drug Exemption filed by Hybritech, Inc (Table 1). Patients were enrolled in the study based on the following criteria: (1) previous curative resection of primary, recurrent or metastatic colorectal carcinoma; and no radiographic or endoscopic evidence of disease recurrence or distant metastasis (Group A); or (2) strong clinical suggestion of disease recurrence based on symptoms and/or elevated serum CEA level (Group B). In addition, all patients must have had: a performance status of 70% or more on Karnofsky's scale, no prior exposure to murine

Mabs, no current chemotherapy or radiation therapy for at least 3 wk prior to and 1 wk after  $^{111}\text{In}$ -ZCE 025 administration and no participation in other Phase I/II diagnostic or therapeutic clinical trials.

Fifteen patients were clinically and radiographically free of recurrences following potentially curative resections of colon/rectal adenocarcinomas (Group A). Postoperative serum CEA levels measured by a radioimmunoassay kit (Abbott Laboratories, North Chicago, IL) within 1 wk prior to the first Mab infusion ranged from 2.0 ng/ml to 6.4 ng/ml (mean  $\pm$  s.d.  $3.23 \pm 1.58$ , normal up to 3.5 ng/ml in nonsmokers and up to 5.0 ng/ml in smokers). Four patients (3, 10, 11 and 14) had elevated serum CEA levels upon entering the study. Their postoperative CEA values were not significantly different from their preoperative CEA levels and were considered not indicative for tumor recurrences. Thirteen patients were enrolled in this study 2–9 mo following resection of their tumors. Patient 10 was 36 mo postoperative resection of rectal adenocarcinoma and was enrolled because of an elevated CEA (6.4 ng/ml). Patient 23 was enrolled 24 mo following successful resection of right lung metastases that developed 2 yr post-resection of sigmoid adenocarcinoma.

Ten patients (Group B) with negative CT scans or other imaging modalities presented with symptoms suggestive of recurrent disease. Nine of these patients (nos. 1, 5, 12, 13, 15, 19, 20, 21 and 25) had mild (4.1 ng/ml) to significantly elevated (72 ng/ml) postoperative serum CEA levels, while Patient 24 was enrolled in the study because of progressive pelvic pain, but a normal CEA level (2.0 ng/ml). All patients enrolled in this study had a Karnofsky Scale greater than 70 and had not received murine Mabs previously. Pre-study evaluation included medical history and physical examination, chest radiographs and colonoscopy. Computerized tomography of the liver, abdomen and pelvis after oral administration of dilute barium with and without intravenous contrast was obtained on an 8800 scanner (GE Medical Systems, Milwaukee, WI) at 1-cm cross sections throughout the liver and 1.5-cm cross sections in the lower abdomen and pelvis. These evaluations were usually performed within 1–6 wk before each Mab infusion and were repeated every 6 mo during the first 2 yr of patient follow-up. Within 1 wk after each Mab infusion, complete blood counts with differential, chemistry panel, liver and kidney function tests and urinalysis were performed. These tests were repeated 3–7 days following each Mab infusion.

After signing an informed consent as required by our Institutional Human Subjects Review Committee, each patient received approximately 5.5 mCi (203 MBq) of  $^{111}\text{In}$ -labeled to 2.0 mg of ZCE 025 in addition to 38.0 mg unlabeled ZCE 025 in 50 ml, co-infused intravenously over 1 hr. This procedure was repeated every 4–6 mo, except that during the second and subsequent Mab infusions, 1.0 mg unlabeled ZCE 025 was administered intravenously as a test dose first, followed 1 hr later (if no adverse reactions were elicited) by the labeled and unlabeled Mab. As a safety measure, diphenylhydramine (25 mg in 50 ml saline) was co-infused in the contralateral arm during the third and subsequent Mab infusion cycles. Vital signs were monitored every 15 min for the first hour, and then each 30 min for the following 3 hr.

### Radioimmunoscintigraphy

Planar and emission computed tomography were obtained 2–3 days and 5–7 days following  $^{111}\text{In}$ -ZCE 025 infusions. Anterior and posterior images of the head, chest, abdomen, and pelvis

were acquired on a large field of view gamma camera Siemens ZLC LFOV (Siemens Medical Systems, Iselin, NJ) equipped with a medium-energy parallel-hole collimator, and peaked at both the 174 and 247 keV photon energies of  $^{111}\text{In}$ , with 20% window. Acquisition time was 7.5 min for each view. Data were stored in a  $128 \times 128$  byte matrix on a Picker PCS Plus Computer System (Picker International, Highland Heights, OH).

