RadioimmunoPET: Detection of Colorectal Carcinoma with Positron-Emitting Copper-64-Labeled Monoclonal Antibody

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Detection of tumor foci may be improved by combining the selective tumor-targeting properties of a monoclonal antibody with the superior sensitivity and contrast resolution of PET.

Methods: An anti-colorectal carcinoma monoclonal antibody (MAb 1A3) was labeled with $^{64}$Cu, a positron-emitting radionuclide, by use of a bifunctional chelate (bromoacetamidobenzyl-TETA) and evaluated in 36 patients with suspected advanced primary or metastatic colorectal cancer. After radiopharmaceutical injection (5-20 mg protein, 10 mCi $^{64}$Cu), PET was performed once or twice, 4 to 36 hr later. All patients had CT scans and 18 patients were also studied with $^{18}$F-fluorodeoxyglucose (FDG) PET.

Results: In 29 patients, one or more tumor sites (n = 56) were proven, in 5 patients the absence of active tumor was confirmed and in the remaining 2, tumor status is not yet confirmed. Of the 56 confirmed tumor sites, 40 were detected by MAb-PET as foci of increased activity (sensitivity 71%). The positive predictive value of MAb-PET was excellent, ranging from 89% (40/45) to 96% (43/45), depending on the ultimate classification of three image-positive, but as yet unconfirmed tumor sites. Also, MAb-PET detected 11 new occult tumor sites, including 9 small abdominopelvic foci less than 2.0 cm in diameter that were not detected by CT or MRI. There were no complications, but significantly elevated HAMA titers were found in 28% of the 29 patients tested 1 to 12 mo after injection. There was no apparent dose-related effect from 5 to 20 mg MAb 1A3.

Conclusion: These Phase I/II results suggest that PET with radiolabeled MAbs (radioimmunoPET) may have important applications in clinical oncology, particularly for detecting smaller colorectal tumor foci in the abdomen or pelvis and for determining accurate dosimetry.

Key Words: radioimmunoPET; copper-64-labeled MAb 1A3; colorectal cancer


Radioimmunoscintigraphy (RIS) with monoclonal antibodies (MAbs) labeled with several different radionuclides has shown definite clinical utility in patients with colorectal carcinoma and other types of cancer (1-5). Although RIS provides some advantages compared with conventional imaging studies (e.g., CT), available methods are still not optimal. PET offers several advantages relative to conventional scintigraphy, including better contrast resolution, direct correction for attenuation and the ability to perform accurate quantification of tissue uptake of the radiopharmaceutical (6-8). Hence, coupling of the tumor-targeting specificity of a MAb with the imaging advantages of PET should yield a technique ["radioimmunoPET" (MAb-PET)] with considerable clinical value.

The positron-emitting halogens, $^{121}$I and $^{18}$F, have been coupled covalently to MAbs, and these radiolabeled MAbs have been used for detecting tumor sites in animal models and in a few patients (9-12). Until recently, however, MAb-PET had not been performed with a MAb labeled by chelation of a positron-emitting metal radionuclide, a method that would allow easier radiopharmaceutical preparation (13). This article is the complete Phase I/II study of our efforts to explore the combination of such a radionuclide ($^{64}$Cu; $T_{1/2} = 12.8$ hr) and a MAb directed against colon carcinoma (MAb 1A3) for detecting tumor foci by PET in patients with colorectal cancer.

