

# Preoperative Scintigraphy and Operative Probe Scintimetry of Colorectal Carcinoma Using Technetium-99m-88BV59

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We report a pilot study of radioimmunoscintigraphy (RIS) and operative gamma probe scintimetry (OPS) using a  $^{99m}\text{Tc}$ -labeled anti-cytokeratin human monoclonal antibody (MAB) ( $^{99m}\text{Tc}$ -88BV59) in patients with newly diagnosed, recurrent or metastatic colorectal cancer. **Methods:** Twelve presurgical patients with biopsy- or contrast radiographic-proven colorectal cancer or recurrent colorectal carcinoma were studied. After chest roentgenography and abdominopelvic CT,  $^{99m}\text{Tc}$ -88BV59 was administered intravenously, planar and SPECT external imaging was performed 3 to 6 hr after injection and planar imaging was performed 18 to 24 hr after injection. Surgery was performed immediately after late planar imaging. OPS of a standardized list of sites to document background radiation activity and of tumor sites, resection margins and tumor beds was performed. **Results:** The patients had 23 histologically proven tumor sites. Overall sensitivity for CT, planar RIS, SPECT, surgery and OPS was 43%, 61%, 78%, 96% and 91%, respectively. SPECT was superior to CT for imaging extrahepatic abdominal and pelvic disease. OPS detected all liver and extrahepatic abdominal tumor sites and correctly predicted histological tumor-free margins and tumor beds in all cases. OPS did not identify tumor deposits that the surgeon could neither see nor feel. No patient demonstrated human anti-human immune responsiveness 1 and 3 mo after  $^{99m}\text{Tc}$ -88BV59 infusion. **Conclusion:** Technetium-99m-88BV59 is a safe, effective radioimmunoconjugate for colorectal cancer imaging, with superior sensitivity as compared to CT.

**Key Words:** radioimmunoscintigraphy; gamma detection probe; human immunoconjugates; colorectal carcinoma; scintimetry

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**C**olorectal carcinoma is resectable for cure in only 70% of patients at the time of initial diagnosis. Tumor relapse can be expected in approximately one-third of those pa-

tients whose original surgery was potentially curative. Unfortunately, recurrent disease too often escapes detection until the patient is beyond salvage (1-3).

As many as 25% of colorectal cancer recurrences are isolated locoregional treatment failures (4), suggesting that microscopic tumor was left at the surgical site despite the surgeon's adherence to the time-honored principles of oncological surgery. An additional 15% to 20% of treatment failures are resectable pulmonary or hepatic metastases (4); some of these are certainly present but undetectable at the time of initial diagnosis.

Radioimmunoscintigraphy (RIS) is becoming a valuable addition to the oncologist's diagnostic armamentarium. In patients with colorectal cancer, RIS using xenogeneic radiolabeled monoclonal antibodies (MAbs) of various antigenic specificities is superior to conventional imaging studies for detection of pelvic and extrahepatic abdominal disease and is complementary to standard imaging for evaluation of the liver (5-8).

The clinical utility of many immunoconjugates is limited by cross-species immunogenicity and radioisotope shortcomings. Intravenous administration of murine MAbs stimulates production of human anti-mouse antibodies (HAMA) which interfere with subsequent RIS and pose an allergic risk to affected patients (9,10). RIS with  $^{111}\text{In}$ -immunoconjugates is limited by hepatocellular metabolism and reticuloendothelial cell uptake of the isotope (5). Iodine-123, an otherwise superb imaging isotope, is difficult and expensive to manufacture. These problems are being addressed in several ways, among them the development of human MAbs for diagnostic imaging of cancer and the use of  $^{99m}\text{Tc}$ , a safe, inexpensive, readily available radioisotope with excellent imaging characteristics that can be conjugated easily to immunoglobulins.

The inverse square law is a potential limiting factor in preoperative detection of surgically occult colorectal cancer by RIS (11). Operative probe scintimetry (OPS), in which the gamma detector is placed in direct contact with at-risk tissues and organs, has been under study since 1984, when Aitken et al. (12) reported the use of a polyclonal

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<sup>131</sup>I-labeled anti-CEA immunoconjugate for this purpose. Martin and colleagues (13–15) have developed the Radioimmunoguided Surgery (RIGS®) system which utilizes <sup>125</sup>I-labeled anti-TAG-72 MAbs to detect colorectal and ovarian carcinoma. Indium-111- and <sup>99m</sup>Tc-immunoconjugates have recently been employed for detection of surgically occult tumors using a gamma detection probe (GDP) in the operating room (16–18). Technetium-99m methylene diphosphonate has been used with a GDP at surgery to localize radionuclide bone scan abnormalities for biopsy (19).

