

Pilot Trial Evaluating an ^{123}I -Labeled 80-Kilodalton Engineered Anticarcinoembryonic Antigen Antibody Fragment (cT84.66 Minibody) in Patients with Colorectal Cancer

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ABSTRACT

Purpose: The chimeric T84.66 (cT84.66) minibody is a novel engineered antibody construct (V_L-linker-V_H-C_H3; 80 kDa) that demonstrates bivalent and high affinity ($4 \times 10^{10} \text{ M}^{-1}$) binding to carcinoembryonic antigen (CEA). The variable regions (V_L and V_H) assemble to form the antigen-combining sites, and the protein forms dimers through self-association of the C_H3 domains. In animal models, the minibody demonstrated high tumor uptake, approaching that of some intact antibodies, substantially faster clearance than intact chimeric T84.66, and superior tumor-to-blood ratios compared with the cT84.66 F(ab')₂ fragment, making it attractive for further evaluation as an imaging and therapy agent. The purpose of this pilot clinical study was to determine whether ^{123}I -cT84.66 minibody demonstrated tumor targeting and was well tolerated as well as to begin to evaluate its biodistribution, pharmacokinetics, and immunogenicity in patients with colorectal cancer.

Experimental Design: Ten patients with biopsy-proven colorectal cancer (6 newly diagnosed, 1 pelvic recurrence, 3 limited metastatic disease) were entered on this study. Each received 5–10 mCi (1 mg) of ^{123}I -labeled minibody i.v. followed by serial nuclear scans and blood and urine sampling

over the next 48–72 h. Surgery was performed immediately after the last nuclear scan.

Results: Tumor imaging was observed with ^{123}I -labeled minibody in seven of the eight patients who did not receive neoadjuvant therapy before surgery. Two patients received neoadjuvant radiation and chemotherapy, which significantly reduced tumor size before surgery and minibody infusion. At surgery, no tumor was detected in one patient and only a 2-mm focus was seen in the second patient. ^{123}I -labeled minibody tumor targeting was not seen in either of these pretreated patients. Mean serum residence time of the minibody was 29.8 h (range, 10.9–65.4 h). No drug-related adverse reactions were observed. All 10 patients were evaluated for immune responses to the minibody, with no significant responses observed.

Conclusion: This pilot study represents one of the first clinical efforts to evaluate an engineered intermediate-molecular-mass radiolabeled antibody construct directed against CEA. cT84.66 minibody demonstrates tumor targeting to colorectal cancer and a faster clearance in comparison with intact antibodies, making it appropriate for further evaluation as an imaging and therapy agent. The mean residence time of the minibody in patients is longer than predicted from murine models. We therefore plan to further evaluate its biodistribution and pharmacokinetic properties with minibody labeled with a longer-lived radionuclide, such as ^{111}In .

INTRODUCTION

The use of radiolabeled antibodies for tumor imaging and therapy continues to be an active area of investigation. Radiolabeled antibodies to TAG-72, prostate-specific membrane antigen, and carcinoembryonic antigen (CEA; Refs. 1–3) have been approved by the Food and Drug Administration for tumor imaging, and recently two radiolabeled antibodies directed against CD20 (4, 5) have been approved by the Food and Drug Administration for therapy in the treatment of non-Hodgkin's lymphoma. Numerous radiolabeled antibodies have been and continue to be evaluated in clinical trials. A Phase III trial is currently evaluating a ^{90}Y -labeled antihuman milk fat globule-1 as adjuvant therapy in patients with ovarian cancer after surgery and first-line chemotherapy. In addition, several antibodies have demonstrated antitumor activity in the clinic, and these may also be of interest as radiolabeled agents in the future (6).

Intact antibodies demonstrate sufficient tumor uptake to be useful as imaging and therapy agents. However, blood circulation times can be prolonged, increasing background activity and radiation dose to normal organs, thus limiting the imaging or therapeutic potential of these agents. Radiolabeled antibody fragments, usually enzymatically produced, continue to be evaluated in patients. These lower-molecular-mass constructs have

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much faster clearance rates, giving improved tumor-to-blood ratios. However, antibody uptake in the tumor is reduced. In addition, fragments below ~60 kDa are often filtered through the glomerular system, leading to significant kidney activity.