Digital images of the liver were processed and displayed in nine windows varying from lightest to darkest intensity in order to enhance image contrast between "hot liver lesions" and normal liver background (26). Indium 111-ZCE 025 SPECT of the liver was obtained 2–3 days postinfusion and SPECT images of the lower abdomen and pelvis were obtained 5–7 days later using a single-head large field of view camera (General Electric 400T, GE Medical Systems, Iselin, NJ) fitted with a medium-energy collimator. Sixty-four projections were acquired over 360 degrees using a circular orbit. Average acquisition time was 20 sec per stop for liver SPECT and 40 sec/stop for pelvic SPECT. Data was acquired on a  $64 \times 64$  word matrix. Images were reconstructed using a modified Shepp Logan filter (number 4) with a slice thickness of 1 pixel each.

Follow-up  $^{99m}\text{Tc}$ -sulfur colloid liver spleen SPECT scans (8.0 mCi) were obtained because of suspicious findings on the Mab scans in eight patients. Also,  $^{99m}\text{Tc}$  bone scans were obtained in four patients. Indium-111-ZCE 025 scans were blindly interpreted by an experienced nuclear medicine physician. Radio-

graphs and CT scans were interpreted by a radiologist without the benefit of pertinent clinical information, except that patients were being evaluated for possible recurrent colorectal carcinomas.

### Human Anti-Mouse Antibody Formation

The presence of human anti-mouse (HAMA) was tested in patient's sera obtained at 1, 3, and 6–8 wk following each Mab infusion. Preinfusion samples were available for comparison, with each patient serving as his/her own control. HAMAs were detected by a solid-phase enzyme-linked immunosorbent assay (ELISA) described previously (27). Wells of microtiter plates were coated with mouse IgG<sub>1</sub> (ZCE 025). Serial dilutions of human sera were added to the wells, washed and incubated with horseradish peroxidase-conjugated goat anti-human IgG. A color substrate solution was added to each well and the plate read at 405 nm on an automatic ELISA Reader (Dynatech Instruments, Inc., Santa Monica, CA). Patients were determined "HAMA-positive" if there was an increase in the optical density of the post-treatment values compared to the pre-treatment values by at least a two-fold dilution at each time period. For example, a patient with baseline HAMA titers of 1:160 would not be considered positive unless post-infusion HAMA titers measured 1:640 or higher.

### RESULTS

To date, seven patients have received four infusions of  $^{111}\text{In}$ -ZCE 025; five have received three infusions, and

**TABLE 1**  
Characteristics of Patients Undergoing Repeated  $^{111}\text{In}$ -ZCE 025 Administrations and Imaging

Patient no.	Sex	Age	Site of initial carcinoma	Duke's staging	Serum CEA (ng/ml)		Interval (in months) between initial surgery and first infusion	Duration of follow-up (in months) since first infusion	No. of Mab infusions
					Pre-op	at Mab first imaging			
<b>Group A</b>									
2	M	69	Cecum	B1	3.3	2.0	6	30	4
3	M	65	Hepatic flexure	B2	4.5	5.8	6	30	2
4	M	68	Rectum	C1	4.8	2.0	1.0	30	2
6	M	62	Cecum	B2	2.0	2.0	6.5	27	3
7	M	53	Transverse colon	C1	3.6	2.0	8	31	4
8	M	70	Sigmoid colon	C2	5.4	2.0	2	30	4
9	M	37	Rectum	C2	9.7	2.8	5	23	3
10	M	67	Rectum	B2	5.4	6.4	36	24	2
11	M	72	Sigmoid colon	B1	4.3	6.0	7	28	3
14	M	62	Sigmoid colon	A1	3.9	4.3	2	26	4
16	F	70	Rectum	B3	8.6	2.2	9	24	3
17	M	62	Cecum	A1	3.1	3.3	4	24	2
18	M	74	Descending colon	B2	18.2	2.0	9	24	3
22	F	69	Ascending colon	B1	3.1	3.4	7	25	2
23	M	69	Sigmoid colon	B2	2.8	2.0	24	18	2
<b>Group B</b>									
1	M	71	Sigmoid colon	C1	2.0	3.6	29	30	4
5	M	56	Descending colon	C2	14.5	37.0	11	5	2
12	M	51	Rectum	C2	3.1	9.7	31	29	4
13	M	55	Sigmoid colon	C2	3.5	72.0	26	27	4
15	M	76	Ascending colon	B2	5.5	13.3	72	18	2
19	F	50	Sigmoid colon	C1	2.8	9.6	36	27	2
20	M	64	Ascending colon	C2	2.9	16.0	14	14	2
21	M	65	Splenic flexure	B2	2.2	4.6	5	15	2
24	F	35	Sigmoid colon	B2	2.0	2.0	8	17	2
25	M	68	Sigmoid colon	B2	2.1	4.1	11	19	2