A new human <sup>99m</sup>Tc-labeled radioimmunoconjugate is under investigation for RIS in patients with colorectal cancer (20–23). We report the results of a pilot study using this antibody for RIS and OPS in patients undergoing surgery for primary or recurrent colorectal carcinoma.

## PATIENTS AND METHODS

Twelve patients (four men and eight women, aged 49–78 yr) were studied between October 1991 and July 1992. Seven of these patients had newly diagnosed colorectal carcinoma. One patient presented with a second primary colon cancer 102 mo after undergoing anterior resection of a Stage II rectal carcinoma. Two patients presented with locally recurrent rectal cancer 30 and 38 mo after sphincter-sparing resection of the original primary cancer. One patient was referred 1 mo after resection of a sigmoid carcinoma for resection of a solitary large right hepatic metastasis. The remaining patient presented with an elevated and rising serum CEA 18 mo after curative anterior resection and postoperative adjuvant chemo-radiotherapy for Stage III rectal carcinoma.

In six patients, the original primary lesion was situated in the rectum. The disease arose in the right colon in two patients, the distal transverse and descending colon in two patients and the sigmoid colon in two patients. One patient had synchronous primary cancers in the distal transverse and descending colon at the time of presentation for the study.

### Radioimmunoconjugate

The MAb 88BV59H21-2 (Organon Teknika/Biotechnology Research Institute, Rockville, MD) was administered to 11 of the 12 patients studied. It is a human IgG<sub>3k</sub> class immunoglobulin with specificity for intracellular cytokeratins found in adenocarcinomas. This antibody reacts with 88% of colorectal carcinomas, 80% of ovarian carcinomas and 100% of breast cancers (23). Patient 12 received human MAb 88BV59H21-2V67-66, a product of a subclone of the cells producing 88BV59H21-2. This antibody has been found to be biochemically identical and immunochemically indistinguishable from MAb 88BV59H21-2 in vitro. The MAbs were radiolabeled with <sup>99m</sup>Tc using stannous chloride and saccharic acid as described elsewhere (24). The amount of MAb injected ranged from 6.0 to 10.0 mg per patient. The MAbs had a specific activity of 2.9 to 5.5 mCi/mg of antibody, the total administered <sup>99m</sup>Tc activity ranging from 24.3 to 38.0 mCi per patient. The percent <sup>99m</sup>Tc bound to antibody ranged from 94.0% to 99.6% and endotoxin concentration in the radioimmunoconjugate preparation was less than 1 EU/ml in all cases, as determined by Limulus Amebocyte Lysate assay (Cape Cod Associates, Woods Hole, MA).

### Operative Gamma Detection Probe

The GDP used in this study (C-TRAK®, Care Wise Medical Products, Morgan Hill, CA) consists of a sodium iodide crystal

coupled to a photomultiplier, enclosed in tungsten alloy shielding. The technetium probe measures 12.5 cm in length and 1.9 cm in diameter, has a 30° angled head to facilitate access to confined areas such as the pelvis or perihepatic spaces, and is optimized for the <sup>99m</sup>Tc energy photopeak (140 ± 10 keV). The probe has a detection efficiency of 1225 cts/μCi·min as measured with a <sup>99m</sup>Tc source of 0.5 cm length and 1 mm diameter placed 3.5 cm away from the face of the collimator along the central axis (no scattering medium between source and probe). Probe response to <sup>99m</sup>Tc activity is linear up to 60 μCi in a volume of 0.11 cc.

The data were recorded on a printer and transcribed contemporaneously to patients' study charts by a research nurse or nuclear medicine technologist.

### Study Protocol

This prospective study included presurgical patients with a diagnosis of colorectal carcinoma based on biopsy or contrast radiography, or recurrent colorectal cancer as evidenced by biopsy or elevated and rising serum CEA levels. Eligibility criteria included a minimum age of 18 yr, Karnofsky performance status of 70% or greater, a life expectancy of at least 1 mo, serum bilirubin and creatinine levels of less than 2.0 mg/dl and peripheral blood leukocyte and platelet counts of at least 3000/mm<sup>3</sup> and 100,000/mm<sup>3</sup>, respectively. Cytotoxic chemotherapy or other antibody preparations could not have been administered within the 2 wk preceding <sup>99m</sup>Tc-88BV59H21-2 infusion. Patients who were pregnant or lactating, those who had had another primary malignancy (except in situ carcinomas or nonmelanoma skin cancer) and those with central or peripheral nervous system disease were excluded. The protocol was approved by the University of Miami Institutional Review Board, and written informed consent was obtained from all patients prior to participation.

Baseline evaluation included a detailed history and physical examination, complete blood count with differential, serum electrolytes, BUN, creatinine, calcium, phosphate, bilirubin, glutamic oxaloacetic transaminase, alkaline phosphatase, CEA and urinalysis.

