Intraoperative Detection of Colorectal Cancer With Radioimmunoguided Surgery and CC49, a Second-generation Monoclonal Antibody

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Radioimmunoguided surgery (RIGS) has been employed intraoperatively in cases of colorectal cancer to assess the extent of local tumor spread and metastatic disease. This technique uses radiolabeled monoclonal antibodies (MAbs) directed against tumor-associated antigens, and a hand-held gamma-detection probe to detect the radiolabel fixed to tumor tissue. Recently introduced is an MAb directed against tumor-associated glycoprotein (anti-TAG), CC49. Sixty patients were entered into the initial study. Eighteen of 21 (86%) primary tumors were localized by the CC49 MAb and the gamma-detecting probe. Twenty-nine of 30 (97%) recurrent tumors were localized. Antibody dose did not affect localization. Specimens were divided into tissue types I through IV, based on antibody localization and hematoxylin and eosin (H&E) staining: type I, RIGS (−) and histologically (−); type II, RIGS (−) and histologically (+); type III, RIGS (+) and histologically (−); type IV, RIGS (+) and histologically (+). Type IV tissues were further classified by whether they were grossly apparent, IVa, or grossly inapparent, IVb (occult). Occult tumor found by RIGS and confirmed by H&E staining (type IV) had localization ratios similar to RIGS-positive, histology-negative tissue (type III). Traditionally found cancer (type IV) had significantly higher ratios. In 12 of 24 patients (50%) with primary tumors and 14 of 30 patients (47%) with recurrent tumors, RIGS with CC49 altered the planned operative procedure. Radioimmunoguided surgery with CC49 provides useful, immediate intraoperative information not available by other techniques.

RADIOIMMUNOGUIDED SURGERY (RIGS, Neoprobe Corp., Worthington, OH) has been employed in the treatment of colon and rectal cancer. This technique is a method of finding cancer during surgery by use of a hand-held gamma detector (the Neoprobe 1000 instrument, Neoprobe Corp.) that locates preadministered, radiolabeled, cancer-specific monoclonal antibodies (MAbs). Initial studies of the intraoperative use of the RIGS system to detect colorectal cancers have shown improved assessment of tumor spread and alterations in surgical management based on added information gained by the RIGS system.[1,2] Widespread use of the technique, however, has been limited by the inefficiency of available targeting MAbs. Although the first-generation anti-TAG (tumor-associated glycoprotein) antibody B72.3 has been useful in developing the RIGS system, it does not target primary tumors or lymph nodes efficiently. Recently, a modified, second-generation anti-TAG antibody, CC49, has been developed for use with the RIGS system. The initial experience with the use of CC49 for RIGS surgery on patients with primary and recurrent colon and rectal cancer is reported here.

Methods

Sixty patients at the Ohio State University Hospitals entered into the study were believed to have either primary or recurrent cancer of the colon or rectum. The only patients excluded were those known to have metastatic disease outside the abdomen. Pretreatment of any type did not result in exclusion. Preoperative evaluation included measurement of carcinoembryonic antigen levels, endoscopy, chest x-ray, computed tomography (CT) scan of the abdomen and pelvis, and CT scan of the chest for patients with recurrent disease or abnormal chest x-rays. All patients signed a written consent approved by the Human Subjects Review Board at The Ohio State University. CC49 was obtained from the Cancer Therapy Evaluation Program of the National Cancer Institute. All patients were entered into the Cancer Therapy Evaluation Program protocol #T90-0038.

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Accepted for publication March 5, 1992.

Saturated solution of potassium iodide, 10 drops twice daily, was started 2 days before injection and continued for 3 weeks after injection to minimize radioactive uptake in the thyroid gland. Part of the study involved dose ranging, with each dose cohort consisting of 20 patients. These cohorts were 2 mCi ^{125}I radiolabeled to 10 mg, 1 mg, or 0.2 mg of CC49 administered intravenously over a 5-minute period. Radiolabeling of CC49 was done with the radioiodination method as previously described.[3] Briefly, CC49 was added to the reaction tube containing 50 μg IODO-GEN (Pierce Chemical Co., Rockford, IL), followed by the ^{125}I as sodium iodide. The reaction mixture was buffered with sodium phosphate and allowed to react for 10 minutes. Unbound ^{125}I then was removed from the mixture by size exclusion chromatography. Quality assessment tests were performed before patient administration.