thirteen have received two infusions. Adverse reactions were encountered in 4 of 25 patients (16%) who received two or more Mab infusions. These reactions, which usually developed within 12 hr from the second infusion, were reported by phone to the physician. In three patients, side effects consisted of skin rashes and pruritis and resolved spontaneously. By contrast, a more severe form of reaction was witnessed in one patient following the fourth infusion. It consisted of bronchospasm, flushing, and generalized skin rash developing within 5 min of the full (38.0 mg) Mab dose. These reactions responded promptly to intramuscular Benadryl® with no long-term sequelae. In all patients, postinfusion laboratory tests (CBC, liver, kidney and bone marrow functions) were unchanged from preinfusion values.

### Indium-ZCE 025 Imaging Results

Positive Mab scans suggestive of tumor recurrence and/or metastases to distant organs (liver, extra-abdominal lymph nodes and bones) were detected in 11 patients, 10 from Group B, and only 1 from Group A (Patient 9). This patient's first and second scans, performed 3 mo apart, were negative and he was clinically disease-free. The third scan, performed after serum CEA climbed to 9.0 ng/ml, was positive for local recurrence in the perineum, confirmed surgically and also mediastinal lymphadenopathy which was confirmed radiologically.

Sixteen separate lesions could be identified by planar

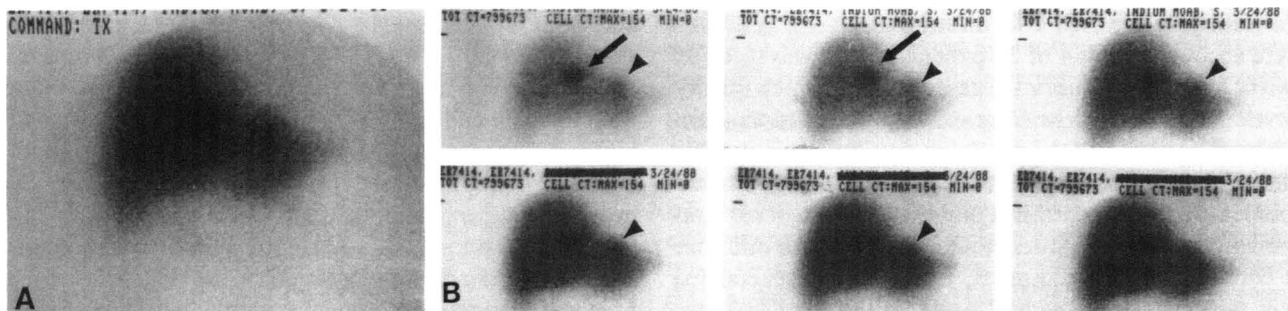
and/SPECT images. The location of these lesions are summarized in Table 2. "Hot" spots suggestive of liver metastasis were identified in two patients after the first Mab infusion and in another two patients after the second infusion. Figure 1 shows the anterior projection images of Patient 5 who presented with elevated CEA level and negative radiographic work-up. The first Mab scan obtained 3 mo prior to this study was negative. The subsequent scans clearly show a lesion in the right lobe of the liver and suggests the presence of another lesion in the left lobe. Initially, all four patients had negative CT and <sup>99m</sup>Tc-sulfur colloid scans of the liver at the time of the Mab infusion, but these studies became positive 4 mo later in two patients who did not have surgery. One of these patients is represented in Figures 2 and 3, which demonstrate a lesion in the right lobe of the liver on the Mab scan (Fig. 2) preceding positive findings on CT scans obtained 4 mo later (Fig. 3).