All known tumor sites were measured on physical examination or by appropriate endoscopic or radiographic investigation. Preoperative electrocardiograms and chest roentgenograms were obtained, and all patients had abdominopelvic CT within the 4 wk preceding antibody infusion.

The radioimmunoconjugate was diluted in 60 cc normal saline containing 1% human serum albumin, and an intravenous test dose of 2.0 cc (300 μg) was administered prior to infusion (over a 15–30-min period) of the remaining tracer. Vital signs and temperature were measured preinfusion and closely monitored for 2 hr thereafter. Serum samples for human anti-human antibody (HAHA) formation were taken prior to infusion and 4 and 12 wk postinfusion.

The detection of HAHA was performed using a solid-phase enzyme-linked immunosorbent assay (ELISA) (23). The assay was designed to measure anti-idiotypic and anti-isotypic reactivity against the 88BV59 MAb with a sensitivity limit of 50 ng/ml.

Preoperative RIS was performed using a large field of view camera with a parallel-hole/LEAP collimator with a 20% window centered at 140 keV. Planar images were obtained 3–6 hr and 18–24 hr after radioimmunoconjugate infusion. Abdominal and pelvic SPECT images were obtained at 6–8 hr using a matrix size of at least 63 × 64, 360° orbit, 20-sec acquisitions and sampling every 6°.

Patients were taken to surgery following acquisition of the late planar RIS images, approximately 24 hr after radioimmunoconjugate infusion. For all patients, the surgeon reviewed the findings

of conventional radiographic investigations and RIS preoperatively. A standard laparotomy was performed in which all abdominal organs, peritoneal surfaces and major lymph node groups were visualized and palpated. The GDP, covered with a sterile probe sheath, was used to take triplicate 10-sec background counts of the superior and hilar surfaces of both lobes of the liver, the kidneys, spleen, small and large bowel, bone marrow (fifth lumbar vertebra) and distal infrarenal aorta (blood pool). Triplicate 10-sec counts were then obtained from all deposits of disease and from sites of increased activity seen on preoperative RIS. In all patients, adjacent normal tissues were counted for comparison; differences of more than three standard deviations between tumor and normal tissue counts were taken as significant, as specified by Waddington et al. (25). For liver lesions, OPS tumor-to-background ratios (TBR) which were significantly ( $p < 0.01$ ) greater or less than unity were considered positive. The decision to consider photopenic lesions as true-positives was based on the rationale that both antibody localization of tumor and nonspecific imaging of lesions would be relevant in surgical practice.

Anatomical areas at risk for micrometastatic involvement by tumor were then scanned with the GDP. Following resection of known disease, the tumor bed and resection margins in the patient were scanned. Finally, the tumor in the resected specimen was counted ex vivo and compared to adjacent normal tissue; the specimen margins and the regional lymph nodes resected with the tumor were also counted.

#### Immunohistochemical Assay for Antigen Expression in Resected Tissues

Purified MAbs were biotinylated using NHS-biotin (BNHS). Antibodies at 1.0 mg/ml were incubated with BNHS at an antibody-biotin molar ratio of 1:75 for 4 hr at room temperature in the dark. Free biotin was removed by passage over a PD-10 column (Pharmacia, Piscataway, NJ) equilibrated with phosphate-buffered saline (PBS). Immunoreactivity, as compared to nonbiotinylated MAb, was determined by titration on a colon tumor xenograft panel.

Immunohistochemical testing of MAb on tissues submitted from the studied patients was performed using a direct immunoperoxidase procedure. Five to 7  $\mu$ m cryostat-cut sections of fresh-frozen tissue were air-dried overnight at room temperature and stored at  $-70^{\circ}\text{C}$  until stained. Slides were rehydrated by immersion in PBS and endogenous peroxidase was quenched by incubation in 0.3% (v/v)  $\text{H}_2\text{O}_2$  in PBS. Nonspecific binding of biotin or avidin reagents was blocked using Avidin-Biotin Blocking reagents (Vector Laboratories, Burlingame, CA) following the manufacturer's instructions. Sections were blocked for 30 min with normal human IgG at 0.5 mg/ml diluted in Hanks' balanced salt solution and bovine serum albumin. Dilutions of biotinylated 88BV59 or normal IgG control were then incubated on the sections for 2 hr at room temperature. Sections were washed with PBS and incubated with ABC peroxidase following the manufacturer's instructions. After washing with PBS, color was developed with DAB for 5 to 10 min. The sections were then counterstained with Harris hematoxylin, dehydrated in alcohol, cleared with xylene and mounted with Permount (Fisher Scientific, Fair Lawn, NJ). Sections were viewed microscopically for the presence of dark brown staining.