The specific purpose of this study was to determine whether an intact MAb labeled with $^{64}$Cu would localize in tumor sites and be cleared from normal tissues rapidly enough to allow tumor imaging. MAb 1A3, developed in our laboratories, is a murine MAb (IgG1,k) that binds to a lipid antigen enriched in human colon carcinoma cells and has excellent immunospecificity for colorectal adenocarcinoma (5,14). RIS with $^{111}$In-labeled MAb 1A3 has been moderately successful for detecting tumor deposits in patients with colorectal cancer (5). Preclinical studies (15) with intact MAb 1A3 labeled with $^{64}$Cu by the bifunctional chelate, 6-bromoacetamidobenzyl-1,4,8,11-tetraazacyclodecane-N,N,N',N"-tetraacetic acid (BAT) demonstrated it to be a stable radiopharmaceutical with excellent...
immunoreactivity that localized well in GW39 human colon carcinoma tumors carried in hamsters. The estimated human radiation dosimetry with this radiopharmaceutical was judged to be acceptable [critical organ (lower large intestine) dose = 5.19 rem/10 mCi; effective dose equivalent = 2.66 rem/10 mCi)]. Because of the high renal uptake (and correspondingly high renal radiation dose) with ⁶⁴Cu-labeled F(ab')₂ fragments, we restricted this initial study to intact MAb.

MATERIALS AND METHODS

We initiated clinical studies with ⁶⁴Cu-benzyl-TETA-MAb 1A3 (13) after filing an investigational new drug application with the U.S. Food and Drug Administration and after obtaining approval of the protocol by the Institutional Review Boards of both the Jewish Hospital of St. Louis and Washington University School of Medicine. Each patient who participated in this trial gave written informed consent. Between July 1992 and June 1994, we studied 36 patients (27 men, 9 women, aged 26–80 yr) with histologically proven adenocarcinoma of the colon or rectum who were thought, at the time of enrollment, to have advanced disease with at least one primary, recurrent or metastatic site. Tumor was confirmed in 21 patients by biopsy and in 8 patients by unequivocal findings on CT, MRI and/or PET with ²⁻¹¹-F]-fluoro-2-deoxy-D-glucose (FDG, 10 mCi). In five patients, the absence of tumor at the time of study was confirmed by biopsy (one patient) or by a stable clinical course without evidence of tumor on CT for periods of 11–15 mo following the imaging study. The true disease status in two patients is not yet confirmed.

Monoclonal Antibody

Clinical-grade MAb 1A3 was produced with our hybridoma cell line from serum-free medium by Invitron Corp. (St. Louis, MO) using proprietary methods. The p-nitrobenzyl-TETA was synthesized according to the method of Moi et al. (16). Bromoacetamidobenzyl TETA was prepared from nitro-benzyl-TETA as described by McCall et al. (17) and conjugated to MAb with previously described techniques (15). Copper-64 was produced at the Missouri University Research Reactor as described (18) and purified at Washington University by evaporation of the ⁶⁴CuCl₂ to dryness and reconstituting it in 0.1 N HCl. The 2-Iminothiolane (2-IT) and Sephadex G-25/50 were purchased from Sigma Chemical Co. (St. Louis, MO). Triethanolamine hydrochloride was purchased from Aldrich (Milwaukee, WI). Ultrapure ammonium dihydrogen phosphate, anhydrous sodium acetate (NaOAc) and 99.9999% pure HCl (minimum 35% by assay) were purchased from Johnson Matthey (Waralilly, MA) and ammonium citrate (puriss) was purchased from Fluka (Ronkonkoma, NY). All solutions were made using distilled deionized water (Milli-Q®; >18MΩ resistivity). Sephadex G-25/50 was equilibrated in 0.1 M ammonium phosphate, pH 8, or 0.1 M ammonium citrate, pH 5.5 or 6.5, as described elsewhere (19).