Seven of 11 patients with positive <sup>111</sup>In-ZCE 025 findings had exploratory laparotomy. Three of these patients had surgical exploration twice (Patients 13 and 24), while Patient 1 had three exploratory surgeries. This patient's first Mab scans (Fig. 4A-B) suggested the presence of a lesion in the anterior abdominal wall on the left side which was not evident on physical examination and was clearly delineated on a concurrent abdominal CT scan (Fig. 4C) where this mass was thought to be a portion of herniated bowel through the lateral rectus muscle. In retrospect, the

**TABLE 2**  
Characteristics of Patients with Positive <sup>111</sup>In-ZCE 025 Scans and Confirmation

Patient no.	First Mab Infusion		Second Mab Infusion		Third Mab Infusion		Fourth Mab Infusion					
	CEA (ng/ml)	Scan findings	Confirmation	CEA (ng/ml)	Scan findings	Confirmation	CEA (ng/ml)	Scan findings	Confirmation			
1	36.3	Anterior abdominal wall recurrence (160)	S, H	2.2	Negative (2560)	E, R	38.1	Negative	C, A, S	906	Metastasis to the ant. abdominal wall	S & H
5	37.0	Negative (40)	E, R	184.0	Two liver metastases (160)	S, H	—	—	—	—	—	—
9	2.8	Negative (640)(1)	E, R	3.9	Negative (640)	E, R	11.8	Perineal recurrence mediastinal LN mets (640)	S, H, R	—	—	—
12	9.7	Negative (160)	E, R	30.3	Possible pelvic recurrence (160)	C, A	68.0	Progression of pelvic recurrence (640)	C, A	247	Further progression of pelvic recurrence (160)	C, A
13	72	Anterior abdominal wall recurrence (160)	S, H	9.2	Negative (2560)	C, E, R	29.5	Bilateral inguinal lymphadenopathy (10240)	C	41.0	Negative (81920)	E, R
15	13.3	Bone metastases to L4 vertebra (40)	B, R	16.9	Bone metastases to L4 vertebra (2560)	B, R						
19	3.6	Mediastinal LN mets (160)	R, P, H	3.1	Mediastinal LN mets (20480)	R, C						
20	16.6	Abdominal LN mets (160)	S, H	39.0	Multiple bone metastases (1280)	B, R						
21	4.6	Intraluminal recurrence (160)	E, S, H	11.5	Multiple liver mets (160)	R						
24	2.0	Liver metastases	S, H	2.0	Negative	E, R S, H						
25	4.1	Liver metastases	R, L/S		Progression of liver metastases	R, L/S						

Numbers in parentheses indicate the amount of HAMA titers in sera obtained 1-4 wk before each Mab infusion. E = endoscopy; R = radiography, including chest x-ray and/or CT scans of the abdomen and pelvis; S = surgery; H = histopathology; C = clinical follow-up; A = serum CEA levels; P = percutaneous biopsy; B = radionuclide bone scan; LN = lymph node; and L/S = liver/spleen scan.



**FIGURE 1.** Patient 5. (A) Anterior projection of the liver obtained 3 days after the second infusion of  $^{111}\text{In}$ -ZCE 025. (B) Multigrade intensification image showing a lesion in the right lobe of the liver (arrow) and possibly another lesion in the left lobe (arrow head), was neither seen on the first Mab scan nor the regular scan in (A). Intraoperatively, this lesion measured 2.5 cm in diameter and histologically was a metastatic well-differentiated adenocarcinoma.

CT scans were interpreted positive for a mass in that region, since it could be separated from underlying bowel by a thin facial layer. A repeat fourth Mab scan (Fig. 5) was performed approximately 27 mo after the first one, because of very high CEA levels (906 ng/ml) and again showed metastatic involvement in the left anterior abdominal wall. Interim Mab scans 2 and 3 were negative.

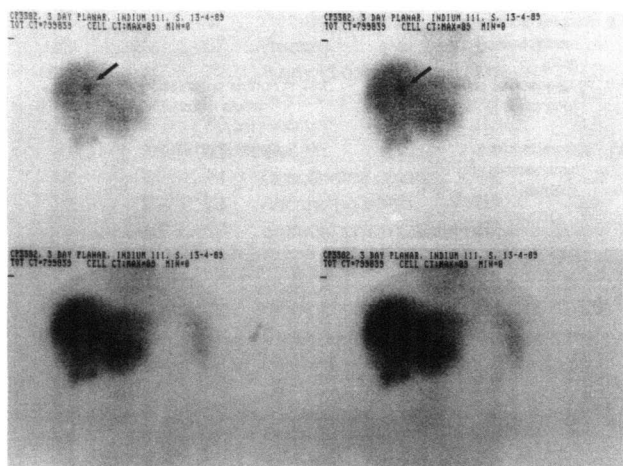
Surgery was suggested by a progressive rise in serum CEA levels in six patients or for symptoms suggestive of local recurrence in one patient (no. 24). In this patient,  $^{111}\text{In}$ -ZCE 025 Mab scan could not identify pelvic tumor recurrence, but detected a solitary lesion in the right lobe of the liver not previously suspected because of a normal serum CEA and a normal CT scan of the liver. This lesion was removed during surgery. The remaining four patients with positive Mab findings refused surgery; three were treated with chemotherapy and one patient (no. 15) refused all forms of treatment.