After injection, a rapid decline of the radiolabeled CC49 level in the blood and a slower uptake in normal and tumor tissue occur. After 12 to 18 hours, MAb levels in blood and normal tissue equalize, and over the next few weeks slowly decline, whereas levels of radiolabeled MAb bound to tumor remain high. Operations were performed when the precordial count taken by the gamma detector had decayed to <20 counts per 2 seconds, indicating optimal clearing of ^{125}I-labeled MAb from the blood background, generally 2 to 3 weeks after injection. All patients underwent preoperative examination by a radiation oncologist and an oncologic urologist to determine whether placement of radiation seeds (brachytherapy) or associated urologic procedures needed to be addressed during operation. A bowel preparation was performed.

The surgical procedure was initiated with a xipho-pubic midline incision. A traditional exploration was performed by inspection and palpation, with careful attention to the presence of metastatic or recurrent cancer. Particular attention was given to evaluating the liver, gastrohepatic ligament, celiac area, retroperitoneal area, colon, small bowel mesentery, pelvis and infrarenal retroperitoneal vena cava, and aorta. In patients undergoing second-look procedures, the anastomosis also was inspected to rule out an anastomotic recurrence. After traditional exploration, the findings were recorded, including an assessment of the extent and resectability of disease. Therapeutic decisions were made and recorded by the RIGS nurse present in the operating room.

Next, a RIGS exploration was performed with the handheld, gamma-detecting probe. The probe contains a radiosensitive cadmium telluride crystal that relays radioactive counts through a preamplifier to emit an auditory signal and a digital readout. The intensity and the frequency of the signal are directly proportional to the level of radioactivity detected. A tumor-to-normal-tissue ratio of 2:1 has been considered to be significant because it is the value at which the audible signal in tumor is clearly different from that in normal tissue. The system was first "squelched" on the infrarenal aorta (*i.e.*, the threshold level of the signal processor is adjusted to eliminate the sound made when the probe is over normal tissue). If readings did not correlate with the precordial count, the aorta was rechecked manually for periaortic disease in multiple areas. Any grossly apparent sites of tumor were probed to determine whether tumor localization had occurred. A systematic search with the probe then was conducted of the following sites: the liver, gastrohepatic ligament, celiac area, colon, small bowel mesentery, pelvis, retroperitoneal vena cava, and aorta. Individual sites were counted three times each with the gamma-detecting probe and averaged for a final count. Counts were reported as ratios of RIGS-targeted tissue to normal tissue. Localization was not considered to have occurred unless the tumor-to-normal-tissue ratio was at least 1.5, with a total minimum count of at least 20 per 2 seconds. Biopsies were taken of lymph nodes and any other suspicious areas. In general, a complete RIGS examination takes 10 to 15 minutes in experienced hands. After RIGS exploration, the findings and assessments were recorded again, and the therapeutic plan was revised accordingly.

After operation, the surgeon constructed a drawing identifying tumor locations found by both traditional visualization and palpation, and by the RIGS system. All data were collected on specialized RIGS data sheets. All tissues were subjected to routine hematoxylin and eosin (H&E) histologic evaluation. Statistical analyses were performed using the nonparametric Mann-Whitney U-test.[4]

Results

A total of 60 patients were injected with CC49 and entered into the study. Six injected patients (three whose surgery was canceled and three whose tumors were found on exploration not to be of colorectal origin) were excluded from the study, resulting in a total of 54 patients for evaluation, 23 women and 31 men. The mean age was 59 years (range, 33 to 87 years). Twenty-four patients had primary colon or rectal cancers and 30 patients had recurrent tumors. There were no injection-related complications.

Ratios of RIGS-positive to normal tissue showed localization of the CC49 MAb. Of the 24 patients with primary tumors, 21 had tumors present at the time of surgery; in three patients, tumor had been removed either through a colonoscope or during an initial exploration performed before referral for definitive surgical management. Eighteen of 21 (86%) primary tumors were localized by the CC49 MAb and readily identified by the gamma-detecting probe. The variations in antibody dose given did not affect primary tumor localization (Table 1). All but one patient

TABLE 1. *CC49 Dose* Versus *Localization for Primary Colorectal Cancer*

Dose	No. of Patients	GDP Mean Ratio of Tumor/Normal Tissue	Range of Ratios
0.2 mg	7	10.7	1.0–21.0
1.0 mg	8	6.5	1.0–36.7
10.0 mg	6	9.7	4.8–27.5

GDP, gamma-detecting probe.

with recurrent cancer (29/30, 97%) had tumor localization by CC49. Neither preoperative carcinoembryonic antigen levels nor primary tumor location correlated with the likelihood of tumor localization by CC49.