A radiolabeled immunoconstruct with enhanced properties for imaging or therapy would potentially be of intermediate molecular mass, with tumor uptake comparable to intact antibodies, but with clearance times that are more rapid than the intact IgG, giving improved therapeutic ratios and imaging capabilities. In the present study, we report the initial clinical results evaluating a radiolabeled anti-CEA minibody. The minibody is a genetically engineered construct of intermediate molecular mass (80 kDa), with retained high affinity to the target antigen that, when compared with the parent intact antibody in animal models, demonstrates comparable tumor uptake and substantially faster clearance.

MATERIALS AND METHODS

Antibody Preparation. Human/murine chimeric T84.66 minibody (cT84.66 minibody) is an anti-CEA engineered bivalent fragment (V_L -linker- V_H - C_H3) with high affinity ($K_a = 4.0 \times 10^{10} \text{ M}^{-1}$) and specificity to CEA. Details of its production, characterization, purification, and radiolabeling have been reported previously (7–11). Briefly, preparation of the radiolabeled dose involved incubation of ^{125}I at a ratio of 5–10 mCi to 1 mg in the presence of Iodogen, followed by purification by size exclusion high-performance liquid chromatography. All administered doses demonstrated radiolabeling efficiencies of >90%, endotoxin levels <1 unit/ml, and immunoreactivity >95%. An Investigational New Drug application for ^{125}I -labeled (^{125}I -cT84.66) minibody is on file with the Food and Drug Administration.

Clinical Trial Design. The objectives of this pilot study were to evaluate the tumor-targeting properties, immunogenicity, pharmacokinetics, and safety of administration of ^{125}I -cT84.66 minibody. Patients were 18 years of age or older, had colorectal cancer, and were about to undergo planned surgical exploration or resection. Tumor CEA production was confirmed by either an elevated serum CEA or positive tumor immunostaining. The following studies were performed before antibody administration: complete blood count and platelet count with differential, SMA-18, urinalysis, pregnancy test if appropriate, plasma CEA levels, computed tomography scans of relevant anatomical locations, chest X-ray, and electrocardiogram. Computed tomography scans were performed using a single slice GE HiSpeed CTi scanner with i.v. and oral contrast routinely given. Barium enema or colonoscopy was also performed to assess disease location and extent. All studies were performed within 6 weeks of antibody infusion.

For each patient, a 100- μg anaphylaxis test dose of ^{125}I -cT84.66 minibody was first administered i.v. This was followed 15 min later by administration of the remaining ^{125}I -cT84.66 minibody imaging dose (5–10 mCi/mg) i.v. over 25 min. Blood samples were taken immediately postinfusion; 15 min, 30 min, and 1, 2, and 4 h after infusion; and at the time of each scan. Twenty-four-h urine collections were performed daily postinfusion up to the time of planned surgery. Spot planar and whole-body imaging studies were performed at 2–4 h, 18–24 h, and

48 h after antibody administration with a Toshiba 901 gamma camera with SPECT capability. SPECT scans were performed at 18–24 h postinfusion. Patients underwent planned surgical exploration 1–2 days after antibody infusion. Biopsies and resection of tumor and adjacent structures were undertaken as medically indicated.

Imaging Analysis. Imaging analysis was performed on a lesion-by-lesion basis. All scans were read in a blinded fashion by a radiologist experienced in antibody imaging (D. Y.). Scan results were then compared with known sites of disease as defined by sites ≥ 1.0 cm identified on computed tomography scans or sites identified at surgery that were histologically positive for cancer. Lesions were then scored as either true positive, false negative, false positive, or true negative. Photopenic areas in the liver were not considered as positive lesions.

Analysis of Human Antiminibody Antibody Response. Serum human antichimeric minibody response to cT84.66 minibody was assayed before infusion and at 2 weeks and 1, 3, and 6 months postinfusion by a double-capture solid-phase quantitative radioimmunoassay described previously (12). Briefly, patients' sera were diluted 1:4 in normal saline, and 100 μl of each dilution were pipetted into duplicate glass tubes. To each tube 100 μl of ^{125}I -cT84.66 minibody (~100,000 cpm) was added. Polystyrene beads coated with cT84.66 minibody were then added to the tubes, incubated at room temperature for 90 min, and then washed. The beads were counted on a gamma scintillation counter. Serial dilutions of a goat antihuman Fc antiserum of known concentration were used to generate a standard curve from 12.5 to 200 ng/ml. Bovine serum albumin (1%) in PBS was used as a negative control. A sample was scored as positive if it was >12.5 ng/ml.

