Diagnostic Imaging of Colorectal Carcinoma with Radiolabeled Monoclonal Antibodies

by:

George H. Hinkle, M.S., R.Ph., BCNP, FASHP
David L. Laven, N.Ph., CRPh, FASHP, FAPPM

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Editor
and
Director of Pharmacy Continuing Education

William B. Hladik III, M.S., R.Ph.
College of Pharmacy
University of New Mexico

Associate Editor
and
Production Specialist

Sharon I. Ramirez, Staff Assistant
College of Pharmacy
University of New Mexico

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DIAGNOSTIC IMAGING OF COLORECTAL CARCINOMA
WITH RADIOLABELED MONOCLONAL ANTIBODIES

STATEMENT OF OBJECTIVES

The primary goal of this continuing education lesson is to increase the reader's knowledge and understanding of diagnostic imaging in the detection and treatment of colorectal carcinoma with radiolabeled monoclonal antibodies.

Upon successful completion of this course the reader should be able to:

1. List examples of several different radiopharmaceuticals that have been utilized for localization and/or therapy of various cancers.
2. Name the radiolabeled monoclonal antibody that was the first to receive FDA approval for the radioimmunodetection of cancer.
3. List several indications for employing radioimmunodetection methodologies in the evaluation of cancer.
4. State which types of cancers might undergo radioimmunotherapy using radiolabeled monoclonal antibodies based on current investigative initiatives.
5. Describe several factors which contribute to the design and selection of monoclonal antibodies for radioimmunodetection of cancer.
6. Compare and contrast the sensitivity, specificity, and positive and negative predictive values of the two commonly-used radiolabeled monoclonal antibodies that have been extensively investigated for the detection of colorectal carcinoma.
7. Explain the meaning of the acronym HAMA and describe how HAMA manifests itself in patients undergoing testing using radiolabeled monoclonal antibodies.
8. Describe the normal biodistribution pattern and elimination characteristics of OncoScint®CR/OV-In following administration to humans.
9. State the chemistry principles that contribute to the preservation of immunoreactivity of OncoScint®CR/OV-In.
10. Describe the radiolabeling techniques and storage conditions for OncoScint®CR/OV-In.
11. Describe the value of performing computed tomography relative to the use of radiolabeled monoclonal antibodies for detection of colorectal carcinoma.
12. State the value range of CEA content in normal patients as compared to those with colorectal carcinoma.
13. Describe the effect that cigarette smoking can have on the determination of CEA levels in patients.
14. Describe the type of antibody and antigenic determinant that pertains to IMMU-4.
15. Describe the radiolabeling technique and storage conditions for ImmuRAID™-CEA (Tc-99m).
16. State what the elimination characteristics are for ImmuRAID™-CEA (Tc-99m) following administration to humans.
17. Compare the techniques for performing chromatographic assessment of both OncoScint®CR/OV (In111) and ImmuRAID™-CEA (Tc99m).
INTRODUCTION

The detection and treatment of cancer has long been a major focus in the development of new radiopharmaceuticals and techniques in nuclear medicine practice. Over the years, many radiopharmaceuticals have been utilized for the localization and/or therapy of various cancers. These include Tc-99m medronate, Ga-67 gallium citrate, Tc-99m sulfur colloid, Tc-99m sodium pertechnetate, I-131 or I-123 sodium iodide, I-131 metaiodobenzylguanidine, I-131 iodomethylnorcholesterol, Tl-201 thallous chloride, Tc-99m sestamibi and the PET radiopharmaceuticals.

The diagnostic procedures currently utilized for cancer detection, including common nuclear medicine imaging studies, are not based on any specific tumor cell characteristic for localizing properties, and therefore lack specificity for tumor cells. This results in the known limitations of the common radiology procedures including x-ray, nuclear medicine, magnetic resonance imaging, computed tomography and ultrasound. For example, the limitations associated with the traditional diagnostic modalities is a major reason less than two-thirds of patients with recurrent colorectal cancer are diagnosed before they become symptomatic.