Conjugation of Benzyl-TETA-MAb and Radio-labeling

Conjugation of BAT to MAb 1A3 was accomplished by previously described methods (15). Briefly, a solution of MAb 1A3 in 0.1 M ammonium phosphate, pH 8, was incubated with excess BAT and freshly prepared 2-IT in 50 mM triethanolamine. Molar ratios of BAT:MAb were 20:1, and the corresponding molar ratios of 2IT:MAb were 10:1. The final chlorato-to-MAb ratio was 1:81 as detailed previously (15). The solution was incubated at 37°C for 30 min and purified by gel-filtration chromatography using centri-
of MAb were counted in a Picker-Pace Autogamma well counter along with aliquots of the injectate. The results were expressed as percent injected dose in the circulation by performing a least-squares fit of these data to a bi-exponential function in order to attain the best possible correlation coefficient with the fitted curve constrained to pass through 100% at t = 0 hr. Urine was collected and the percent injected dose excreted in the urine during the first 20 to 24 hr postinjection was calculated by counting of urine samples and aliquots of the injectate. HAMA titers were determined on blood samples obtained from all patients preinjection and at several times postinjection (Immunistrip HAMA, Immunomedics, Warren, NJ).

PET Imaging and Image Analysis

Multiple transmission and emission PET scans (each 10 and 30 min in duration, respectively) of 3-5 contiguous 16.2-cm axial fields were performed with a Siemens (Des Plaines, IL) ECAT EXACT scanner to cover the pelvis, abdomen and chest. Transmission scans were obtained immediately before or after each emission scan. Imaging was performed at times from 3 to 36 hr after injection of MAb as follows: All patients underwent imaging 18-24 hr postinjection. In addition, 17 patients were also studied 3-6 hr, and 3 patients were studied 24-36 hr postinjection. Catheter drainage of the urinary bladder was not required for MAb-PET. Images were reconstructed as transaxial sections (47 for each scan) and then combined into volume images of the torso for viewing in transaxial, coronal, and sagittal planes or as maximum-pixel, volume-rendered reprojecction images.

All PET images were evaluated qualitatively by two experienced nuclear medicine physicians (FD, BAS) who were unaware of the clinical data, including results of other imaging studies. Based on knowledge of the normal biodistribution of the radiopharmaceutical, foci of abnormal increased 64Cu-MAb accumulation were scored as definite tumor (21 foci), probable tumor (19 foci) (all categorized as representing tumor) or equivocal (8 foci) (see below). Following completion of the blinded image analysis, the PET images were correlated with the clinical, radiographic, and surgical findings to provide a final interpretation for data analysis. There were no major disagreements in image interpretations between the two observers. The final interpretations corresponded closely to the initial blinded analyses (91% site-by-site agreement); seven probable tumor foci were reclassified as definite on unblinded analysis. Five sites in the liver or lung with abnormally decreased tracer accumulation were scored as negative for tumor. The eight foci classified as equivocal on the blinded readings were reclassified for final interpretations by consensus (BAS, FD, GWP) with final scoring of five foci as positive and three as negative.

All tumor sites and benign lesions were confirmed by biopsy or by strong clinical and radiographic evidence (CT, MRI and/or FDG-PET). Most (24/33) of the tumor foci in the abdomen and pelvis were proven by biopsy, but clinical and radiographic evidence were the basis for confirmation of 9 of 15 hepatic metastases and all 8 pulmonary tumors. The presence or absence of tumor could not be established for three positive foci on MAb-PET, and these foci were excluded from further analysis. Two of these excluded foci (in two different patients) could not be validated as tumor from available data at the time of, or shortly after, the MAb-PET study, however, both patients developed widespread metastatic disease 4 to 6 mo after MAb-PET study. The third small focus, which was strongly positive on both FDG-PET and MAb-PET, has not yet been confirmed histologically or by clinical follow-up for 4 mo. One patient thought to have mediastinal lymph node metastases from a primary rectal tumor had sarcoidosis proven by biopsy. Other lesions (11 sites), originally thought to be colorectal cancer by clinical criteria, were confirmed as benign by remaining stable clinically (history, physical examination, CEA level) and radiographically (CT/MRI) for 11-15 mo following the imaging study. Many of the patients referred for this study were considered to represent difficult diagnostic problems following conventional evaluation, and this patient selection bias no doubt accounted for the fact that 5 of the 36 patients (14%) were misdiagnosed clinically as having metastatic or recurrent colorectal tumor prior to MAb-PET.