Pharmacokinetic Analysis and Dosimetry Estimates. Blood and urine samples were counted for ^{125}I activity on a gamma counter, and serum samples were processed on a high-performance liquid chromatography size-exclusion Superose 6 column. Given the mean uptake values for blood (percentage of injected dose per gram), curves were fit to a sum of two exponential functions of time, when possible, by use of the ADAPT II software program (13). Mean blood residence time was calculated (14) from the decay-corrected data.

For those organs seen in both projections, ^{125}I activity in normal organs was estimated by use of parallel-opposed nuclear images to construct the geometric mean uptake as a function of time. Otherwise, single-view images were acquired. All resultant curves for ^{125}I activity *versus* time were corrected for background and patient attenuation. Attenuation was estimated by use of a separate series of experiments evaluating gamma camera efficiency in counting a planar ^{125}I phantom source as a function of tissue-equivalent absorber thickness. Given the geometric mean or single-view uptake values and measured blood and urine activity, a compartment modeling analysis was performed to estimate residence times for ^{125}I activity in blood, urine, liver, and the whole body. Details of this compartmental model have been published previously (15). ^{125}I radiation doses to normal organs based on biodistribution were estimated (16) by use of the MIRDOSE3 program and standard organ sizes (17). Red marrow radiation dose estimates were performed with the AAPM algorithm (18) based on blood residence times determined from the compartmental model.

Table 1 Summary of patients and lesions analyzed

Patient	Lesion at surgery	Serum CEA (ng/ml)	Size* (cm)	Imaging			
				CT	¹²³ I-labeled minibody	MRI	PET
1	Rectosigmoid primary	22	7.5	TP	TP		
2†	No tumor: pathology-based complete response of rectal primary after preoperative therapy	<2.5	NA	FP	TN		
3	Liver metastasis	20.9	7.5	TP	TP		TP
4	Abdominal wall metastasis	11.4	10.0	TP	TP		
	Peritoneal implant		0.9	FN	FN		
5	Presacral region‡	18.1	3.0	FN	TP	TP	TP
6	Left pelvic mass	<2.5	15.0	TP	TP	TP	
	Right pelvic node		2.2	FN	FN	TP	
	Omental implant		2.0	FN	TP	FN	
	Peritoneal implants (10 lesions)		~0.5	FN	FN	FN	
7	Presacral mass	121.6	8.2	TP	TP		
8†	Rectal primary	<2.5	0.2	FN	FN		
9	Presacral/Perianal recurrence	<2.5	3.5	FN	TP		TP
10	Colon primary	<2.5	4.7	FN	FN		

* Greatest dimension of surgically resected lesion.

† Received preoperative chemotherapy and pelvic radiotherapy.

‡ Includes presacral anastomotic recurrence and PET-positive sacral uptake.

Abbreviations: CEA, carcinoembryonic antigen; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; TP, true positive; NA, not applicable; FP, false positive; TN, true negative; FN, false negative.

RESULTS

Ten patients with colorectal cancer were enrolled on this pilot study and received ¹²³I-cT84.66 minibody. Six patients presented with a newly diagnosed primary tumor, one with a local pelvic recurrence, and three with limited metastatic disease. Six patients were male, and four were female; their age range was 35–84 years. Serial nuclear scans and blood and urine collections for pharmacokinetics were carried out to 18–24 h in all 10 patients, with 7 patients having scans and samples out to 48 h. All patients underwent planned surgery immediately after the last scan, allowing for intra-operative correlation of sites of disease with sites of visualized antibody uptake. Before planned surgery, two patients had completed neoadjuvant chemotherapy and pelvic radiation to allow for anal-sphincter-preserving resection of their primary rectal cancer.

Imaging Results. All patients underwent computed tomography and minibody scanning before surgery. Although not required by this protocol, before surgery two patients underwent magnetic resonance imaging scanning and three patients underwent positron emission tomography scanning. The results of preoperative imaging are shown in Table 1 and compared with surgical findings. Of the eight patients who did not undergo neoadjuvant chemoradiotherapy, seven demonstrated antibody targeting to known disease sites. In the two patients (patients 2 and 8) who received preoperative radiation and chemotherapy, no tumor was detected in one patient and only a 2-mm residual focus was found in the second patient at surgery. A tumor was not imaged by either ¹²³I-labeled minibody or computed tomography before surgery in this patient.