## RESULTS

Twenty-two colorectal carcinoma lesions were histologically confirmed in 11 of the 12 patients at the time of surgical exploration and OPS. In the twelfth patient, no

demonstrable tumor could be found at the time of laparotomy and OPS, despite an elevated serum CEA and abnormal radiolocalization in the pelvis on planar RIS and SPECT studies. Recurrent pelvic tumor presented 10 mo later (see below).

There were six hepatic metastases, eight abdominal lesions outside the liver and nine pelvic lesions. The extrahepatic abdominal and pelvic lesions included nine primary cancers (one patient had two synchronous primaries), three local recurrences, one regional nodal metastasis and four transcoelomic metastases. Four of the 12 patients had primary disease only, three had local recurrences only, one patient had a primary cancer plus regional nodal and distant disease, three patients had primary cancer plus distant metastases and one patient had a solitary hepatic metastasis.

The surgical experience with OPS demonstrated that the technique is quickly mastered and generally adds only 20–25 min to the operative time. OPS did not add more than 35 min to the duration of surgery for any patient.

#### Lesions

Only one patient had pulmonary metastases, which were imaged on preoperative chest roentgenogram, chest CT, planar RIS and SPECT; all results are shown in Tables 1 and 2.

Table 3 summarizes the OPS raw tumor and background counts and ratios for all confirmed lesions and false-positives in vivo. Ex vivo counting of confirmed tumors and adjacent normal tissues corroborated the in vivo findings, with TBR being somewhat higher ex vivo than in vivo for extrahepatic abdominal and pelvic lesions due to the reduced radiation background. The  $^{99\text{m}}\text{Tc}$ -88BV59 immunconjugate specifically targeted the tumor, as qualitatively demonstrated in Figure 1.

Of the three hepatic metastases imaged on planar RIS and the four imaged on SPECT, two were photopenic; both had fill-in (increased radiolabeling) on the late planar images. All six liver lesions were palpable or visible to the surgeon, measuring 1.0–8.0 cm (median 2.5 cm) in greatest dimension. Four of the six metastases were significantly

**TABLE 1**  
Number of Lesions Detected by CT, Planar RIS, SPECT, Standard Surgical Exploration and OPS

	Liver				Abdomen				Pelvis			
	TP	FP	TN	FN	TP	FP	TN	FN	TP	FP	TN	FN
CT	3	5	10	3	2	0	9	6	5	0	5	4
Planar	3*	5	10	3	5	0	9	3	6	0	5	3
SPECT	4*	4	11	2	6	1	8	2	8	0	5	1
Surgeon	6	0	15	0	8	1	8	0	8	1	4	1
OPS	6†	1	14	0	8	1	8	0	7	1	4	2

\*Two of these lesions were photopenic.

†Four of these had TBR  $< 1.0$  (photopenic).

TP = true-positive; FP = false-positive; TN = true-negative; FN = false-negative; OPS = operative probe scintimetry.

**TABLE 2**  
Assessment of Colorectal Cancer Detection by Each Diagnostic Modality\*

	CT (%)	Planar (%)	SPECT (%)	Surgeon (%)	OPS (%)
<b>Liver</b>					
Sensitivity	50	50	67	100	100
Specificity	67	67	73	100	93
PPV	38	38	50	100	86
NPV	77	77	85	100	100
Accuracy	62	62	71	100	95
<b>Abdomen</b>					
Sensitivity	25	63	75	100	100
Specificity	100	100	89	89	89
PPV	100	100	86	89	89
NPV	60	75	80	100	100
Accuracy	65	82	82	94	94
<b>Pelvis</b>					
Sensitivity	56	67	89	89	78
Specificity	100	100	100	80	80
PPV	100	100	100	89	88
NPV	56	63	83	80	67
Accuracy	71	79	93	86	79
<b>Overall</b>					
Sensitivity	43	61	78	96	91
Specificity	83	83	83	93	90
PPV	67	74	78	92	88
NPV	65	73	83	96	93
Accuracy	65	73	81	94	90

\*All calculations were done counting photopenic liver lesions detected by planar RIS, SPECT or OPS as true-positives.

PPV = positive predictive value; NPV = negative predictive value.

less radioactive than adjacent normal liver on OPS (Table 3).

Preoperative planar RIS and SPECT were much superior to CT for detection of extrahepatic abdominal disease, chiefly because of greater sensitivity in imaging transcoelomic metastatic tumors. All eight lesions were identified at surgical exploration and by OPS; OPS was superior to both planar RIS and SPECT.

SPECT, surgical exploration and OPS were superior to CT and planar RIS in the pelvis. SPECT detected more lesions than CT or planar RIS.