Ten of the 16 (62.5%) positive sites by Mab were confirmed as cancerous by surgery (eight lesions) or biopsy (two lesions). Longitudinal radiographic follow-up studies confirmed the presence of liver metastasis in two patients

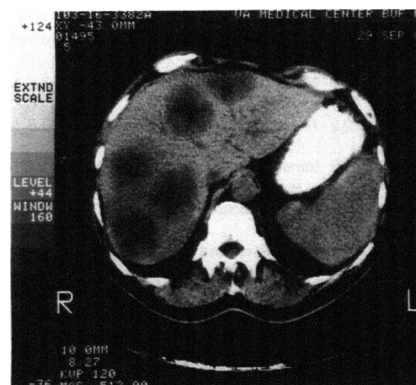
and bone metastases in the remaining two. In one patient with positive Mab uptake in the inguinal lymph nodes, physical examination revealed enlarged, non-tender, and freely mobile lymph nodes. A repeat physical examination performed 6 wk later was entirely negative, and a repeat Mab scan performed 4 mo later was also negative. In another patient (no. 12) with a positive lesion in the pelvis detected on the second Mab scan, follow-up CT scans did not confirm the Mab findings. However, increased size and radioactivity accumulation in that lesion was demonstrated on two subsequent Mab scans performed 6 mo apart. This patient refused exploratory laparotomy because of rapid progression of CEA from a value of 9.7 ng/ml immediately preceding the first negative Mab scan to values of 30.3, 68.0 and 343 ng/ml prior to the second, third, and fourth Mab scans, respectively, and is currently being treated with weekly 5-fluorouracil administrations.

As shown in Table 2, eight positive sites were detectable following the first Mab infusion, four were detected following the second, three following the third and one following the fourth infusion.

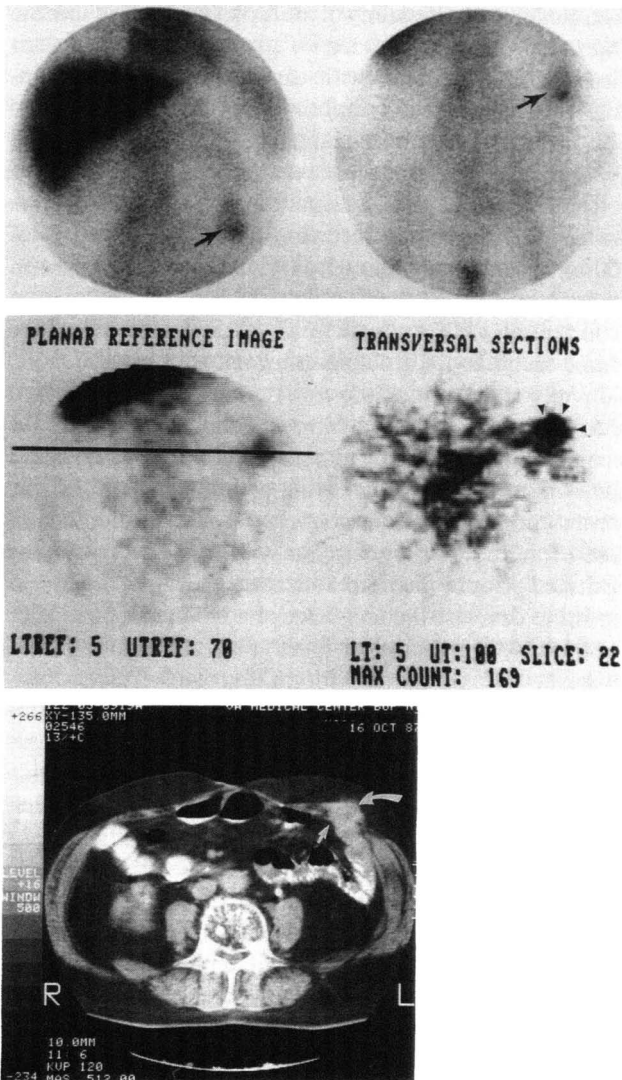
In the remaining 14 patients in Group A, no recurrences were detected by repeated  $^{111}\text{In}$ -ZCE 025 scans, endoscopy, CT scans, periodic clinical evaluations and serum CEA determinations up to 36 mo from the initial scans. Two of the 14 patients with negative scans underwent exploratory laparotomy within 2.5 mo from the



**FIGURE 2.** A multigrade intensification image of the liver 3 days after the second Mab infusion to Patient 21, showing a focal area of increased  $^{111}\text{In}$ -Mab in the right lobe of the liver (arrow).