We determined the ability of the minibody to detect tumor by comparing scan results with findings at surgery for all patients, with the exception of patient 5, for whom comparisons were made to magnetic resonance imaging and positron emission tomography scans. Patient 5 had a previous history of rectal

cancer and presented with findings indicating of a presacral recurrence, including right sacral pain and sciatica, an increasing CEA, and abnormal findings in the right presacral region seen on magnetic resonance imaging and positron emission tomography scans. At surgery, biopsies of this region were not performed. Radiation therapy was directed to the right presacral region and produced resolution of pain.

Tumor imaging by ¹²³I-cT84.66 minibody was primarily observed for lesions ≥ 1.0 cm. For lesions ≥ 1.0 cm confirmed at surgery, 8 of 10 were imaged by ¹²³I-labeled minibody compared with computed tomography, which imaged only 5 of 10 such lesions. Tumor imaging was observed with ¹²³I-labeled minibody but not with computed tomography for three infiltrative lesions: a presacral recurrence (patient 5; Fig. 1), a perianal recurrence (patient 9; Fig. 2), and an omental implant (patient 6).

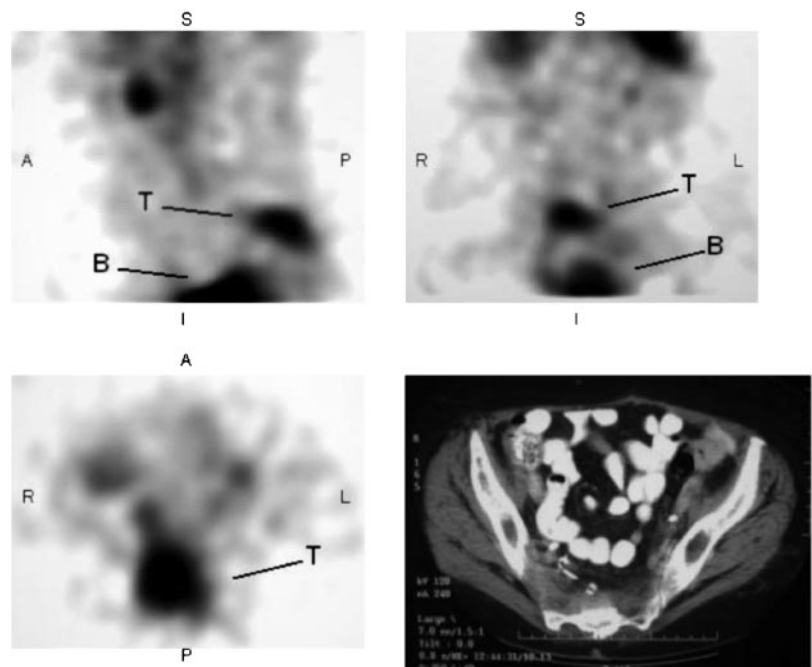
Small i.p. implants ≤ 1.0 cm found at surgery (patients 4 and 6) were not detected by either computed tomography or ¹²³I-labeled minibody scans. The one liver metastasis in this study (patient 3) was imaged by the minibody, with activity seen primarily in the viable rim of the lesion (Fig. 3).

No adverse side effects were observed.

Pharmacokinetic Analysis. Mean blood residence time for ¹²³I-cT84.66 minibody was 29.8 h (range, 10.9–65.4 h). This was a faster clearance than for ¹¹¹In-labeled intact cT84.66 (98.3 h; Ref. 19) and was comparable to that for ¹²³I-cT84.66 F(ab')₂ (20.3 h),¹⁰ which were evaluated in previous clinical trials. Mean blood residence time was substantially longer for the minibody in humans than in an animal model (7.2 h; LS-174T human-colon-cancer-bearing nude mice). This trend was also observed with the ¹²³I-cT84.66 F(ab')₂ construct and with

¹⁰ Our unpublished data.

Fig. 1 Patient 5 with presacral recurrence of rectal cancer. Sagittal (*top left*), coronal (*top right*), and axial (*bottom left*) SPECT views of pelvis at 18 h shows targeting of ^{123}I -labeled minibody to presacral recurrence (bladder activity also seen). Axial computed tomography image (*bottom right*) shows no obvious tumor recurrence. A, anterior; P, posterior; S, superior; I, inferior; R, right; L, left; B, bladder; T, tumor.



^{111}In -labeled diethylenetriaminepentaacetic acid-intact cT84.66 (Fig. 4). There was no correlation between preinfusion serum CEA level and blood residence time (Fig. 5).

High-performance liquid chromatography serum tracings out to 20 h postinfusion showed primarily a single peak, indicating no significant degradation of circulating ^{123}I -labeled minibody (Fig. 6). Radiation absorbed-dose estimates are shown in Table 2.