Radiolabeled antibodies that bind specifically to tumor associated antigens have been shown to be useful in the diagnosis and staging of a variety of cancers (1). Monoclonal antibodies (MAbs), single species of antibody molecule derived from individual
cells maintained in cell culture in order to provide a reproducible production method for MAbs of high specificity and affinity, can be infused into patients for the identification of the anatomic distribution of a cancer using a technique called radioimmunodetection (2-4). CYTOGEN Corporation's OncoScin® CR/OV (satumomab pendetide), the first radiolabeled MAb to gain FDA approval, is used to localize colorectal and ovarian carcinomas. A number of other radiopharmaceuticals in the form of radiolabeled MAbs directed against a variety of specific tumor associated antigens or markers will enable the detection of other tumor types after FDA approval. In addition, the use of radiolabeled bioactive peptides which bind to specific cell surface receptors is under investigation at a number of institutions across the country.

**DIAGNOSTIC APPLICATIONS OF RADIOLABELLED MAbs**

During the past decade, external imaging with radiolabeled MAbs has proven to be successful for tumor localization, particularly in the detection of colorectal and ovarian carcinomas (5-12). These studies not only demonstrated the clinical usefulness but also the safety of single and repeat infusions of murine MAbs (13,14).

In December of 1992, the U.S. Food and Drug Administration granted final approval for the routine clinical use of the first radiolabeled MAb in this country, indium In-111 satumomab pendetide (OncoScin® CR/OV-In, CYTOGEN Corp., Princeton, NJ). This new radiopharmaceutical, also known as CYT-103, In-111 B72.3 and In-111-GYK-DTPA B72.3 MoAb, is currently approved for detection and localization of colorectal and recurrent ovarian carcinomas.

It is believed other new radioimmunopharmaceutical approvals will follow in the near future to improve the nuclear medicine practitioner's ability to diagnose these and other forms of cancer. Radioimmunodetection offers a number of advantages over conventional diagnostic modalities including cross-sectional imaging tests such as computed tomography (CT) and magnetic resonance imaging (MRI). Radioimmunodetection can be used to survey the entire body but not routinely viewed by CT and/or MRI. In addition, radioimmunodetection provides biological information about tumors as well as the anatomic site.

When radioimmunodetection is used in the evaluation of cancer, indications include the detection of occult disease in those patients with high clinical suspicion, the staging of the extent and degree of disease pre- and post- therapy, as an adjunct to other diagnostic tests that are indeterminate, the assessment of tissue viability, the assessment of the potential role of therapy using radiolabeled MAbs and the follow-up of patients with known disease that present with new symptoms indicating increased metastatic involvement (15).

**THERAPEUTIC APPLICATIONS OF RADIOLABELLED MAbs**

Although the use of radiolabeled MAbs for treatment is not progressing as quickly as radioimmunodetection, the technique holds much promise as adjuvant therapy in the treatment of hematological malignancies such as lymphoma as well as solid tumors including colorectal, ovarian, prostate, brain, lung and breast (16). In addition to the hope of killing solid and hematologic tumor cells, radioimmunotherapy should prove useful as an adjuvant to other forms of therapy and in the reduction of recurrence from small, undetected residual tumor after surgical excision.

**PHARMACIST INVOLVEMENT IN THE PREPARATION AND USE OF MAbs**

Nuclear pharmacists are well trained to provide expertise in designing and developing product dosage forms and radiolabeling schemes, preparing the final product and testing to assure its quality, calculating radiation dose estimates, and assuring regulatory compliance. In many settings, including biotechnology firms developing these radioimmunopharmaceuticals, government agencies, centralized nuclear pharmacies and established research institutions, the nuclear pharmacist has added the necessary expertise to bring a number of radiolabeled MAbs to the investigational testing stages and eventually to clinical use.

**SELECTION OF RADIONUCLIDES FOR DIAGNOSIS**

Radionuclides which decay with the emission of gamma photons without particulate radiation are preferred in radioimmunodetection. For external imaging with instrumentation found in most nuclear medicine departments, gamma emissions of 100-200 keV energy range are ideal. Radionuclides which have been used include indium-111, technetium-99m, iodine-123 and iodine-131.