Comparison with FDG-PET

FDG-PET was performed for routine clinical assessment of tumor status in 18 patients enrolled in this study (16 studies within 2 wk of MAb-PET and 2 within 2 mo). As in the MAb-PET imaging protocol, multiple contiguous FDG-PET scans (each consisting of a 30-min emission and a 10-min transmission scan) were performed to include the chest, abdomen and pelvis. All patients fasted for at least 4 hr before the FDG injection and were hydrated with 0.9% saline solution intravenously during the imaging procedure. Furosemide (20 mg) was given 20 min after FDG administration to facilitate clearance of renal activity. A Foley catheter was placed in the urinary bladder to minimize urinary radioactivity on pelvic scans. The FDG-PET images were interpreted in standard clinical fashion (i.e., with access to all other relevant clinical data). The foci with definitely abnormal or probably abnormal FDG uptake were compared with the MAb-PET results.

### RESULTS

#### Imaging

Thirty of the 36 patients had original primary cancers in the rectum or sigmoid colon; the remaining 6 had cancers elsewhere in the colon (4 ascending, 1 transverse, 1 descending).

Of the 29 patients with confirmed tumor sites, 25 had at least one known tumor site detected by MAb-PET as a focus of increased radioactivity (86% patient sensitivity) (Table 1). All positive sites were evident by 18-21 hr after injection, and there was no further improvement in sensi-

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total no.</th>
<th>Number with increased activity</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor confirmed</td>
<td>29</td>
<td>25</td>
<td>86</td>
</tr>
<tr>
<td>Benign process confirmed</td>
<td>5</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>Tumor status indeterminate</td>
<td>2</td>
<td>2</td>
<td>?</td>
</tr>
<tr>
<td>Confirmed tumor sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>7</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Recurrent</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Metastatic</td>
<td>39</td>
<td>23</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>40</td>
<td>71%</td>
</tr>
</tbody>
</table>
Foci in the three patients who had more-delayed images. In the 17 patients also studied 3–6 hr postinjection, the tumor foci had much less or no activity on the early images, and the change in activity distribution, apparent during comparison of the early and later images often made it easier to detect small lesions.

Imaging results of MAb-PET were negative in five patients with lesions initially thought to be metastatic foci by conventional work-up but eventually proven to be benign processes by biopsy (one patient) or by clinical and radiological follow-up of 11–15 mo duration (four patients).

Of the 56 proven sites of tumor involvement (Table 1), 40 were detected as foci of increased uptake (lesion sensitivity, 71%). All 17 primary and recurrent sites were clearly visualized, but only 59% (23/39) of the metastatic sites were detected (Figs. 1–3). Additionally, four hepatic lesions and one pulmonary lesion (all scored as negative) were seen on MAb-PET as regions of relatively decreased uptake. The sensitivity of MAb-PET was best in the abdomen and pelvis (Fig. 4), in which 100% of lesions were detected as positive foci, including all 19 lesions <3 cm in maximum diameter. Only 5 of the 15 hepatic metastases were clearly seen as foci of increased uptake and only 2 of the 8 known pulmonary metastases were positive, even though one negative lesion was quite large (>6 cm). Nonspecific tissue background activity was not a problem in the lung but was problematic in the liver.

In addition to detecting 29 of the 45 previously known sites of tumor, MAb-PET visualized 16 additional foci of increased activity in all patients. Of these 16 scintigraphic abnormalities, 4 were subsequently proven by biopsy to represent tumors, 7 were considered to represent tumors by clinical assessment, 2 were proven false-positive results, and 3 are likely tumor but unconfirmed at this time. Thus, based only on the positive foci proven to represent sites of tumor involvement (40 sites) and all positive foci (45 sites), the

**FIGURE 1.** Posterior volume-rendered reprojecion PET image, obtained after injection of 64Cu-MAb 1A3 (20 mg, 11 mCi), shows a biopsy-proven focus (arrow) of recurrent carcinoma in a man originally treated by low anterior resection, radiation therapy and chemotherapy for rectal carcinoma.