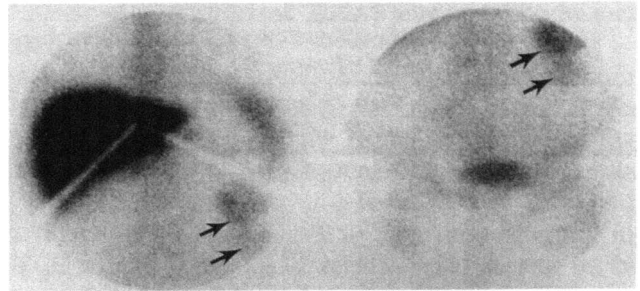


**FIGURE 3.** Computerized tomograms of the liver with contrast obtained 3 mo following the second Mab scan now showing multiple lesions in the liver.



**FIGURE 4.** Patient 1. (A) Anterior planar projection of the abdomen and pelvis obtained 5 days after the first Mab infusions showing an area of increased Mab accumulation in the left lower quadrant (arrow) suggestive of metastatic involvement of the anterior abdominal wall. (B) Transaxial SPECT image showing the anterior location of this lesion (arrowhead). (C) Abdominal CT with contrast showing as a 2-cm rounded soft-tissue mass located between the left lateral rectus muscle (curved arrow). A thin facial layer appears to be separating this mass from underlying bowel (short arrow).

second Mab infusion. Indications for surgery were bowel obstruction secondary to adhesions (Patient 2) and cholecystectomy for acute cholecystitis (Patient 10). No tumors were found by palpation and inspection during these operations. Also, two patients from the second group underwent surgery for relief of bowel obstruction. Patient 13 had surgery approximately 6 mo following the fourth Mab scans (no tumor found) and Patient 24 had surgery 2 mo following the second Mab scan, which was also negative. In both patients, no tumor was found intraoperatively.



**FIGURE 5.** Anterior planar projection of the abdomen and pelvis 5 days following the fourth infusion of  $^{111}\text{In}$ -ZCE 025 Mab to same patient as in Figure 4. Positive Mab localization is seen in the left hypochondrial area, suggestive of recurrent tumor (arrows). Intraoperatively, metastatic moderately well-differentiated adenocarcinoma measuring  $7 \times 5 \times 3.5$  cm was found in the jejunum. Transmural and peri-intestinal adipose tissue involvement and direct extension into the anterior abdominal wall were also found.

### HAMA Development

Serum samples were available from 24 patients for HAMA evaluation. Pre-Mab infusion samples were negative ( $\leq 1:160$  titer) in 20 of 24 patients and positive in four patients. Those four patients remained positive during successive Mab infusions (Table 3). Of the 20 patients with baseline negative HAMAs, 6 (30%) became positive 2–4 wk following the first infusion, while 9 of 14 (64%) patients became positive after the second infusion. Two patients had modest HAMA titers ( $1:40 - \leq 1:160$ ) after the third infusion, and three patients had increased HAMA titers after the fourth infusion. All HAMA titers reported were obtained approximately 3–4 wk following each Mab infusion. Increase in HAMA titers from baseline levels following repeated Mab infusion did not interfere with successful detection of metastasis in three patients. Patient 1 (Fig. 5) is a representative example, whereas an increase in HAMA titers from  $1:160$  baseline to  $1:2560$  immediately preceding the fourth Mab administration was inconsequential in the detection of this patient's metastases by immunoscintigraphy.

### Patient Status and Follow-up

To date, 13 of the 14 patients from Group A who had negative Mab scans are alive; 12 have no clinical or radiographic evidence of disease recurrence 18 to 31 mo following the first Mab scan. Four patients have received adjuvant chemotherapy with 5-Fluorouracil and leucovorin under a separate adjuvant chemotherapy trial at our institution. One patient (no. 10) died from cardiac arrest 8 mo after the second Mab scan, while another patient (no. 2) developed transitional-cell carcinoma of the urinary bladder. Local exploration during surgical resection of this patient's bladder cancer showed no evidence of abdominal recurrence. Of the 11 patients with positive Mab scans (one from Group A and 10 from Group B), 6 patients have died, 4 due to progression of metastatic disease (Patients 9, 20, 21, and 25) and 2 from complications