Along with the types and energies of emissions, another important consideration is the half-life of the radionuclide. Whole, intact IgG MAbs which may require longer clearance times prior to imaging should be radiolabeled with long half-life radionuclides such as indium-111 or iodine-131 (17,18). MAb fragments
including F(ab')2 molecules or monovalent Fab which have faster clearance rates from the blood may be radiolabeled with short half-life radionuclides such as technetium-99m or iodine-123. Presently, there appears to be no optimal form of the MAb (whole or fragment) for all diagnostic applications. The goal is to choose the most appropriate form of the MAb and the radionuclide for the disease state being evaluated.

PREPARATION OF RADIOLABELED MAbS

Radiolabeling of a MAb with the appropriate radionuclide for imaging must produce an in vitro and in vivo stable bond with the MAb at a site on the protein structure that does not interfere with antigen binding. Biotechnology companies, recognizing the specific abilities and expertise of the end-users of their products, are developing final products that are adaptable to a reagent kit method. This type of reagent kit is necessary in order to provide a short half-life, finished product at the time of patient use. The MAb (and other raw materials) should be developed and manufactured into a reagent kit that will simplify the radiolabeling process. Specifically, these kits should require little manipulation during preparation and should involve activities the nuclear pharmacist (in the clinical setting) conducts routinely.

RADIOIMMUNODETECTION OF COLORECTAL CARCINOMAS WITH INDIUM-111 LABELED MAbS

A number of biotechnology companies have been conducting several clinical investigations in the U.S. to assess the efficacy of radioimmunodetection and radioimmunotherapy. Patients have been studied with several different MAbS radiolabeled with several different radionuclides. The safety and efficacy of these preparations have proven acceptable.

Two of these MAbS have been radiolabeled with In-111 and investigated for their ability to detect colorectal carcinoma. They include the MAb B72.3, which recognizes and binds a tumor-associated glycoprotein antigen called TAG-72 (19,20), and MAb ZCE-025 which is an anti-carcinoembryonic antigen antibody (21,22). These antigens are expressed by the majority of colon and rectal adenocarcinomas, which contributed to the success of these agents in detecting tumors. Similar sensitivities were found in the multicenter clinical trials conducted with these two different MAbS. Table 1, which lists the performance statistics compiled from the literature, indicates a slightly lower specificity for the anti-CEA MAb, probably due to the ability of this MAb to react with 20-30% of normal colon mucosa (7,9,23,24). Adverse events were noted in only 4% of patients for both products. None of these were serious or life threatening and most resolved with no treatment. This low incidence of adverse events confirmed the safety of single administration of radiolabeled murine MAbS in humans.

Table 1. Radioimmunoscintigraphy statistics of two In-111 labeled MAbS used for detection of colorectal carcinoma

<table>
<thead>
<tr>
<th>MAb B72.3</th>
<th>MAb ZCE-025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>70%</td>
</tr>
<tr>
<td>Specificity</td>
<td>90%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>97%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>72%</td>
</tr>
</tbody>
</table>

In-111 ZCE-025 MAb

MAb ZCE-025 is an IgG1, murine monoclonal antibody produced against CEA. Hybritech Inc. has produced a monoclonal anti-CEA ZCE-025 antibody kit (Hybri-CEAker®) that includes four components used in the radioimmunopharmaceutical preparation. The kit reagents include: indium In-111 citric acid solution, neutralizing buffer, unmodified MAb ZCE-025 and MAb ZCE-025 conjugate. The MAb conjugate contains a chelating agent tightly bound to the protein for radiolabeling with the In-111 radiometal.

RESULTS FROM CLINICAL TRIALS WITH In-111 ZCE-025 MAb

This radiolabeled antibody conjugate has been used to conduct a number of clinical investigations which led to the company filing for product approval (21,22). The rate of adverse events involving the imaging agent was reported to be 3.7% after single administration. The majority of these reactions were considered to be mild allergic-type reactions which resolved with no drug intervention. Severe adverse events involving hypotension and respiratory distress (readily controlled with drug therapy) occurred only rarely (0.8% of all patients). There were no deaths reported in over 1000 patient administrations.