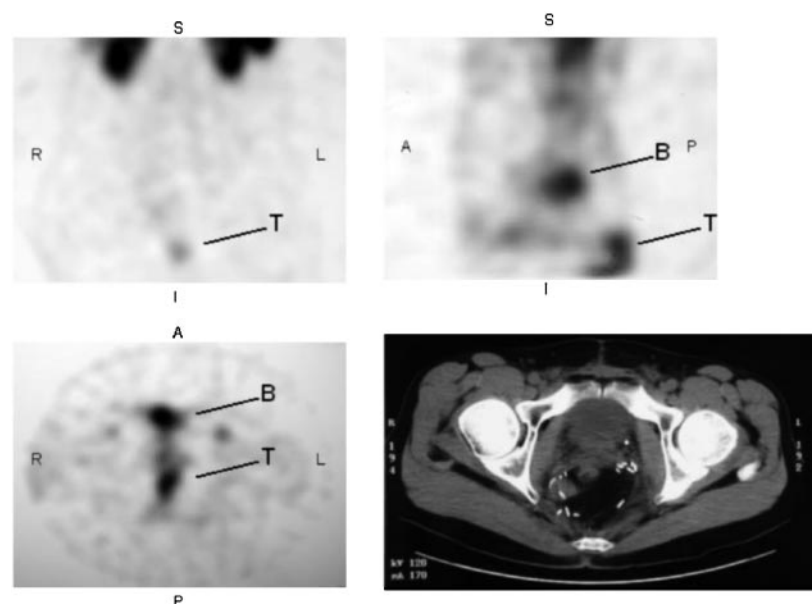
Immunogenicity. Human antiminibody antibody response was assayed by a previously reported double-capture

radioimmunoassay (12). Assays were performed for all 10 patients: 4 patients out to 1 month, 1 patient out to 3 months, and 5 patients out to 6 months. No significant human antiminibody antibody responses were observed.

DISCUSSION

CEA is a 180-kDa glycoprotein that was among the first tumor markers to be identified (20). This antigen is well characterized with respect to both its molecular nature and its tissue

Fig. 2 Patient 9 with presacral/perianal recurrence of rectal cancer. Coronal (*top left*), sagittal (*top right*), and axial (*bottom left*) SPECT views of pelvis at 24 h show targeting of ^{123}I -labeled minibody to infiltrative presacral/perianal recurrence. Lesion not seen by computed tomography (*bottom left*). A, anterior; P, posterior; S, superior; I, inferior; R, right; L, left; B, bladder; T, tumor.



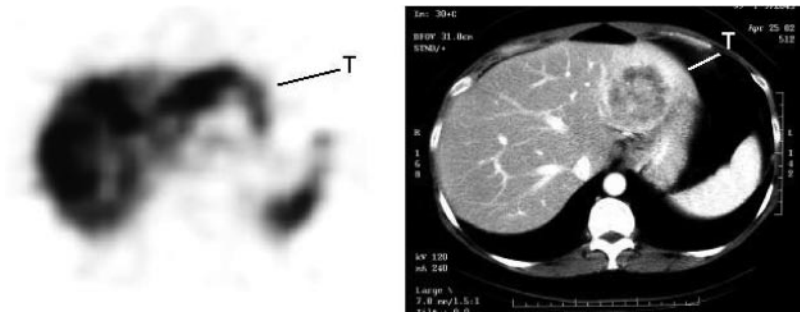


Fig. 3 Patient 3, a 39-year-old female with 7.5-cm left lobe solitary liver metastasis seen on computed tomography (right) with contrast enhancing rim and necrotic center. Left, axial SPECT view at 24 h. ^{123}I -labeled minibody targeting seen as photopenic area of center of lesion and uptake to anterior rim of lesion. T, tumor; R, right; L, left