**FIGURE 2.** Transaxial PET images of the pelvis, obtained after injection of 64Cu-MAb 1A3 (7 mg, 10 mCi), in a man with biopsy-proven recurrent rectal carcinoma. CT (not shown) was equivocal for tumor, but MAb-PET clearly showed a 2-cm recurrent tumor (long arrow in panel A) as well as a nodal metastasis (long arrow in panel B) superior and lateral to the tumor. Blood-pool activity is seen anteriorly in both external iliac vessels (short arrows).

**FIGURE 3.** Transaxial PET image of the pelvis (A) and at the level of the diaphragm (B), obtained after injection of 64Cu-MAb 1A3 (5 mg, 10 mCi), in a man with primary carcinoma in the sigmoid colon (arrow in panel A) and metastases in the lung (long arrow in panel B) and liver (short arrow in panel B).

**FIGURE 4.** Sensitivity for detection of all confirmed tumor sites by MAb-PET. Results are presented for tumors > 3 cm in diameter (diagonally lined bars); for tumors < 3 cm in diameter (solid bars); and for tumors of all sizes (stippled bars).
positive-predictive value of a focus of increased activity on MAb-PET was at least 89% (40/45). If the additional three foci considered likely to represent tumor are also considered as true-positive results, the positive predictive value would be 96% (43/45).

In 18 of the 29 patients with confirmed tumor, CT either did not demonstrate the tumor site(s) or was indeterminate for the presence of tumor. The MAB-PET images in these 18 patients were scored as definitely positive for tumor. The major discrepancies in CT and MAB-PET results were in the abdomen and pelvis where MAB-PET was 100% sensitive (33/33 lesions) and CT only 48% (16/33 lesions). For hepatic and pulmonary metastases, however, CT was more sensitive, detecting 17 of 22 lesions (77%) versus 7 of 22 (32%) detected by MAB-PET.

In the 18 patients also examined by FDG-PET (Table 2), all 21 sites of tumor (9 abdominal-pelvic, 8 hepatic, 4 pulmonary) were detected by FDG-PET. Only in the abdomen and pelvis was MAB-PET equally sensitive. There were, however, seven sites in four patients that did not represent colorectal cancer that were definitely positive by FDG-PET but negative by MAB-PET (Fig. 5). This apparently better specificity of MAB-PET for colorectal tumors was clinically helpful in several patients. When the results of the two studies were the same, the presence or absence of tumor was confirmed, but the combination of a negative MAB-PET and positive FDG-PET suggested an inflammatory lesion or other primary tumor (Table 2, Fig. 5).

Both FDG-PET and MAB-PET were particularly helpful for detecting smaller tumors, those less than 2 cm in maximum diameter (Table 3). There were 15 such sites evaluated by MAB-PET; 10 of these were also studied by FDG-PET. CT and/or MRI failed to detect most of these sites (80%) and were particularly insensitive for lesions in the abdomen and pelvis, the areas in which both MAB-PET and FDG-PET were extremely sensitive.

**Blood and Urine Clearance**

Clearance of $^{64}$Cu from the blood in the first 24 hr was similar to that seen with $^{111}$In-labeled-MAB 1A3 (5). Normalizing both datasets to intersect 100% ID at $t = 0$, the fractions remaining in the blood were 60.9% ± 3.7% after 21 hr for $^{64}$Cu and 60.3% ± 7.7% after 23 hr for $^{111}$In. Urinary clearance was also similar, with 2.9% ± 2.6% ID of $^{64}$Cu excreted in 21 hr (versus 2.3% ± 0.3% ID of $^{111}$In in

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**TABLE 2**

Results of MAb-PET versus FDG-PET in Eighteen Patients Who Had Both Studies

<table>
<thead>
<tr>
<th>Tumor sites</th>
<th>FDG-PET</th>
<th>MAB-PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen/Pelvis</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>38</td>
</tr>
<tr>
<td>Lung</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>False-Positive sites for colorectal cancer</td>
<td>Abdomen</td>
<td>Sarcoïd (1)*</td>
</tr>
<tr>
<td></td>
<td>Mediastinum</td>
<td>Sarcoïd (2)</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>Lung, primary (1)</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>?Lesion (1)</td>
</tr>
<tr>
<td>PPV for colorectal cancer</td>
<td>All</td>
<td>21/28 (75%)</td>
</tr>
</tbody>
</table>

*Value in parentheses is number of sites.