When evaluating the drug for its ability to detect surgically confirmed colorectal carcinoma, the clinical studies showed In-111 ZCE-025 more effective than conventional CT at detecting tumor. The evaluation
of the data on a per lesion basis in patients with both
primary and recurrent disease yielded a detection rate
of 90% with 82% accuracy and 90% positive predict-
ive value. The drug also detected 64% of metastatic
lesions in the abdomen compared with 37% for CT.
The combination of both radioimmunodiagnosis and
CT was significantly better for the localization of
abdominal and liver lesions than when CT was used
alone (24).

On a per patient basis, radioimmunodiagnosis with
In-111 ZCE-025 had an overall detection rate of 72%
compared with 58% for CT alone. The combined use
of the two modalities increased this sensitivity to 86%
(24).

Currently, this drug is awaiting U.S.F.D.A. ap-
proval with over ten clinical protocols completed on
the safety and efficacy of the product. Figure 1
includes images of the abdomen and pelvis in a col-
rectal cancer patient five days post infusion of In-111
ZCE-025. A lesion in the right lobe of the liver and
one in the cecum are easily seen at the arrow points.

SATUMOMAB PENDETIDE

Satumomab pendetide (OncoScint® CR/OV
-CYTGEN Corp.) is a diagnostic imaging agent for
determining the extent and location of extrahepatic
malignant disease in patients with known colorectal or
ovarian adenocarcinoma. Satumomab pendetide is an
immunoconjugate produced from the murine mono-
clonal antibody B72.3 that binds to a tumor-associated
glycoprotein antigen (TAG-72) which is expressed by
a variety of adenocarcinomas. Because the TAG-72 is
a pancarcinoma antigen found on many different tumor
types, satumomab pendetide is reactive with 94% of
colorectal adenocarcinomas, all of the ovarian adeno-
carcinomas for which it was tested, as well as a
variety of other adenocarcinomas. The drug is limited
to single use only due to the chance for an allergic-
type of reaction upon repeat infusion in those patients
with circulating levels of human anti-mouse anti-
bodies, or HAMA. It is not indicated as a screening
test for ovarian or colorectal cancer. Satumomab
pendetide is supplied as a kit for radiolabeling with
indium-111 (In-111). The radiolabeled product
is referred to as OncoScint®CR/OV-In.

The radiolabeled In-111 satumomab pendetide is
administered as an intravenous infusion over five
minutes and should not be combined with other
medication during administration. The recommended
adult dosage is 1 mg of the antibody conjugate
radiolabeled with 5 mCi of In-111. Whole body
images and dosimetry studies suggest that the primary
organ of metabolism is the liver. However, the
principal route of elimination is through the urine with
13% of the administered radioactivity being excreted
within 72 hours. The radiolabeled drug displays both
a monoexponential and a biexponential pattern of
elimination with a biological clearance of
approximately three days. Optimum imaging time is
between 48 and 72 hours after infusion. However,
imaging has been completed as early as 24 hours and
as late as 120 hours after administration.

In-111 SATUMOMAB PENDETIDE
FORMULATION

The non-radioactive, "cold" kit contains the intact,
whole, murine IgG monoclonal antibody, B72.3, that
has been formulated with a linker-chelate for
radiolabeling with radioactive metals. OncoScint®CR/OV
is prepared by the site-specific conjugation of the linker-chelator glycyl-tyrosyl-
(N-E-diethylenetriaminepentaacetic acid)-lysine
(GYK-DTPA) to the oxidized carbohydrate component
of MAb B72.3.

CYTOGEN Corporation's unique, patented
radiolabeling of the antibody molecule distant from
the antigen binding site preserves the immunoreactivity
of the monoclonal antibody in the radiolabeled
OncoScint®CR/OV-In form. This method provides a
more controlled, site-specific, covalent attachment of
many chelate molecules on the Fc portion of the MAb
structure. This technique increases the number of
radioactive atoms that can be bound to the protein
(specific activity) while preserving the
immunoreactivity of the MAb by keeping the
chelate-radiometal combination at a distance from the
antigen binding site of the MAb. When compared to
the random placement of the chelate-radiometal
complex, site-specific radiolabeling through the use of
a linker-chelate structure allows the stable attachment
of the chelate-radiometal far removed from the antigen
binding end of the MAb. In addition, the ability to
attach the chelate to the MAb molecule and radiolabel
at some later time enables the radiopharmaceutical kit
technology, common with technetium-99m agents, to
be used with an indium-111 labeled MAb.