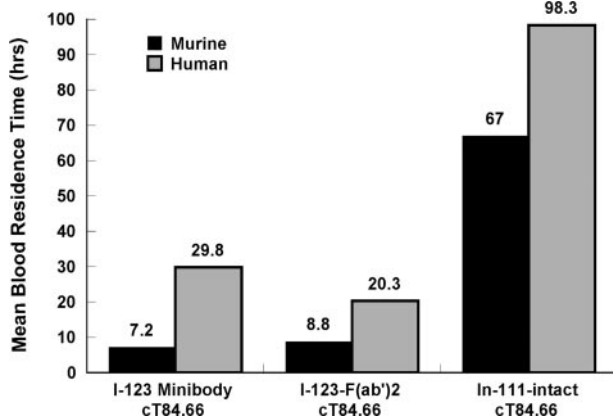


Fig. 4 Mean blood residence times for three radiolabeled anticarcinoembryonic antigen antibody constructs evaluated in LS-174T human-colon-cancer-bearing athymic mice and subsequently in patients. ^{123}I -labeled minibody (current study) and ^{123}I -cT84.66 F(ab')₂ (our unpublished data) were evaluated in patients with colorectal cancer about to undergo surgery. ^{111}In -labeled diethylenetriaminopentaacetic acid-cT84.66 intact antibody was evaluated in patients with anticarcinoembryonic antigen-positive malignancies, primarily colorectal cancer (12).

distribution in humans (21, 22). CEA expression in normal tissues is largely restricted to the intestinal epithelium but is also seen in other sites, such as the testes (23). This very limited normal tissue distribution combined with the widespread occurrence of CEA in tumors, particularly colorectal tumors, has made CEA a prominent target for experimental monoclonal antibody-based radionuclide imaging (radioimmunoscinigraphy) of colorectal cancer (24–26).

Early work with polyclonal anti-CEA antibodies and ^{131}I labels (24–26) was sufficiently encouraging to prompt further trials using murine monoclonal anti-CEA antibodies labeled with radioiodine or radiometals such as ^{111}In (27–29). Beatty *et al.* (30, 31) evaluated the murine anti-CEA monoclonal antibody, mT84.66, an IgG1 with high affinity for CEA ($K_a = 2.6 \times 10^{10} \text{ M}^{-1}$) and no cross-reactivity to normal tissues, labeled with ^{111}In in patients with colorectal cancer. Successful tumor imaging was seen in 67% (18 of 27) of primary colorectal cancers (28, 30–32).

Murine antibodies have the disadvantage of being recognized as foreign by the patient's immune system, which can lead to the formation of human antimouse antibodies in more than 50% of patients after single administration (35–38). The forma-

tion of human antimouse antibodies can hasten blood clearance and thereby compromise the imaging or therapeutic efficacy of subsequently administered antibody (37, 39). Investigators have recently evaluated human/mouse chimeric and humanized antibodies, which have demonstrated decreased immunogenicity (40–44). cT84.66 is a human/murine chimeric IgG1 monoclonal antibody developed at this institution with high affinity ($K_a = 1.16 \times 10^{11} \text{ M}^{-1}$) and specificity to CEA (9, 33, 34). cT84.66 was initially evaluated radiolabeled with ^{111}In in two pilot biodistribution/imaging trials, which entered 30 patients with CEA-producing malignancies of various histologies (12, 45). Both studies demonstrated tumor targeting, imaging sensitivity comparable to other intact anti-CEA monoclonals, no allergic reactions, and decreased immunogenicity compared with murine monoclonals. Although radiolabeled intact antibodies have successfully imaged tumors, limiting factors include slow clearance of the antibody, which contributes to background activity and normal liver uptake for ^{111}In -labeled preparations. This has led to the investigation of smaller, enzymatically produced fragments [F(ab')₂ and Fab']. In animal models, radiolabeled antibody fragments demonstrated faster clearance and higher tumor-to-background ratios than the intact antibody, which translated to improved imaging results in several clinical trials (46–49). In addition, the immunogenicity of these frag-