Of the three OPS false-positives, one occurred in a patient with recurrent rectal cancer. In this patient, palpation and OPS scanning of the pelvis after removal of the rectum revealed a suspicious hot mass in the prostate and base of the bladder. Biopsies were negative for tumor, but bladder outlet obstruction persisted postoperatively. Transurethral resection of the prostate was complicated by profuse bleeding, necessitating a transvesical surgical procedure at which a very vascular prostatic leiomyoma was removed. The second false-positive was a hot proximal ileocolic lymph node detected by OPS scanning in a patient with carcinoma of the caecum. This node looked and felt reac-

**TABLE 3**  
OPS Tumor-to-Background Ratios for Histologically Confirmed Colorectal Cancer Lesions

Patient no.	Lesion	Size (cm)	Tumor*	Background*	TBR	OPS Result
1	Rectal 1°	6.0	168	77	2.18	TP
	Hepatic	5.0	653	828	0.79	TP
	Hepatic	4.0	536	781	0.69	TP
2	Rectal 1°	3.2	211	175	1.21	TP
	RLN	3.0	234	139	1.68	TP
	Hepatic	1.0	2066	1665	1.24	TP
	Hepatic	1.0	1477	1749	0.84	TP
	Hepatic	1.0	1711	1558	1.10	TP
3	Colon 1°	3.5	265	62	4.27	TP
	Colon 1°	3.0	99	40	2.48	TP
	Ser Imp†	0.5	198	72	2.75	TP
	Omentum	1.5	49	13	3.77	TP
	Cul de sac	0.5	180	174	1.03	FN
4	Colon 1°	2.3	86	25	3.44	TP
5	Rectal Rec‡	3.0	118	49	2.41	TP
6	Colon 1°	1.5	92	51	1.80	TP
7	Rectal Rec	2.2	46§	29§	1.59	TP
8	Colon 1°	2.5	280	57	4.91	TP
	Cul de sac	1.0	182	233	0.78	FN
9	Rectal 1°	6.5	114	69	1.65	TP
10	Hepatic	8.0	1191	1702	0.70	TP
11	Colon 1°	2.3	125	43	2.91	TP
12	Rectal Rec	Micro	1	1	1.00	FN

**OPS False-Positives**

5	Liver	2.0	581	1140	0.51	FP
6	RLN	1.0	440	177	2.48	FP
7	Prostate	2.0	46	26	1.77	FP

\*Mean 10-sec OPS counts.

†Serosal implant.

‡Locoregional recurrence in patient with history of rectal cancer.

§Ex vivo counts—pelvis too narrow to permit reliable in vivo counts.

¶Raw data lost: TBR = 1.0 as specified by surgeon.

1° = primary; RLN = regional lymph node; TBR = tumor-to-background ratio; TP = true-positive; FN = false-negative; FP = false-positive.

tive to the surgeon, but its benign nature was confirmed on histopathology and the antigen expression assay was negative. The third false-positive was a 2-cm fibrous scar in the anterior inferior surface of the medial segment of the left lobe of the liver. Biopsy revealed only pericholangitis which was tumor antigen-negative.

The surgeon detected eight of the nine confirmed lesions in the pelvis; OPS failed to demonstrate a significant TBR for a 0.5-cm and a 1.0-cm pelvic peritoneal tumor deposit in two patients due to high background from the adjacent sacrum and pelvic sidewalls.

**Patients**

In the patient operated upon for hepatic metastatic disease, OPS and intraoperative hepatic ultrasonography confirmed that the disease was unilobar and therefore resect-



**FIGURE 1.** Ex vivo image on a standard external gamma camera of resected colon containing primary cancer. Specimen was cut open longitudinally prior to imaging. Technetium-99m-88BV59 tumor targeting is apparent in this scintigram.

able; preoperative planar RIS and SPECT had suggested the presence of bilobar disease.

In one patient operated upon for rectal carcinoma, SPECT and surgical exploration suggested the presence of a neoplastic mass in the region of the ileocaecal valve. OPS was negative, and histopathology on the mass, removed in a limited ileocaecal resection, confirmed the presence of chronic lymphadenitis only. The antigen expression assay was also negative.

