The use of radiolabeled anticancer antibodies to detect cancer sites by external scintigraphy has had a relatively long history. With the advent of monoclonal antibodies (MAbs), which precluded the need for purifying the antibodies by laborious purification steps, there was a surge of interest and efforts to develop these reagents for both imaging and therapy applications (1). Today, many thousands of patients have received different forms and doses of MAbs for various purposes, and four MAb-based products have been licensed for manufacture and sale in the U.S. (2,3). This article describes the most recent MAb product to be approved in the U.S. for colorectal cancer imaging, including discussions of using this agent and its therapeutic counterpart in several cancer types.

**Key Words:** immunoscintigraphy; radioimmunodetection; cancer; lymphoma; CEA


Why is a $^{99m}$Tc-labeled Fab' fragment of an antibody a desired imaging agent? The first radiolabeled antibodies used for cancer imaging consisted of whole IgGs labeled with $^{131}$I, both with purified polyclonal antibodies and even after the introduction of MAbs (1,4). Subsequently, whole IgG antibodies were labeled with $^{123}$I, $^{111}$In and $^{99m}$Tc (5). It became apparent that the short half-lives of $^{123}$I (13 hr) and $^{99m}$Tc (6 hr) required a faster targeting agent, such as a fragmented form of the antibody. Indium-111 had a severe disadvantage of binding to normal liver tissue and to a lesser extent to spleen and bone marrow. The monovalent Fab' fragment is devoid of the immunogenic Fc portion and is of only 50,000 molecular size (Fig. 1). This allows tumor targeting within minutes to hours and clearing from the blood pool and other tissues within 24 hr. Good tumor-to-background ratios can be achieved within 24 hr, and often within 2-5 hr. This makes tumor imaging feasible on the same day that the reagent is injected intravenously and allows sufficient counts of $^{99m}$Tc to be concentrated early so that SPECT can be used to enhance image contrast and improve lesion resolution. It was also found that a dose of 1 mg of Fab' could target tumors as well as the same dose of the bivalent $F(ab')_2$ form, and that the 1 mg dose of either fragment was as optimal as a dose that is 10 times higher (6). Thus, 1 mg of a specific antacingeembryonic antigen (CEA) MAb Fab' fragment became the antibody form of choice for radiolabeling with $^{99m}$Tc.

Why use $^{99m}$Tc as the radiolabel? This isotope is used in over 70% of all nuclear medicine procedures, is readily available through a generator at low cost and has an excellent photon energy for conventional gamma cameras. These features are not matched by any of the other radionuclides available commercially. However, the use of $^{99m}$Tc in antibody immunoscintigraphy, or radioimmunodetection (RAID), requires simple and stable labeling methods. Additionally, there has been a long development of labeling methods, both with chelates and as direct conjugation procedures (7). The product licensed as the anti-CEA Fab' involves a one-step, direct, instant labeling method of high stability and requiring no postlabeling purification (7,8).

Finally, why use CEA as the antibody target for cancer imaging? CEA was described by Gold and Freedman (9) in 1965 as a glycoprotein antigen elaborated by colorectal cancer and has been used routinely since the mid-1970s as a serum...
marker for monitoring disease status in patients who have
diverse epithelial tumors, such as gastrointestinal carcinomas
(esophageal, gastric, colonic, rectal, pancreatic and biliary),
mammary, lung, medullary thyroid, uterine (cervical and endo­
dometrial), ovarian and bladder carcinomas (10,11). It is not
specific for these cancers, since it can be elevated in the blood
of patients with some benign and inflammatory conditions of
the same organs (12), but is definitely increased significantly
in malignant as compared to normal or benign tissues (13,14).
Anti-CEA antibodies do not have access to increased intersti­tial
levels of CEA under normal conditions, such as when the
basement membrane of the intestinal mucosa is intact. The
normal mucosa does contain and shed CEA from its surface. It
is only when this basement membrane is compromised by an
invading carcinoma that CEA is shed into the blood and when
circulating CEA antibodies can target to the neoplasm. The
presence of CEA in the blood was thought originally to be a
reason to preclude the binding of CEA antibodies to tumor
sites, because the antigen in the blood would complex to the
injected antibody and thus preclude tumor targeting (15). Our
clinical studies of CEA radioimmunodetection proved this pre­
diction to be false, and even when some complexes were
formed in the blood of the patients injected with whole anti­
CEA IgG antibody labeled with 131I, tumor targeting and
imaging was successful (4,16,17). These findings verified the
usefulness of CEA antibodies for this purpose and provided
direct clinical evidence for the prospects of targeting cancers
with radiolabeled antibodies either for imaging or therapy.