**TABLE 3**  
Human AntiMouse Antibody Titers in Patients' Sera  
Following Repeated Infusions of <sup>111</sup>In-ZCE 025

Patient no.	Baseline*	First infusion†	Second infusion	Third infusion	Fourth infusion
1	160	160	2560	2560	NA‡
2	160	640	160	2560	160
3	40	640	160		
4	160	160	40,960		
5	40	40	160		
6	160	160	655,360	40,960	
7	160	160	2560	160	81,920
8	640	2560	10,240	1280	NA
9	640	640	640	640	
10	160	160	10,240	81,920	
11	160	160	2560	640	
12	160	160	160	640	160
13	160	160	2560	10,240	81,920
14	2560	2560	640	163,840	NA
15	40	40	2560		
16	160	40	160	2560	
17	640	640	163,840		
18	160	640	163,840	655,360	
19	160	160	20,480		
20	160	640	40,960		
21	160	160	10,240	10,240	
22	160	160	160		
23	160	320	1280		

\* Baseline values obtained 1–3 days before first Mab injection.

† HAMAs present in serum samples obtained 3–4 wk following each Mab infusion.

‡ NA = not available.

Grading System: HAMA titers 1:40–1:160 (–), >1:160–1:2560 (+), >1:2560–1:40,960 (++) , >1:40,960–1:655,360 (+++).

following surgery to resect metastases from the anterior abdominal wall (Patient 1) or the liver (Patient 5). Five patients are still active; two without evidence of disease recurrence following four Mab infusions (Patient 13) or two Mab infusions (Patient 24), while the remaining three patients have recurrent and/or metastatic disease and continue to receive chemotherapy.

## DISCUSSION

The results of this study indicate that the administration of four separate doses of 40 mg of ZCE 025 at 4–6-mo intervals were associated with an increased incidence of side effects, although none of these reactions were serious or life-threatening. This study also demonstrates that recurrent colorectal carcinomas can be detected by immunoscintigraphy following repeated administration of <sup>111</sup>In-ZCE 025 despite positive HAMA titers.

The relative safety of multiple infusions of murine Mabs in diagnostic amounts has been reported previously (28) and is redemonstrated in this larger study. Several therapeutic trials with unlabeled murine monoclonal antibodies in patients with a variety of malignancies also have dem-

onstrated the relative safety of multiple infusions of murine Mabs. Schroff et al. reported no adverse reactions in their clinical trials of patients with chronic lymphocytic leukemia, cutaneous T-cell lymphomas, or melanoma treated with mouse Mab at doses ranging from 1 to 200 mg bi-weekly (29). The lack of adverse reactions was also reported by Douillard and associates in patients with gastrointestinal carcinoma receiving Mab 17-1A at doses of 100 to 200 mg, even though HAMAs were detectable in the sera of 40% of their patients (30). Other reports by Frodin et al. (31), or Sears et al. (32,33), also have confirmed the safety of multiple infusions of murine Mab.

In this study minor adverse reactions (skin rash and pruritis) were reported by three patients following the second administration of murine Mab. These patients did not exhibit adverse signs during or following the 1.0 mg pre-test tracer dose given intravenously 1 hr before the full dose of antibody. Pretreatment with diphenhydramine instituted prior to the third and subsequent Mab infusions tended to decrease the incidence of adverse reactions after the third (0/13 patients) or fourth (1/7 patients) infusion. However when compared with the rate of adverse reactions (4.7%) following a single intravenous infusion of <sup>111</sup>In-ZCE 025 (21,22), administration of four doses of 40 mg of ZCE 025 had a cumulative incidence (16%) of side effects. In our opinion, these side effects represented acute allergic reactions, which could neither be predicted nor avoided by prescreening by injecting 1 mg of antibody intravenously to patients. However the development of adverse reactions did not correlate with the presence of HAMAs which were detected in the sera of 30% and 64% of patients following the first and second infusions respectively. As previously described, human anti-murine immunoglobulin responses developed within 1–3 wk following murine Mab administration and either returned to baseline levels, increased in titer or remained unchanged (34). In general, positive HAMA titers were detectable up to 12 mo following the last infusion in some patients. The solid-phase ELISA assay used in this study is specific only for human IgG anti-mouse IgG antibodies and does not detect human IgM response. Also, we have not attempted to identify anti-idiotypic, anti-isotopic, or anti-species antibodies, which have been shown to develop following single or multiple infusions of murine Mabs (27,29,35).