Preoperative planar RIS and SPECT had suggested the presence of recurrent pelvic tumor in the rectal cancer patient in whom the only evidence on conventional testing was an abnormal and rising serum CEA level. At laparotomy, no tumor was found, the only pathology being a cytologically benign, OPS-negative ovarian cyst. Ten months postoperatively, she presented with a perineal abscess between the anus and posterior vaginal fourchette, which was biopsied during incision and drainage because of her persistent abnormal CEA. Adenocarcinoma consistent with a rectal primary was found in the submitted tissue, and the patient subsequently underwent salvage abdominoperineal resection of the rectum en bloc with the posterior wall of the vagina. Pathological examination revealed multiple small tumor nodules in the distal rectovaginal septum, none of which directly

**TABLE 4**  
OPS Normal Tissue Radioactivity 24 Hours after  
Administration of  $^{99m}\text{Tc}$ -88BV59H21-2\*

Tissue	Mean $\pm$ s.d.	Median	Range
Liver			
Right lobe			
Dome	1223 $\pm$ 285	1196	856–1565
Hilar	922 $\pm$ 201	835	632–1248
Left Lobe			
Dome	1196 $\pm$ 294	1209	665–1731
Hilar	820 $\pm$ 243	719	523–1205
Kidneys			
Right	2359 $\pm$ 940	2337	1232–3657
Left	2161 $\pm$ 764	2319	1180–3222
Spleen	480 $\pm$ 404	343	152–1582
Transverse colon	21 $\pm$ 6	22	12–31
Proximal jejunum	41 $\pm$ 31	31	7–120
Lumbar spine	200 $\pm$ 82	192	83–330
Distal abdominal aorta	472 $\pm$ 502	242	110–1652

\*All data are expressed as counts per 10 sec. The transverse colon and first loop of jejunum distal to the ligament of Treitz were preferentially counted as their intraperitoneal anatomy allows OPS to be performed with the GDP pointed away from the peritoneal cavity. Splenic measurements were variable, with the GDP being positioned on the hilar or parietal surface of the organ as best suited the anatomical circumstances of the individual patient.

involved the perineal skin or the rectal or vaginal mucosa. These likely represented recurrences due to residual tumor emboli in the lymphatics of the rectovaginal septum from the original primary cancer.

OPS correctly predicted the histological status of all surgical resection margins and of the tumor bed in the 11 patients in which tumor was histologically confirmed and excised at the time of the OPS laparotomy.

Three patients had OPS false-positive findings, as noted above. OPS did not reveal any tumor deposits which could be neither seen nor palpated by the surgeon in these 12 patients.

#### Technetium-99m-88BV59H21-2 and OPS

The background radioactivity detected by OPS 24 hr after administration of this radioimmunoconjugate is detailed in Table 4. Normal tissue background  $^{99m}\text{Tc}$  activity at this time point was uneven and, in the liver, kidneys, spleen and aorta, quite high. Interpatient variability likely related to factors such as small differences in the time intervals (less than 1 hr) between MAb injection and OPS, intraoperative fluid administration, differences in organ size and thickness (especially the liver and spleen) and patients' body habitus and adiposity.

Triplicate 10-sec counts for tumor deposits, adjacent normal tissues and normal tissue survey yielded highly reproducible data, with very small standard deviations in individual patients.

#### HAHA

No HAHA reactivity was found in any patient at any time before or after administration of the MAb.

## Toxicity

Four patients developed Grade I fever and/or chills shortly after injection of  $^{99m}\text{Tc}$ -88BV59H21-2, which resolved without incident. This toxicity was not endotoxin-related, but was likely due to a contaminant cytokine which has subsequently been eliminated by modification of the purification scheme for the current MAb 88BV59H21-2V67-66. Patient 12, to whom the newer MAb was administered, experienced no fever or chills.

## Antigen Expression in Submitted Tissues

Fresh-frozen tissue samples from 11 of the 12 patients were assayed for the presence of antigen by direct immunoperoxidase staining with 88BV59. Antigen was present in all tissues containing tumor (all true-positive and false-negative lesions) and all tumor tissue staining was of moderate to strong intensity. All normal tissues and noncancerous lesions (true-negatives and false-positives) demonstrated a negative staining pattern, which is recognized as staining limited to the brush border and superficial glands in noninvolved colon tissue, with no staining of nonepithelial tissues.

## DISCUSSION

Intraoperative gamma probe detection of cancer should demonstrate superior sensitivity as compared to preoperative RIS because of the inverse square law. Ideally, OPS should detect tumor deposits which would otherwise be missed by the surgeon. A new tumor detection system which facilitates complete surgical excision of tumor should result in fewer local recurrences and, perhaps, improved disease-free survival and cure rates.

Krag et al. (17), using an  $^{111}\text{In}$ -labeled murine MAb and C-TRAK GDP in 11 patients with colorectal cancer, found three occult scalene nodal metastases in one patient. Kuhn et al. (16) detected occult abdominal tumors with the same GDP in 3 of 11 colorectal cancer patients using another murine  $^{111}\text{In}$ -immunoconjugate, and these findings changed the treatment regimen of these individuals. In both studies, OPS detected more lesions than preoperative RIS.