Specimens located by the RIGS system were divided into four tissue types (Table 2), depending on whether or not they were identified as neoplasm and by which method. Type I is tissue that is RIGS negative (*i.e.*, no MAb localization detected by the gamma probe) and histologically negative (*i.e.*, not identified with H&E staining). Type II tissue is RIGS negative and histologically positive. Type III tissue is RIGS positive (*i.e.*, MAb localized in tissue as detected by the gamma probe) and histologically negative. Type IV tissue is both RIGS positive and histologically positive. Type IV tissues are divisible into two subgroups: IVa tissue was grossly apparent and IVb tissue was grossly inapparent (occult metastasis).

The specimens were grouped into those tissues found by traditional techniques (*i.e.*, inspection and palpation) and those tissue found only by the RIGS system (*i.e.*, occult tissue). Table 3 shows 79 separate specimens removed from 30 recurrent cancer patients. Nine specimens removed were histologically confirmed occult tumor sites found by the RIGS system (Type IVb). Sixteen specimens removed were RIGS positive, but histologically negative (Type III). The mean localization ratios of these groups were 7.7 and 7.4, respectively. There was no significant difference between the localization rates of these groups ($p = 0.73$). In contrast, 45 specimens found by traditional means were histologically confirmed RIGS-positive tissues. The mean ratio of this group was 23.8, a significantly higher localization ratio than either of the above groups ($p < 0.01$, $p = 0.015$).

Table 4 gives data from 55 specimens removed from 24 primary cancer patients (excluding specimens taken from the primary tumors). Five histologically confirmed occult sites and 40 histologically negative sites were positive by the RIGS system. The mean localization ratios were 18.9 and 4.8, respectively. Ten specimens of grossly positive and histologically confirmed RIGS-positive tissues had a mean ratio of 20.9. This group had significantly greater ratios than the RIGS-positive, histologically negative group ($p = 0.0013$). Primary tumor location had no effect on the results.

Table 5 shows the changes in operative procedure made as a result of RIGS exploration. In the 24 patients with primary disease, there were 16 changes in the therapeutic plan for 12 patients (50%). One patient had a rectal resection based on RIGS-identified positive tissue. Eight patients underwent gastrohepatic ligament dissection to remove RIGS-positive lymph nodes. Two patients underwent additional bowel resection. Radioimmunoguided-surgery–identified positive tissue also resulted in a retroperitoneal node dissection, an aortic node dissection, and two iliac node dissections. In one patient, planned placement of a hepatic arterial pump was abandoned after finding RIGS-positive, extrahepatic disease. For the 30 patients undergoing second-look surgery for recurrent disease, RIGS findings changed 17 operative procedures in 14 (47%) patients. Three planned liver resections were abandoned because of RIGS-located, extrahepatic occult tumor. Three patients underwent additional gastrohepatic ligament dissection because RIGS-targeted lymph nodes were found in the periportal area. Five patients had brachytherapy added and directed by information provided by the RIGS system. Two patients had iliac node dissection, and one patient underwent re-resection of her previous anastomosis because RIGS-positive nodes were found in the adjacent mesentery. One patient had a more extensive liver resection than originally planned, one underwent a cystectomy, and one underwent a vaginectomy after additional RIGS-positive tumor was found.

Discussion

Intraoperative use of the RIGS system has changed considerably since it was first introduced in 1986. Most of the initial work was done with the anti-TAG antibody B72.3. CC49 is a second-generation anti-TAG antibody developed by Schlom and associates at the National Cancer Institute for use with the RIGS system.[5] CC49 is a murine monoclonal antibody of the IgG_1 subclass that recognizes a 200,000 to 400,000 molecular-weight tumor-associated glycoprotein, TAG-72. TAG-72 is expressed on certain human colon carcinoma cell lines, but not significantly on normal tissues. CC49 is one of two antibodies derived from the anti-TAG antibody B72.3 that exhibits a relative specificity for the human colorectal cancer antigen, TAG-72. It has been suggested that the affinity of

TABLE 2. *RIGS Tissue Categories*

Tissue Type	Definition
Type I	RIGS negative, histology negative
Type II	RIGS negative, histology positive
Type III	RIGS positive, histology negative
Type IV	RIGS positive, histology positive