Prior to use, OncoScint®CR/OV is radiolabeled by
the addition of a sterile, nonpyrogenic solution of
buffered indium In-111 chloride. Indium-111 is the
required radionuclide for radiolabeling
OncoScint®CR/OV. The physical half-life of 67 hours
is optimal since it closely matches the biologic half-life
of the radiolabeled antibody product and enables
nuclear medicine images to be obtained from two to
five days after administration.

The indium In-111 chloride is supplied by the user
and currently is only available from one supplier,
Amersham-MPI. It is buffered with the sodium
acetate buffer solution supplied in the
OncoScint®CR/OV kit.
Figure 1. Images of pelvic (top four) and abdomen (lower four) in a colorectal cancer patient 5 days post injection.
The use of other In-111 based radiopharmaceuticals, such as indium In-111 oxine, is strictly prohibited. To insure successful radiolabeling, buffered indium In-111 chloride with a radioactive concentration of approximately 5 mCi per 0.5 ml at the time of calibration is used. Radiolabeling generally results in greater than 98% radiochemical purity and the radiolabeled product is stable in vitro and in vivo (25).

OncoScint®CR/OV kits have a 24 month expiration time and should be stored upright at temperatures between 2°C and 8°C but not frozen. After radiolabeling (Table 2), In-111 satumomab pendetide may be stored at room temperature and should be used within eight hours because it contains no preservative.

In-111 SATUMOMAB PENDETIDE CLINICAL USE

In-111 Satumomab Pendetide is a diagnostic imaging agent indicated as an adjunct for determining the location and extent of extrahepatic malignant disease in patients with known colorectal or ovarian cancer. Clinical studies suggest this radioimmunopharmaceutical should be used after completion of standard diagnostic tests when additional information regarding disease extent could aid in patient management. This drug should not be used in patients who are hypersensitive to this or any other product of murine origin or to indium In-111 chloride. In addition, since insufficient safety and efficacy data regarding repeat administration of this product exists, the drug is currently limited to single use only.

Allergic reactions, including anaphylaxis, can occur in patients who receive murine antibodies. Although serious reactions of this type have not been observed in clinical trials after In-111 satumomab pendetide administration, medications for the treatment of hypersensitivity reactions should be available during administration of this agent. Mild adverse events were reported in 4% of the patients who received a single infusion of the drug during clinical trials; no deaths were reported. The most common adverse event was fever which occurred in less than 2% of the patients.

Clinical trial results indicate that optimal diagnostic images are obtained between 48 and 72 hours after infusion. Patient variability has been reported and interpretable images have been obtained as early as 24 hours and as late as 120 hours. Normal biodistribution patterns show radioactivity in the liver, spleen and bone marrow of most patients, with localization in bowel, blood pool, kidneys, urinary bladder, male genitalia and breast nipples in women also reported.

Table 2. Radiolabeling of OncoScint®CR/OV (satumomab pendetide) kit.

| The kit contains: |
| One 6 ml vial containing 1 mg MAb conjugate in 2 ml phosphate-buffered saline. |
| One 2 ml vial of 0.5 M sodium acetate solution, pH 6.0. |
| One 0.22 μm Millex® GV low protein binding filter. |
| One package insert and two identification labels. |

| Your facility will need to supply: |
| One 5 mCi/0.5 ml vial of indium In-111 chloride (INDICLOR™ -Amersham/MPI) |
| Three sterile 18G to 20G needles. |
| One sterile 1 ml tuberculin syringe. |
| One sterile 3 ml syringe. |
| One sterile 10 ml syringe. |
| Vial shield. |

1. Remove the OncoScint®CR/OV kit from the refrigerator approximately 30 minutes before radiolabeling in order to bring the contents of the vials to room temperature. OncoScint®CR/OV is a protein solution that may develop particulates which will be removed by filtration later in the preparation.

2. After cleaning the rubber stopper of each vial with an alcohol wipe, use the 1 ml tuberculin syringe to add 0.5 ml of sodium acetate solution to the contents of the indium In-111 chloride vial. Mix well.