The RIGS<sup>®</sup> system, in which a TBR of 1.5 or more is considered significant, has been used extensively at Ohio State University where it was developed (13–15). The low energy of  $^{125}\text{I}$  precludes preoperative scanning, a disadvantage in the view of some. In colorectal cancer patients, RIGS<sup>®</sup> findings changed the surgical procedure in 25%–50% of patients with primary disease and in 47% of those operated upon for recurrent disease (13,14). Moreover, upstaging of disease as a result of RIGS<sup>®</sup> findings led to chemotherapy being administered postoperatively to 11 of 36 patients (13). The incidence of RIGS<sup>®</sup>-positive, histopathology-negative lesions (mainly lymph nodes), however, was substantial, and the significance of this has been a point of contention. Whereas many would consider these to be false or unconfirmed positive findings, Arnold et al. (13,14) suggested that the presence of tumor antigen in RIGS<sup>®</sup>-positive, histiocytic nodes is evidence of metastasis missed by histopathology. In support of this view, they

cited a study, published in abstract form, demonstrating a correlation between TAG-72 localization in lymph nodes and poor clinical outcome (26). More recently, it has been demonstrated that grossly normal RIGS<sup>®</sup>-positive lymph nodes harbor lymphocytes which are sensitized to autologous tumor and have a significantly increased CD4:CD8 ratio due to CD4<sup>+</sup> cell pleocytosis. These observations suggest the possibility of isolating this lymphocyte population for adoptive immunotherapy in the future (27).

It is clear from the foregoing that OPS with murine  $^{111}\text{In}$ - and  $^{125}\text{I}$ -labeled MABs can detect tumor deposits which would otherwise escape the surgeon's attention.

Technetium-99m-88BV59 is an effective radioimmunoconjugate for imaging colorectal cancer (20–22) and is among the first antibodies of human origin used for this purpose. Planar RIS and SPECT were superior to CT in assessing abdominal disease, and were complementary to CT in the pelvis. These findings are consistent with earlier experiences with 88BV59 (20–22).

From the standpoint of immunogenicity,  $^{99m}\text{Tc}$ -88BV59 offers a significant advantage over radiolabeled murine whole antibodies. Readministration of murine immunoconjugates results in the development of HAMA in 26%–100% of patients; these antibodies pose an allergic risk and can interfere with tumor targeting (6,9,10,28–32). In the present study, adverse effects have been limited to occasional short-lived chills possibly due to low-level cytokine activity, which has subsequently been eliminated by the current MAB purification scheme. There has been no HAMA response in any patients to whom this radioimmunoconjugate has been administered (20–22).

OPS with this radioimmunoconjugate correctly predicted the histological status of all resection margins and tumor beds in this pilot study. In addition, OPS correctly predicted negative histology in a suspicious ileocaecal mass found at surgery which had imaged on planar RIS and SPECT. As the inverse square law might predict, OPS was superior to both planar RIS and SPECT in identifying colorectal cancer deposits in the abdomen and pelvis.

OPS and surgical exploration failed to identify recurrent rectal cancer in one patient presenting with an elevated CEA, in whom preoperative planar RIS and SPECT had shown abnormal localization in the pelvis. Biopsies of the posterior surface of the bladder and the pouch of Douglas, and cytology of an ovarian cyst were all benign, and OPS failed to identify any discrete areas of increased radiation in the pelvis. The pelvic radiation background at 24 hr was quite high (Table 3, Patient 12), and the disease ultimately presented in the distal rectovaginal septum. The recurrence, therefore, was situated below the peritoneal reflection in tissue planes rendered fibrotic by the primary surgery and adjuvant irradiation. These factors in aggregate probably accounted for the inability of the surgeon or OPS to identify the recurrent disease at the time of laparotomy.

Technetium-99m is widely available and inexpensive. Its energy is sufficient for external imaging, unlike  $^{125}\text{I}$ , but low enough to meet rigorous radiation safety standards in

the operating room (33). The time elapsed from injection of radioimmunoconjugate to surgical exploration is much shorter than with  $^{125}\text{I}$ -labeled preparations. Nonspecific hepatic or reticuloendothelial tissue uptake is not as problematic with  $^{99\text{m}}\text{Tc}$  as with  $^{111}\text{In}$  (16,17) or  $^{67}\text{Ga}$  (34), and this radiolabel is not as prone to in vivo deconjugation as radioiodine isotopes. For these reasons,  $^{99\text{m}}\text{Tc}$  is an attractive radiolabel for RIS and OPS applications.