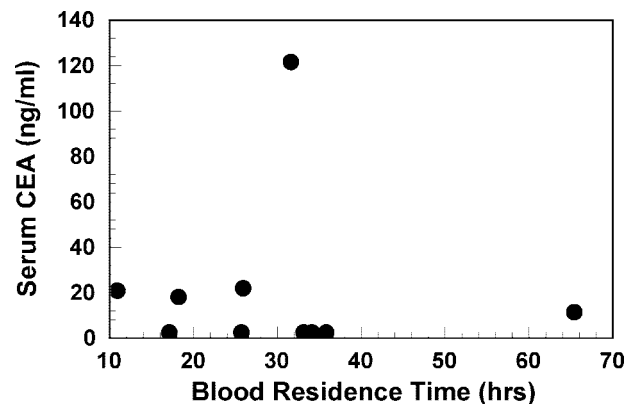
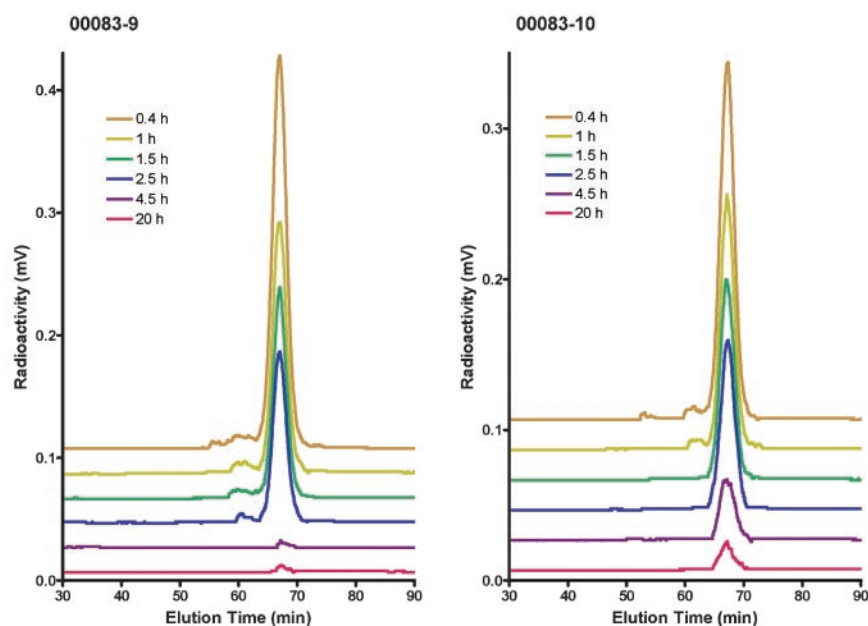


Fig. 5 Serum carcinoembryonic antigen (CEA) level as a function of blood residence time of ^{123}I -labeled minibody. No obvious correlation between serum CEA and blood residence time was observed. Patient 7, with the highest CEA at 121.6 ng/ml, presented with a 8.2-cm recurrence of a rectal cancer.

Fig. 6 Representative serum high-performance liquid chromatography tracings from 0.4 to 20 h after infusion for patients 9 (left panel) and 10 (right panel). Tracings demonstrate a single primary peak out to 20 h, indicating the stability of the circulating ^{123}I -labeled minibody.



ments was diminished given the lack of the Fc portion of the molecule.

^{123}I -labeled antibody fragments to human milk fat globulin (50) and CEA (47, 48) have been used with success to image solid tumors. Goldenberg *et al.* (47) evaluated ^{123}I -labeled $\text{F}(\text{ab}')_2$ and Fab' fragments of the anti-CEA antibody IMMU-4 in 62 patients with colorectal cancer and found that the fragments gave an imaging sensitivity and positive predictive value of 77%. Delaloye *et al.* (48) imaged 86% of all known tumor sites in colorectal cancer patients, also using ^{123}I -labeled anti-CEA $\text{F}(\text{ab}')_2$ fragments. Radiolabeled antibody fragments have also been evaluated clinically as radioimmunotherapeutics with reported success (51–55). For example, Juweid *et al.* (53) observed stable disease of 3–7 months duration and minor responses after therapy with ^{131}I -NP4 $\text{F}(\text{ab}')_2$ anti-CEA. Ychou *et al.* (55) also noted stable disease in three of nine patients up to 12 months duration after ^{131}I -anti-CEA $\text{F}(\text{ab}')_2$ radioimmunotherapy.

The $\text{F}(\text{ab}')_2$ fragment of cT84.66 radiolabeled with ^{123}I has been evaluated in tumor-bearing animals and demonstrated tumor targeting and imaging of LS174T colon cancer xenografts and rapid clearance from the blood ($t_{1/2\alpha} = 0.09$ h and $t_{1/2\beta} = 6.77$ h; Ref. 56). A pilot imaging trial evaluating ^{123}I -labeled cT84.66 $\text{F}(\text{ab}')_2$ fragments in patients with potentially resectable colorectal carcinomas demonstrated imaging of at least one known tumor site in 13 of 19 patients.¹¹

Lower-molecular-mass antibody fragments have much faster clearance rates than intact antibodies, which increases tumor-to-blood ratios, reduces background activity, and makes them attractive as potential tumor-imaging agents. However,

peak uptake and retention times in tumors are often reduced. In addition, fragments smaller than ~ 60 kDa are often filtered through the glomerular system, producing significant kidney activity.