CEA-Scan® (Arcitumomab) in Colorectal Cancer

CEA-Scan® (Immunomedics Inc., Morris Plains, NJ) is the
antigen imaging agent that consists of a specific anti-CEA
MAb Fab' labeled directly with 99mTc and was recently li­
censed by the FDA for detecting recurrent or metastatic colo­
rectal carcinoma in the abdominopelvic region (8,18). Two
prospective, pivotal clinical studies served as the basis for
approval of this product, involving 210 presurgical patients
with proven colorectal carcinoma. Although the patient
population studied consisted of those with proven or suspected
recurrence or spread, a small number of patients with primary
colorectal cancer were also successfully imaged (3).

One milligram of CEA-Scan® labeled with 15–25 mCi 99mTc
was injected intravenously; external scintigraphy was per­
formed at 2–5 and 18–24 hr later. Imaging with standard
diagnostic modalities (SDM), such as CT, roentgenograms,
MRI, ultrasonography, etc., was also performed, and the find­
ings were confirmed by surgery and histology. The sensitivity
(true-positive rate) of CEA-Scan® was statistically superior to
that of SDM (mostly CT) in the extrahepatic abdomen (55%
versus 32%, p = 0.007) and pelvis (69% versus 48%, p = 0.005)
and complementary to SDM in the liver (Table 1). The small­
est lesion depicted by CEA-Scan® was in the order of 0.5 cm,
but hot or rimmed lesions could be seen in the majority of
tumors that were 2 cm or less, due to the penetration of the
tumor by the smaller antibody fragment; much larger tumors
sometimes required some time before “filling in” of activity
was observed (Fig. 2). Of 122 patients with known disease, the

positive predictive value was significantly higher when both
modalities were positive, as compared to when SDM were
positive and CEA-Scan® was negative (98% versus 68%, p <
0.0001), potentially obviating the use of biopsy for histological
confirmation of a suspected lesion when both tests were positive.
When CEA-Scan® was added to SDM, imaging accuracy was
significantly enhanced (93% versus 83% for SDM used alone,
p = 0.0005). In 88 patients with suspected recurrence that was
not disclosed by SDM (occult disease), imaging accuracy was
also enhanced by CEA-Scan® when combined with SDM (61%
versus 33% for SDM alone, p = 0.0004; three patients who
were initially false-negative by CT were reevaluated after
CEA-Scan® and corrected to be true-positive). When evaluat­
ing different organ regions, CT and CEA-Scan® were found to

| TABLE 1
Comparison of Sensitivity of CEA-Scan® and
SDM (Mostly CT) by Body Site* |
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<tr>
<td></td>
<td>Abdomen</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>(n = 69)</td>
<td>(n = 81)</td>
</tr>
<tr>
<td>CEA-Scan™</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. patients</td>
<td>38</td>
<td>51</td>
</tr>
<tr>
<td>%</td>
<td>55.1</td>
<td>63.0</td>
</tr>
<tr>
<td>95% C.I.</td>
<td>42.6–67.1</td>
<td>51.5–73.4</td>
</tr>
<tr>
<td>SDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. patients</td>
<td>22</td>
<td>52</td>
</tr>
<tr>
<td>%</td>
<td>31.9</td>
<td>64.2</td>
</tr>
<tr>
<td>95% C.I.</td>
<td>21.2–44.2</td>
<td>52.8–74.6</td>
</tr>
<tr>
<td>p*</td>
<td>0.007</td>
<td>ns*</td>
</tr>
<tr>
<td>*From Moffat et al. (3).</td>
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1p values determined by McNemar’s test.
2ns = not significant.

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FIGURE 2. (Top) Planar anterior abdomen views obtained at 2
(left), 4 (middle) and 24 hr (right) postinfusion of 30 mCi 99mTc-CEA­
Scan® in a patient with metastatic colon carcinoma. Note that nearly
all liver lesions are initially cold at 2 and 4 hr after infusion, but that
some (arrows) show a substantial filling in 24 hr after the infusion.
(Bottom) A CT scan of the abdomen showing the metastatic liver
lesions.
be equivalent and complementary in the liver, but in the extrahepatic abdomen and pelvis, CEA-Scan® was significantly better than CT (Table 1).