3. Using the 3 ml syringe, withdraw 5-6 mCi of the buffered In-111. After assaying the contents of the syringe in a dose calibrator to insure adequate radioactivity (between 5 mCi and 6 mCi), add the buffered In-111 to the vial containing the MAb conjugate. Swirl gently to mix (DO NOT SHAKE). On one of the labels provided, record the date, time of preparation and radioactivity in the vial. Affix to lead container.

4. Allow the mixture to react at room temperature for 30 minutes.

5. Aseptically attach the 0.22 μm Millex® GV low protein binding filter and a sterile 18G to 20G needle to the sterile 10 ml disposable syringe. Withdraw the OncoScint®CR/OV-In (approximately 3 ml) through the filter into the syringe. Be careful to keep the needle immersed in the solution to avoid air-locking the filter. Do not allow the solution to move back across the filter into the vial.

6. Discard the filter and needle into a radioactive waste container. Aseptically attach a new, sterile 18G to 20G needle to the 10 ml syringe. Transfer approximately 0.1 ml of the preparation to a plastic or glass tube for quality assessment.

7. Place the 10 ml syringe containing the OncoScint®CR/OV-In finished product into a dose calibrator to assay the dosage. The final product for injection should contain not less than 4 mCi.

8. On the second label provided, record the date, time of assay and radioactivity in the syringe. Affix the label to the syringe shield.

9. Discard all used materials in accordance with local, state and federal regulations governing radioactive and biohazardous waste.

Because of the presence of radioactivity in the stool of the large bowel, the administration of a cathartic...
prior to obtaining initial or follow-up images may prove useful. Table 3 provides instructions on the instant thin-layer chromatography (ITLC) procedure used to determine the radiochemical purity of OncoScint®CR/OV-In. This is an optional procedure that may be completed prior to administration of the drug.

Table 3. Quality Assessment of OncoScint®CR/OV-In

The following materials are required:
- ITLC-SG paper, cut into 1 cm x 8 cm strips
- 0.9% sodium chloride solution as the developing solvent
- 0.05 M solution of N-E-dicthylenetriaminepentaaetic acid (DTPA) in water
- Developing chamber for chromatography
- Gamma well counter

1. Mix equal amounts of OncoScint®CR/OV-In and 0.05 M DTPA solution and allow to react at room temperature for 1 minute.

2. Mark the origin of an ITLC-SG strip 1 cm from the bottom. Spot a small drop of the DTPA/OncoScint®CR/OV-In mixture onto the ITLC-SG strip at the origin.

3. Add 0.9% sodium chloride solution to the developing chamber to a depth of approximately 0.5 cm to be certain the origin will not be submerged under the sodium chloride solution.

4. Place the ITLC-SG strip into the developing chamber with the origin at the bottom. Be careful not to bend the strip or allow the strip to adhere to the side of the chamber.

5. Allow the solvent front to migrate approximately 6 cm from the origin of the strip.

6. Using forceps, carefully remove the strip from the developing chamber. Cut the strip in half and place each piece in a counting tube with top and bottom ends of the strip facing downwards in the tube.

7. Measure the radioactivity of both halves of the strip in counts per minute using a gamma well counter.

8. Calculate the percent radiochemical purity by dividing the net counts per minute on the bottom strip (origin) by the net counts on the entire strip and multiplying the result by 100.

   The radiochemical purity should be ≥ 95%. If it falls below 95%, repeat the ITLC procedure twice more. If the results of each repeat procedure are ≥ 95%, the product is acceptable for clinical use. If either of the two repeat results is < 95%, the material should not be used. Contact your technical service representative for further information.

COLORECTAL CANCER PATIENT STUDIED WITH ONCOSCINT®CR/OV-In

Figure 2 shows the radioimmunodiagnostic images of a primary colorectal carcinoma. This anterior pelvis image taken 6 days post infusion shows three areas of disease including the cecum, ascending colon and periaortic lymph node metastasis near the midline.