A more suitable radiolabeled immunoconstruct would potentially be of intermediate molecular mass, with tumor uptake comparable to that of intact antibodies but clearance times that are more rapid, providing improved therapeutic ratios, and imaging capabilities. Recently, efforts have focused on genetic engineering and recombinant DNA technology to further improve antibody characteristics. The minibody is an 80-kDa dimeric engineered antibody fragment (V_L -linker- V_H - C_H3) capable of bivalent binding to antigen. The variable regions (V_H and V_L) assemble to form the antigen-combining sites, and the protein forms stable dimers through self-association of the C_H3 domains. Minibodies have the following properties: (a) high affinity to the target antigen when derived from parent antibodies with high affinity constants in the 10^{10} M^{-1} range; (b) intermediate molecular mass for improved tumor uptake combined with rapid clearance from normal tissues and blood; and (c) chimeric (murine variable light and heavy domains and human C_H3 domain) for reduced immunogenicity. An anti-CEA

Table 2 Absorbed radiation dose estimates for ^{123}I -labeled minibody based on clinical data ($n = 10$)

Organ	Mean (range) absorbed radiation dose (cGy/mCi)
Bone	0.018 (0.014–0.028)
Kidneys	0.13 (0.09–0.16)
Liver	0.071 (0.038–0.12)
Lungs	0.009 (0.007–0.011)
Marrow	0.027 (0.019–0.051)
Spleen	0.20 (0.09–0.42)
Total body	0.012 (0.011–0.016)

¹¹ Our unpublished data.

minibody was produced from the parental cT84.66 antibody (57). In the athymic mouse LS-174T xenograft model, cT84.66 minibody demonstrated faster clearance than F(ab')₂ fragments and high tumor uptake (up to 30% injected dose per gram), approaching that of some intact antibodies. As a result, it demonstrated superior tumor-to-blood ratios and tumor images in animal models compared with the F(ab')₂, making it attractive for further clinical evaluation as an imaging and therapy construct.

This report describes the initial clinical evaluation of radiolabeled cT84.66 minibody as a potential tumor-targeting agent in patients with colorectal cancer and represents one of the first engineered intermediate-molecular-mass anti-CEA constructs to be evaluated in patients. Imaging results from ¹²³I-minibody nuclear scans and computed tomography scans were compared with surgical findings. The minibody was associated with no adverse side effects. Furthermore, immune responses to the minibody were observed. In this pilot study of 10 patients, ¹²³I-labeled minibody imaged 8 of 10 tumors ≥1.0 cm in size, whereas computed tomography imaged 5 of 10. The three lesions not imaged by computed tomography but imaged by the minibody were diffuse, infiltrative, and difficult to definitively identify as mass effects by computed tomography scan. Although the number of patients studied was small, these initial results are encouraging and suggest that the minibody may have a role as a functional tumor-imaging agent to complement computed tomography scanning. Only a few patients had preoperative magnetic resonance imaging and positron emission tomography scans because these were not required for the protocol, making comparisons between minibody imaging and these imaging modalities premature.

In this study ¹²³I was used as the radiolabel given its relatively short half-life of 13 h, which is comparable to the residence time observed for the minibody in tumor-bearing mice (7.2 h). However, blood residence times were more prolonged in the 10 patients, with a mean of 29.8 h and a wide range observed (10.9–65.4 h). Longer blood residence times in patients than in tumor-bearing nude mice have also been observed for other cT84.66 constructs evaluated, including the F(ab')₂ and intact antibody (Fig. 4). These data suggest that a radionuclide with a longer half-life may be better suited to evaluate tumor targeting, biodistribution, and pharmacokinetics of the minibody.

In summary, cT84.66 anti-CEA minibody is a novel genetically engineered immunoconstruct of intermediate molecular mass that demonstrates tumor targeting and potential as an imaging agent to detect viable sites of tumor. In this pilot trial, ¹²³I-labeled minibody detected surgically confirmed active sites of disease that were not detected by computed tomography scans. These lesions were primarily infiltrative and without a clear mass effect. The minibody was well tolerated, and no antimibody responses were observed, making repeat administrations potentially possible. The mean blood residence time (29.8 h) for the minibody was longer than expected based on animal studies, thus making ¹²³I too short-lived a radionuclide for optimum imaging with this construct. A pilot trial with ¹¹¹In-labeled minibody is planned to further assess the tumor imaging, tumor uptake, biodistribution, pharmacokinetics, and imaging/therapeutic potential of this construct.

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