SPECT imaging was found to be important for identifying tumors, especially small ones near major organs of high radioactivity, such as the kidneys (Fig. 3), which are known to metabolize small antibody fragments and peptides. Nonspecific intestinal activity could be seen in some of the later images (after 7 hr) and could be identified by showing a change in location when comparing early and late scans. Only two patients in the series developed an immune antimouse antibody response (HAMA) to CEA-Scan® after a single injection; none of 22 assessable patients developed HAMA after receiving two injections (3). In terms of the role of plasma CEA titer in influencing the CEA-Scan® imaging results, blood values up to 250 ng/ml (where 2.5 ng/ml is the cutoff for normal range) did not show complexation of the injected antibody with circulating CEA, while a blood titer of >2,000 ng/ml showed about 50% complexation yet did not interfere with tumor imaging (Fig. 4). This is probably due to the use of one arm for antigen binding in an antibody Fab’ fragment, as well as the relatively modest affinity of the CEA antibody used in CEA-Scan® (about 10^8 M/1^-1).

The conclusions from these studies are that CEA-Scan® is a same-day imaging method that adds clinically significant information in the assessment of the presence, location and extent of disease in colorectal cancer patients with recurrent or metastatic cancer, and only rarely induces a HAMA response (3).

Since concordant findings of CEA-Scan® and SDM (mostly CT) resulted in the most reliable outcome predictions, as confirmed by surgery, the relative role of these two imaging modalities in the presurgical evaluation of patients being considered for resection of locally recurrent or metastatic colorectal cancer was evaluated in another study. In a blinded analysis of 209 patients with known or suspected colorectal cancer (one less patient than those included in the study of imaging performance already discussed), the accuracy of CEA-Scan®, alone and combined with CT, was compared to that of CT for predicting abdominopelvic tumor resectability by correlating the results with surgical and histopathological findings. CEA-Scan® alone or combined with CT was found to be significantly more accurate for predicting surgical outcome than use of CT by itself (19). When both tests were concordant for resectability, then 100% were truly resectable. When the two tests were discordant, CEA-Scan® was correct substantially more often than CT (Table 2).

These results were true for the entire abdominopelvic cavity or for the liver. This analysis thus led to the conclusion that CEA-Scan® was more accurate than CT for assessing resectability in all patients undergoing evaluation for potentially curative abdominal resection of colorectal cancer, and in a subset of patients with suspected or proven liver metastases. The additional use of CEA-Scan® with CT potentially more than doubles the number of patients who could avoid unnecessary abdominopelvic surgery and could increase those who are potentially resectable for cure by 40%. Given that surgical resection is the only method currently available to cure recurrent or metastatic colorectal carcinoma (3,19), these are profound results which demonstrate the clinical utility of determining the location and extent of colorectal cancer preoperatively.

**CEA-Scan® (Arcitumomab) in Other Cancer Types**

Several carcinomas other than colorectal have been found to express CEA in increased quantities and virtually all of these tumor types have been shown to be targeted by radiolabeled anti-CEA antibodies (4,16,20–24). These include esophageal, gastric, pancreatic, biliary, mammary, lung, medullary thyroid, ovarian, uterine (endometrial and cervical) and urinary bladder carcinomas (Table 3). Of these, our own efforts focused on lung, mammary, medullary thyroid, pancreatic and ovarian carcinomas. Various carcinomas of the lung express CEA and, thus, can be targeted by anti-CEA antibodies (22). CEA-Scan®’s potential value in lung carcinoma patients, as well as in
TABLE 2  
Prediction of Surgical Outcome when CT and CEA-Scan® are Concordant and Discordant

<table>
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<tr>
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<th>Concorance</th>
<th>Discorance</th>
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<tbody>
<tr>
<td></td>
<td>No. patients</td>
<td>No. correct</td>
</tr>
<tr>
<td>Resectable</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>Non-resectable</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>73</td>
</tr>
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*Adapted from Hughes et al. (19).