RESULTS FROM CLINICAL TRIALS WITH ONCOSCINT®CR/OV-In

OncoScint®CR/OV-In is the first MAb-based imaging agent to receive marketing approval in Europe and the United States after extensive clinical testing over the past five years. The drug provided a 74% sensitivity for tumor localization in the pelvic region of colorectal cancer patients. When used in combination with CT imaging, the two diagnostic modalities were found to be complimentary in the detection of colorectal cancer with the OncoScint®CR/OV-In imaging detecting 69% of proven positives, CT detecting 68% and the two modalities combined providing a sensitivity of 88% (25,26). A review of sensitivity by anatomic site indicated radioimmunodetection fared much better than CT in the abdomen and pelvis while CT was twice as good in the localization of lesions in the liver.

The largest U.S. clinical trial of ovarian carcinoma imaging was completed with OncoScint®CR/OV-In. Over 100 ovarian cancer patients were entered in this multicenter trial which compared radioimmunoscintigraphy with CT in order to determine their usefulness in identifying sites of primary and recurrent tumors (27). CT provided an overall
sensitivity of 44% compared to 66% for OncoScint®CR/OV-In imaging with patient management positively affected by radioimmunoscintigraphy in 27% of the patients.

Overall, OncoScint®CR/OV-In provided similar sensitivity and specificity results in ovarian cancer trials (compared to CT) with an even greater degree of detection of occult disease. This latter finding is very important when staging patients and determining a therapeutic approach. Surgeons believe radioimmunodetection has a very important role in the reduction of exploratory laparotomies in those patients who would not benefit.

RADIOIMMUNODETECTION OF COLORECTAL CARCINOMAS WITH TECHNETIUM-99m LABELED MAbs

Carcinoembryonic antigen (CEA), a tumor associated antigen (TAA), was first discovered by Gold and Freedman (28). The molecule bearing this antigenic activity has been described as being a glycoprotein with a molecular mass of approximately 180,000 to 200,000 daltons (29). CEA was initially thought to be a fetal antigen because it was present in high levels in the fetal gastrointestinal tract and not detected in normal colon. With the development of more sensitive assays for CEA, findings indicate that CEA is present in many normal body secretions (30,31). Nevertheless, this particular TAA remains the primary marker for colorectal carcinoma, both as an in vitro serum assay for assessment of disease activity and as a target for the radioimmunodetection of the disease (32). For example, CEA can be identified in the serum of over 95% of patients with disseminated colon carcinoma and approximately 20% of patients with localized disease (33).

Using various immunoassay methods, the CEA content of normal colon may be on the order of 1 μg/g. This value can increase 10-20 fold (and yield mean CEA levels of 5-10 μg/g) as a carcinoma originating from the epithelial cells lining the colon grows through the basement membrane and releases CEA into the extracellular fluid, eventually reaching the blood (34). Serum CEA levels appear to be elevated in proportion to the mass of tumor present. Generally speaking, serum CEA levels that are below 2.5 ng/ml are considered to be normal (35). In patients with resectable primary colorectal cancer, CEA levels of 50 ng/ml can be detected, whereas in patients with metastatic disease, CEA levels of several hundred ng/ml (or infrequently, in low μg/ml range) may occur (35).

With respect to CEA measurement, many questions remain as to whether or not such low levels of the antigen are primarily the result of absorption of minute amounts of secretory CEA from developing carcinoma, or if CEA is produced internally from other sources. Studies conducted by Hansen et al. showed that an increase in normal CEA serum levels could be observed in patients who are cigarette smokers, whereas only minimal increases were observed in patients with acute, nonmalignant chronic gastrointestinal epithelium inflammatory conditions (e.g., pancreatitis, ulcerative colitis, cirrhosis, ulcers, etc.) (35). Such observations appear to support the opinion that the source of serum CEA in healthy individuals is due to absorption of secretory CEA.

TECHNETIUM-99m IMMU-4 MAb

Recently, Immunomedics, Inc (Morris Plains, New Jersey) has been successful in developing an "instant" labeling kit for Tc-99m labeling of the Fab' fragment of the anti-CEA monoclonal antibody, IMMU-4 (otherwise known as NP-4, and ImmuRAID™-CEA) (36).