TABLE 3. *RIGS-Located Tumor* Versus *Traditionally Found Tumor in Patients With Recurrent Colorectal Cancer*

Tissue Type	No. of Specimens	Grossly Apparent	RIGS Findings	Hematoxylin + Eosin Findings	Mean Ratio of Tumor/Normal Tissue*
Traditionally found tumor					
Type IVa	45	Yes	+	+	23.8†
Type II	9	Yes	−	+	1.0‡
Occult tumor					
Type IVb	9	No	+	+	7.7§
Type III	16	No	+	−	7.4‖

* Statistical analyses (Mann-Whitney U test): († *versus* §) p = 0.015; († *versus* ‡) p = <0.01; († *versus* ‖) p = <0.01; (‡ *versus* ‖) p = <0.01; (§ *versus* ‖) p = 0.73; (§ *versus* ‡) p = <0.01.

an antibody may strongly influence its effectiveness, and the affinity constant of CC49 has been determined to be 16.2×10^9/mol/L, eight times that of B72.3.[5,6]

The initial experience reported here has been encouraging. CC49 localized primary tumor 86% of the time, exceeding the 75% localization rate for B72.3. Location of the primary cancer is rarely in doubt, but localization of the MAb in primary tumor during the initial survey with the gamma-detecting probe determines that the antibody specifically targeted the tumor. In patients with recurrent disease undergoing RIGS second-look surgery, CC49 successfully targeted metastatic disease 97% of the time, exceeding the recently reported 63% localization of metastases by the B72.3 MAb.[2] This successful targeting rate for metastatic disease is the most important aspect of the RIGS system, because metastatic disease is often occult and cannot be found by other methods.

The surgical specimens were divided into four types of tissues depending on RIGS localization and H&E histologic evaluation. Type I tissue, that which is both RIGS negative and histologically negative, is clearly nonmalignant. Type IV tissue, that which is both RIGS positive and histologically positive, is clearly malignant. Type II tissue, that which is RIGS negative and histologically positive, is also malignant but does not express the targeted antigen for CC49 and represents RIGS false negatives. Eleven per cent of the specimens removed during recurrent colon and rectal cancer surgery were RIGS false negatives. These type II tissue removed were visible carcinoma that failed to localize with the RIGS system. Localization with the anti-TAG antibody may not occur if the metastatic tumor does not express the TAG-72 antigen or if within a given tumor there are undetectable levels of MAb binding. The actual false-negative rate is difficult to determine, because only tissues grossly involved with tumor or those tissues ordinarily included in the standard resection are available for analysis. Long-term studies of recurrence rates and survival as well as autopsy data are needed to assess the actual false-negative rate. Nonetheless, because RIGS frequently detects occult tumor sites not found on traditional examination, the surgeon who uses the RIGS system will have a lower false-negative exploration than the surgeon who does not use the RIGS system.

The most intriguing group is type III tissue, RIGS positive but histologically negative. Other investigators have considered these to be false-positive specimens,[7] but on further scrutiny, evidence suggests otherwise. CC49 targets the sialomucin antigen, a byproduct of tumor cells. For a lymph node to be RIGS positive, it must have tumor antigens present. A number of findings have suggested that type III tissue is indeed malignant. Some lymph

TABLE 4. *RIGS-Located Tumor* Versus *Traditionally Found Tumor in Primary Colorectal Cancer Patients*

Tissue Type	No. of Specimens	Grossly Apparent	RIGS Findings	Hematoxylin + Eosin Findings	Mean Ratio of Tumor/Normal Tissue†
Traditionally found tumor					
Type IVa	10	Yes	+	+	20.9‡
Type II	0	—	—	—	—
Occult tumor					
Type IVb	5	No	+	+	18.9§
Type III	40	No	+	−	4.8‖

* Excluding 10 specimens taken from primary tumor.

† Statistical analysis (Mann-Whitney U test): (‡ *versus* §) p = 1.0; (‡ *versus* ‖) p = <0.05; (§ *versus* ‖) p = 0.26.

TABLE 5. *RIGS-Influenced Operative Changes*

Operative Changes for Primary Colorectal Patients*	No. of Changes	Operative Changes for Patients With Recurrent Colorectal Cancer†	No. of Changes
Rectal resection	1	Abandoned liver resection	3
Gastrohepatic node dissection	8	Gastrohepatic node dissection	3
Small bowel resection	2	Added or directed brachytherapy	5
Retroperitoneal node dissection	1	Added more extensive liver resection	1
Aortic node dissection	1	Iliac node dissection	2
Iliac node dissection	2	Added reresection of intestinal anastomosis	1
Abandoned plans for hepatic pump	1	Added cystectomy	1
		Added vaginectomy	1
Total operative changes	16		17

* n = 12 patients of 24 total patients.