In a radiation physics study of intraoperative probe detection of radiolabeled MABs, Waddington et al. (25) proposed that a difference of three standard deviations between the means of triplicate background and target counts should be taken as the threshold of significance, as at this level there is only a 0.3% probability that such a difference is due to chance alone. Statistically significant TBR is inversely proportional to the magnitude of the raw background count; when background counts are over 1000, significant localization of activity is denoted by TBR of less than 1.1. The absolute magnitude of a significant TBR is therefore much smaller with OPS than external RIS; even in the RIGS® system in which the  $^{125}\text{I}$  background is relatively low, TBR of only 1.5 represents a difference of three standard deviations. In this pilot study, TBR of confirmed extrahepatic and pelvic tumors ranged from 1.1 to 4.27.

Previous studies of RIS with 88BV59 and other radioimmunoconjugates (23,35–39) have found that this modality is inferior or, at most, only complementary to CT for imaging hepatic lesions. Liver metastases frequently image as photopenic lesions. Despite the fact that most such lesions prove to be metastatic disease (35,38,40), these have not been counted as true-positives in many analyses of RIS because this mode of imaging is not directly related to antibody targeting of tumor. Strictly speaking, however, photopenic lesions do not necessarily represent a primary failure of tumor antigen-antibody interaction (40). Nonspecific hepatic accumulation of radioactivity, large tumor size, tumor necrosis, poor-to-moderate tumor differentiation, relative paucity and/or inhomogeneity of blood flow through hepatic metastases, adverse intratumor interstitial fluid pressure gradients and long interstitial transport distances all contribute to the phenomenon of hepatic antigen-positive colorectal metastases failing to image positively on RIS (40–42). Investigators who do not accept photopenic hepatic RIS lesions as positive nonetheless count CT abnormalities which are not clearly benign cysts as positive. This approach biases their analyses in favor of CT (37) and underestimates the true clinical utility of RIS. In this surgical pilot study, hepatic tumor imaging by either direct immunoconjugate targeting or by nonspecific means was considered clinically relevant.

Two of the three hepatic lesions seen on planar RIS and two of the four seen on SPECT were photopenic on early images; each had fill-in on the later images, indicating  $^{99\text{m}}\text{Tc}$ -88BV59 localization. All lesions were readily apparent to the surgeon at laparotomy, and OPS TBR was significant (greater than unity for two lesions, less for four). All six liver metastases expressed the tumor antigen for

which 88BV59 is specific. An additional liver lesion with a significant TBR less than unity on OPS proved not to be a tumor (and therefore was counted as a false-positive by our criteria) and was antigen-negative. By our criteria, these results are concordant with those of larger series demonstrating that RIS images extrahepatic disease much more accurately and reliably than hepatic metastases.

In this study, surgery was performed 24 hr (four  $^{99\text{m}}\text{Tc}$  half-lives) after  $^{99\text{m}}\text{Tc}$ -88BV59 administration to allow blood pool and normal tissue radioactivity to fall for facilitation of OPS. Even at this time, the background radioactivity of  $^{99\text{m}}\text{Tc}$ -immunoconjugates is quite high and uneven in its distribution, as borne out by our experience (Table 4) and that of Reuter et al. (18). Although statistically significant TBR was 1.1 or less when 10-sec background counts were over 1000 (25), high background may drown out the signal from very small or microscopic deposits of tumor cells. This almost certainly accounted for the inability of  $^{99\text{m}}\text{Tc}$ -88BV59 OPS to detect two small pelvic transperitoneal tumor deposits and the recurrence in the rectovaginal septum in the twelfth patient, and may have precluded detection of microscopic tumor deposits which the surgeon could neither see nor palpate in the patients reported here. These pilot data do not exclude the possibility that this radioimmunoconjugate used under different circumstances can detect surgically occult tumor deposits.

There are several possible strategies for circumvention of the problem of high radiation background with  $^{99\text{m}}\text{Tc}$ -immunoconjugates. Surgery could be performed later after antibody injection, although the tumor signal would be substantially weaker. Whether a net improvement in TBR would be realized has not been established. Technetium-99m-labeled Fab' or  $\text{F(ab')}_2$  MAB fragments, with their shorter tumor targeting and blood-pool clearance times as compared to whole MABs (43), could prove to be advantageous for OPS. The use of bifunctional haptens (44) or avidin-biotin systems (45), in which sequential injection of MAB and radioisotope result in reduced background radioactivity, would theoretically enhance the ability of OPS to detect occult tumor deposits at surgery.

## CONCLUSION

This study provides further evidence of the promise of  $^{99\text{m}}\text{Tc}$ -88BV59 as an imaging agent for colorectal cancer. OPS with this radioimmunoconjugate is more sensitive than planar RIS and SPECT, can assist the surgeon in determining the completeness of resection of colorectal cancer and may be helpful in distinguishing malignant from benign pathology at laparotomy in colorectal cancer patients.

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