patients with other cancer types, may well be to determine the extent of disease, for presurgical staging, by means of a single body survey followed by selected SPECT imaging. This now requires multiple imaging tests to identify tumor sites in the viscera, brain, bone and bone marrow. In breast cancer, there is preliminary evidence that CEA-Scan® can disclose tumors missed by mammography (23) and can help differentiate between benign, abnormal hyperplasia with atypia and carcinoma, thus, having a higher specificity than mammography (24). If substantiated by additional trials, this could decrease the number of unnecessary biopsies being performed at present in 75% of women having a pathological finding on routine mammography. The use of CEA-Scan® in the management of patients with medullary thyroid carcinoma has been extremely encouraging, since it can result in the identification of occult disease suggested only by elevated serum calcitonin and/or CEA (25). Although pancreatic and ovarian carcinomas usually are advanced when initially diagnosed, CEA-Scan® has the potential of better assessing the extent of disease in patients being considered for debulking or aggressive surgery. In ovarian carcinoma, this imaging method may be important to define those patients who are eligible for anti-CEA radioimmunotherapy, as discussed below. An example of identifying a medullary thyroid carcinoma with CEA-Scan® is shown in Figure 5.

Cancer Imaging with Other Antibodies

Many antibodies against diverse human tumor-associated markers have been developed and studied clinically as targeting or therapeutic agents in recent years (1,5). Any of these that demonstrate a sufficient gradient between tumor and normal tissues can, in principle, be used as cancer imaging agents, and antibody Fab' fragments labeled with 99mTc, as in CEA-Scan®, are undergoing clinical evaluation. LymphoScan™ consists of a B-cell-specific (CD22) MAb for detecting and staging non-Hodgkin's lymphomas and has shown promising results in initial clinical trials comparing this agent to 67Ga or other imaging agents or modalities (26–29). An example of lymphoma imaging with LymphoScan™ is shown in Figure 6. Alpha-fetoprotein (AFP) is an oncofetal antigen that is shed into the blood, similar to CEA, which is produced in elevated amounts by testicular and ovarian germ-cell and hepatocellular carcinomas. Anti-AFP antibodies have been shown to target and image these neoplasms (30,31). AFP-Scan™ (Immunomedics Inc., Morris Plains, NJ), which is also a Fab’ labeled directly with 99mTc, is under clinical study to determine its role in the management of patients with these tumor types.

Prospects for CEA Radioimmunotherapy

The successful targeting of CEA-expressing cancers with anti-CEA antibodies has stimulated interest in treating these cancers with radiolabeled anti-CEA antibodies (32). The use of 99mTc-labeled antibodies may have several advantages over other imaging agents. First, the antibody may allow more specific tumor localization, since it can target and image the neoplastic tissue itself. Second, the antibody Fab’ fragment conjugated to 99mTc can be injected intravenously, whereas other imaging agents, such as 124I-labeled antibodies, require intraperitoneal injection. Third, the antibody-targeted radiotherapy may be more effective, since it can deliver a higher radiation dose to the tumor. In fact, some antibodies have been shown to deliver a dose of radiation that is 1000 times greater than that of the same dose delivered by conventional external beam radiation therapy (32). These advantages of radiolabeled antibodies suggest that they may be useful in the treatment of patients with CEA-expressing cancers.
Various lymphomas and leukemias have been particularly responsive to RAIT, even at relatively low radiation doses due to the radiosensitivity of hematological malignancies (41,42). Different antibodies labeled with either 131I or 90Y have shown high response rates when used in patients who have failed conventional treatments, and some of these responses have been durable for up to 2 yr (41,43–45). These findings strengthen our conviction that RAIT at sufficiently high doses can become successful for treating many solid, relatively radioresistant neoplasms, if concomitant toxicities to the bone marrow and possibly other organs can be mitigated or prevented. Examples include autologous bone marrow or stem cell transplantation for controlling myelotoxicity, and possibly the administration of cationic amino acids to reduce renal reabsorption of small fragments and peptides for potentially reducing renal toxicity (46). The era of cancer therapy with radiolabeled antibodies should follow rapidly on the foundations laid by the radiolabeled antibodies used as tumor targeting and imaging agents, once repeated administrations and higher tumor doses and therapeutic indices are achieved.

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Cancer Imaging with Radiolabeled Antibodies: New Advances with Technetium-99m-Labeled Monoclonal Antibody Fab’ Fragments, Especially CEA-Scan® and Prospects for Therapy

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