The development of immune response to the murine Mab in this study did not interfere with successful tumor detection and did not compromise the quality of the image obtained following each infusion, nor did we observe noticeable alteration in organ distribution or increased deposition of <sup>111</sup>In-ZCE 025 in the liver (36). These findings are in disagreement with previously reported studies, particularly those by Perkins et al. (28) and Dillman et al. (37). The differences can be attributed to the choice and/or dose of antibody used in those studies and ours. The Mabs used in Dillman's study were shown to be reactive with granulocytes and erythrocytes with subsequent in-



creased accumulation of the radiolabeled material in soft tissues, liver and spleen. Also, the majority of patients studied by Dillman et al. had very high serum CEA levels which could have cross reacted with the injected Mab, leading to the formation of large immune complexes and subsequent excessive deposition in the liver and thus reduced radioimmunodetection. Patients enrolled in our study had either normal or moderately elevated CEA levels. The lack of radioimmunodetection reported by others can also be attributed to the dose of antibody used. While Perkins et al. and Dillman et al. have used considerably lower amounts of antibodies (0.2 to 1.0 mg) and (3 to 6 mg) respectively, we have used a larger dose (40.0 mg) of ZCE 025 which has been previously identified as optimal in colorectal carcinoma patients (6-9).

The importance of this study lies in the fact that accurate localization of pelvic and extra pelvic tumor recurrences as well as liver metastases is feasible by repeated injections of <sup>111</sup>In-ZCE 025 to patients every 4-6 mo. Indium-111-ZCE 025 scintigraphy was particularly helpful in the group of 11 patients who had strong clinical suspicion for tumor recurrence, but were free of disease by CT scan or other imaging modalities. Only sixteen recurrences were detected by immunoscintigraphy, which were subsequently confirmed by surgery and biopsy in 10 lesions, thus yielding a true-positive rate of 62.5%. Four additional lesions were confirmed by follow-up radiographic studies. The remaining two lesions were clinically compatible with recurrent colorectal carcinomas. Thus, all lesions detected by <sup>111</sup>In-ZCE 025 were compatible with tumors for a positive predictive value of 100% and a sensitivity of 87.5%. Also, no tumors were found in the four patients with negative Mab scans undergoing surgery for reasons other than tumor resections. These data suggest that a negative immunoscintigram means that the patient will remain free of disease, although the maximum 30-mo follow-up is still preliminary. Conversely, a positive Mab scan was associated with recurrence of disease. Our study also suggests a correlation between the presence of symptoms and signs suggestive of recurrent disease and the positivity of subsequent Mab scans. While all patients from Group A had negative scans and no clinical evidence of disease recurrence on further follow-up, patients from Group B and one patient from Group A all had positive scans compatible with disease recurrence and/or distant metastases whenever symptoms, signs or elevated serum CEA levels were identified.

Contrary to the results of Chetanneau (38), no false-positive lesions in the liver were observed in our study. We have not used the computer subtraction techniques previously described (39); rather, we employed a simple, inexpensive multigraded intensification technique developed in our laboratory (26). This technique improved discrimination of liver metastases from background liver uptake and increased the sensitivity of <sup>111</sup>In-ZCE 025 for liver metastases by 20%-30% without the loss of specificity

associated with other background subtraction techniques (40).

In conclusion, our study indicates that repeated intravenous infusion of <sup>111</sup>In-ZCE 025 every 4-6 mo can be performed with an acceptable rate of side effects and that accurate tumor localization can be achieved, even in the presence of HAMAs.

Furthermore, repeated and periodic imaging with <sup>111</sup>In-ZCE 025 may assume a valuable clinical role in the managing of patients with high clinical suspicion of tumor recurrence and elevated serum CEA, particularly for local recurrences that may be indistinguishable from postoperative or postradiation changes on CT scans (3-5,41,42). In that subset of patients, we believe that immunoscintigraphy will become the modality of choice and ought to be performed prior to other conventional radiographic modalities. Repeated immunoscintigraphy could also be used to monitor cancer progression in those patients refusing surgery or in whom surgery is contraindicated or noncurative, although the benefit of immunoscintigraphy in these circumstances requires further investigation. On the other hand, our data do not support repeated and periodic use of <sup>111</sup>In-ZCE 025 in the follow-up of patients with normal CEA levels who are asymptomatic. The low yield of immunoscintigraphy in this particular group of patients, and the relative increased risk of adverse reactions might offset the potential benefit to those patients.

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