PPV = positive predictive value.

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**TABLE 3**

Detection of Colorectal Cancer Lesions Less Than 2.0 cm in Maximum Diameter

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>CT/MRI</th>
<th>FDG-PET</th>
<th>MAB-PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen/Pelvis</td>
<td>0/9</td>
<td>5/5</td>
<td>9/9</td>
</tr>
<tr>
<td>Liver</td>
<td>3/5</td>
<td>4/4</td>
<td>2/5</td>
</tr>
<tr>
<td>Lung</td>
<td>0/1</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Total</td>
<td>3/15 (20%)</td>
<td>10/10 (100%)</td>
<td>11/15 (73%)</td>
</tr>
</tbody>
</table>

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**FIGURE 5.** Anterior volume-rendered reprojection PET images, obtained after injection of FDG (left, 10 mCi) and $^{64}$Cu-MAB 1A3 (right; 10 mCi, 20 mg), in a woman postresection of Dukes’ C rectal cancer 1 yr before enlarged mediastinal and periporal lymph nodes were observed. These nodes were strongly positive on FDG-PET (left panel) but were completely negative on $^{64}$Cu-MAB 1A3-PET (right panel). Subsequent biopsy of the lymph nodes demonstrated sarcoïdosis.
TABLE 4
Results of HAMA Assays

<table>
<thead>
<tr>
<th>MAb dose (mg)</th>
<th>No. of patients</th>
<th>No. negative (0–0.2 μg/ml)</th>
<th>No. weakly positive (0.2–0.4 μg/ml)</th>
<th>No. moderately positive (0.4–1.5 μg/ml)</th>
<th>No. strongly positive (&gt;1.5 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>15</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

24 hr). The administered dose of MAb (5, 10 or 20 mg) had no significant effect on the clearance of $^{64}$Cu from the blood or urine.

**Adverse Reactions and HAMA Studies**

All patients were followed for 4 to 24 mo. There were no complications from $^{64}$Cu-MAb 1A3 infusion detected during this period. Serum samples from 29 patients were tested for HAMA prior to injection of the MAb and all were negative (Table 4). HAMA concentrations in about half of the patients tested at least 1 mo after injection remained negative (<0.2 μg/ml), and about a quarter of the patients had markedly increased titers (>1.5 μg/ml). Titers tended to decrease somewhat after 10–12 mo in the three patients followed that long. The injected dose of MAb did not appear to affect the development of HAMA.

**DISCUSSION**

This study has shown that $^{64}$Cu-benzyl-TETA-MAb 1A3 is a safe radiopharmaceutical that causes no clinically detectable adverse effects in the doses used for this study. Additionally, and most importantly, the ability to obtain PET images of small colorectal tumors in the abdomen and pelvis was most impressive. Even though this was a Phase I/II study, MAb-PET clearly exhibited superior sensitivity for detection of abdominal-pelvic tumors by comparison with conventional clinical detection methods (including CT or MRI). Only FDG-PET appeared to be as sensitive as MAb-PET, but it was not as specific for colorectal tumors. FDG uptake was noted in a primary lung tumor as expected, and false-positive results were seen in some inflammatory lesions as well. Similar false-positive results for inflammatory lesions with FDG-PET have been reported by other investigators (22–24).