† n = 14 patients of 30 total patients.

nodes, which on routine examination appear to be negative for tumor, have undergone cytokeratin staining and have been found to be positive for tumor. These results have been reported in abstract form by other investigators (ASCRS, 1991). In our study, the ratios of counts in lymph nodes *versus* normal tissues were similar in type III (RIGS positive, histologically negative) and type IVb (RIGS positive, histologically positive, or occult) tissue, suggesting that tumor cells were actually present but were missed by the pathologist. Quinlan et al.[8] correlated CC49 uptake in the germinal center of lymph nodes removed from patients with colon and rectal cancer with early death or long-term survival. It was found that CC49 uptake in the staining of the germinal centers of these lymph nodes was a poor prognostic sign.

We have participated in two studies re-examining type III lymph nodes. In the first, a retrospective study, 15 type III lymph nodes were recut and restained with H&E and a mixture of two anticytokeratin antibodies (International Conference on Monoclonal Immunoconjugates for Cancer, 1992). Two of 15 were positive for H&E on recutting and staining and four of 15 had occult metastasis established by cytokeratin staining. An additional four of 15 had staining with CC49 in nontumor elements, indicating tumor breakdown products. In summary, six of 15 (40%) nodes were demonstrated to have occult metastasis, and an additional four of 15 (27%) were exposed to tumor antigen. A second prospective study using serial H&E, cytokeratin staining, and autoradiography demonstrated micrometastasis in 55% of lymph nodes originally labeled RIGS positive, histologically negative (AACR, 1992). Although these studies have not conclusively demonstrated malignancy in 100% of type III nodes, we believe that they demonstrate the superiority of RIGS detection over historic histologic techniques.

From these preliminary results, it appears that the RIGS technology will become an important adjunct to routine H&E staining for the staging and prognosticating of colon and rectal cancers. Use of the RIGS system during surgery had an immediate impact on the surgical procedure in half of all the cases. In many cases, RIGS-positive tissue was found in the gastrohepatic, celiac, or iliac nodes, which are areas not traditionally thought of as common sites for colon and rectal cancer spread. All RIGS-positive tissue was considered to be malignant and was removed when possible. Four of the study patients underwent additional bowel resections based on occult tumor found only by RIGS exploration. Follow-up is currently too short to know if these additional procedures will improve survival.

Radioimmunoguided surgery system findings also contributed to the operative technique in other, less obvious ways. Radioimmunoguided surgery exploration during surgery has resulted in the upstaging of cancers for several study patients. Findings of occult disease also led to the abandonment of hepatic resections in three patients undergoing second-look surgery. Margins of resection were more accurately checked with the gamma-detecting probe than by traditional means. Finally, thickened, scarred, or suspicious tissues were quickly assessed in the operating room with the RIGS method. Ultimately, the true value of RIGS procedures will depend on improvements in morbidity rate, longer disease-free intervals, and increased patient survival.

One concern with any new technology is possible additional cost. Radioimmunoguided surgery exploration adds only 10 to 15 minutes to the traditional exploration. Finding additional occult tumor requiring resection may add to the length of the operative procedure. In some cases, however, RIGS findings have led to the abandonment of a planned liver resection, thereby saving the patient the complications and cost of a long procedure that ultimately would not have resulted in any benefit.

There is no protocol for second-time injections with CC49. All patients who undergo RIGS procedures, however, are followed with periodic examinations, carcinoembryonic antigen, endoscopy, and CT scan if necessary. Any sign of recurrence requires a complete workup. If disease is determined to be limited to the abdomen, and if the patient has had a previous curative procedure (either primary or second-look), re-exploration is rec-

ommended. Those patients who have previously undergone RIGS surgery undergo a traditional re-exploration only.

In summary, RIGS surgery with the second-generation anti-TAG antibody CC49 results in useful, immediate intraoperative information not provided by any other technique. Because the CC49 MAb targets both primary tumor and metastatic disease efficiently, the surgeon can use the RIGS method to improve patient selection for more radical procedures during second-look surgery, perform a more complete tumor ablation, more accurately and immediately stage primary cancers of the colon and rectum, and assess extent of disease outside the regional local site.

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