In assessing metastatic disease of the liver and lung, however, MAB-PET was not as sensitive as either FDG-PET or CT/MRI. MAB-PET did detect some of the hepatic and pulmonary metastases, including some smaller than 3.0 cm, but clearly PET with $^{64}$Cu-labeled intact MAB is not sensitive enough in these areas of the body to lead to significant improvement in their radiological detection. Previous experience (5,14) has shown that there is 1A3 antigen in most of the primary colorectal tumors and in many of the hepatic and pulmonary metastases. It is possible that smaller fragments of MAB 1A3 or, of other MAb, may improve the performance of MAB-PET in the liver and lung.

MAb-PET with $^{64}$Cu-labeled MAb 1A3 compares quite favorably to RIS with other radiolabeled MAb directed against colorectal cancer (1–4), including $^{111}$In-labeled MAb 1A3 (5) and the only FDA-licensed radiolabeled MAb for RIS (2). In our study of RIS with $^{111}$In-MAb 1A3 (5), the overall sensitivity for tumor detection was 76% per patient and 63% per lesion. In the present study, the sensitivity of MAB-PET was 86% per patient and 71% per lesion. A comparison of detectability by tumor site also favored MAB-PET over RIS (abdomen-pelvis; 100% versus 86%; liver 33% versus 20%; lung 25% versus 0%), although our experience is still limited. The positive predictive value also was better for MAB-PET than for RIS (89%–96% versus 83%). In contrast to $^{111}$In-labeled MAb 1A3, little excretion of $^{64}$Cu-labeled MAb 1A3 into the bowel was observed on the MAB-PET images, and, even though an intact MAb was used, imaging was performed within 24 hr postinjection. This is a distinct advantage of the $^{64}$Cu-labeled agent. Nontarget activity in the liver is still too high, however, to allow for satisfactory detection of hepatic metastases. Additionally, blood-pool activity is quite high at the early time when imaging must be performed. The use of an F(ab')2 fragment should significantly decrease blood-pool activity.

When this study was undertaken, the $^{64}$Cu-labeled fragments available to us had such high renal clearance that the radiation dosimetry to the kidney was excessive (15). Refinements in our labeling technique have reduced this dose to an acceptable level (25). These promising results with the animal model suggest that the fragment will produce superior imaging results. Labeling approaches using other linkers (26) may also increase the clearance of the label from the circulation and improve images with $^{64}$Cu-MAb-PET. Use of F(ab')2 fragments, as well as smaller agents, should significantly decrease the activity in the blood pool and may allow for improved detection of hepatic and pulmonary metastasis (27).

The $^{64}$Cu used in these studies was prepared at the Missouri University Research Reactor (MURR), one of a limited number of research reactors capable of producing $^{64}$Cu in the required quantities. With the proper facilities at MURR, it is possible to produce $^{64}$Cu with a specific activity $>$640 Ci/mmol in multi-millicurie amounts daily. This procedure can be adapted to produce curie quantities (Zinn K, personal communication). This would allow for
wider availability of 64Cu-labeled MAbs. Copper-64 is one of a limited number of positron-emitting radionuclides that can be produced both on a nuclear reactor and a cyclotron. Techniques that use enriched targets have been described, whereby 100-mCi quantities of 64Cu can be produced with low-energy biomedical cyclotrons (28,29).

Copper-64 has been shown to have cytotoxic effects similar to 67Cu (30), a radionuclide currently under evaluation in a radioimmunotherapy trial. We have shown the potential of 64Cu as an agent for radioimmunotherapy both in vitro and in vivo (31–34). The potential wide availability of 64Cu, both from reactors and biomedical cyclotrons, and the capability to perform accurate dosimetry estimates using PET imaging, suggests that 64Cu is a radionuclide with great potential for radioimmunootherapy.

Our results clearly show that combining the tumor-targeting specificity of a MAb with the superior imaging capabilities of PET is a promising approach. Continued efforts to improve this approach with faster clearing fragments for diagnostic assessment of colorectal and other cancers, and eventually for the therapy of tumors, are warranted.

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