Reports of recognition and characterization of NP-4, can be found in the literature (36-38). NP-4 is an IgG1 with a kappa light chain and an isoelectric point of 5.7. F(ab')2 is produced from pure IgG by pepsin cleavage, with Fab' being produced by the reduction of F(ab')2.

To date, NP-4 appears to be one of the most specific anti-CEA monoclonal antibodies that has been characterized. It is reactive with only 200,000 dalton CEA, and two variants have been described in the literature, one of which is of 200,000 daltons (29) and the other of a lower molecular weight, 180,000 daltons (39). This lower molecular weight CEA variant has been termed meconium antigen (MA), normal cross reactive antigen II, normal fecal antigen, and CEA low (38). NP-4 is specific for the 200,000 dalton CEA, and is not reactive with non-specific cross-reacting antigen (NCA) or meconium antigen. It complexes to a very limited degree with circulating CEA (37,38). This is an important observation in the use of NP-4 as a potential imaging agent knowing that there can be evidence of elevated circulating CEA levels in some patients with primary or recurrent cancer. In considering fragments compared to the intact IgG, fragments show very minimal signs of complexing with circulating CEA, and do not cross-react with non-specific proteins (37,38).

Tc-99m IMMU-4 KIT FORMULATION

Many of the reagent kits used in nuclear medicine for the preparation of technetium Tc-99m labeled
products utilize tin(II) to achieve the reduction of Tc-99m. Of these, the kit that is most similar to the "instant" kit developed by Immunomedics for the preparation of ImmuRAID™-CEA (Tc-99m) is the kit used to produce Tc-99m Human Albumin Injection described in the European Pharmacopeia Monograph 640 (1989). IMMU-4, the instant kit used in the preparation of Tc-99m labeled IMMU-4, is supplied as a sterile, non-pyrogenic, lyophilized powder in 3 ml vials with an inert atmosphere, with each vial containing approximately 1.25 mg of IMMU-4 anti-CEA monoclonal antibody Fab' fragment. Additionally, each vial contains various other ingredients such as stannous chloride, sodium potassium tartrate, sodium chloride, sodium acetate, sucrose, acetic acid, and hydrochloric acid.

To prepare this product, reconstitution and labeling is achieved by adding 25-30 mCi of Tc-99m sodium pertechnetate in 2 ml sterile, preservative-free normal saline to a properly shielded vial of IMMU-4, and gently swirl and shake for 30-45 seconds to effect dissolution of the vial contents. Approximately five minutes is needed for incubation at room temperature. One milliliter of sterile saline for injection can then be added to the vial to facilitate removal of the labeled product, Tc-99m labeled IMMU-4 Fab' fragment in a solution ready for intravenous injection. A dose of approximately 1 mg of the antibody fragment is administered to patients, with the reconstituted product showing good stability for at least two hours post preparation.

Upon completion of the radiolabeling procedures, the final product should be assayed for free pertechnetate. Instant thin-layer chromatography using ITLC-SG strips (1 cm x 9 cm) is suitable for this purpose. A solution for radiochemical purity testing is prepared by placing 10 µl of the radiolabeled product into an empty vial and adding normal saline up to 1.5 ml. Approximately 10 µl is then applied to the ITCL-SG strip. Spotting of the test sample on the chromatography paper should be at an origin that measures 1 cm from the bottom of the ITLC-SG strip, with the test strip being placed into a developing chamber containing acetone following two minutes of air drying. The solvent should be allowed to migrate to the top of the test strip, with cutting of the strip taking place at a mark 4 cm from the top. The percent activity which is present as free Tc-99m pertechnetate can be determined as follows:

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\text{Free Tc-99m pertechnetate (\%) = \frac{\text{net cpm in upper 4 cm segment}}{\text{net cpm on entire QC strip (9 cm)}} \times 100}
\]

Tc-99m IMMU-4 CLINICAL USE

Clinical trials using this product are continuing, with data analyzed this far indicating a sensitivity of about 80% and a specificity of 95% for detection of lesions in the liver. One hour after infusion, the blood level of this imaging agent in patients was about 62% of baseline. At five hours post injection, this value was reduced to 22% of baseline and at 24 hours, 7% of baseline remained in the blood. The distribution half-life has been determined to be 0.91 ± 0.47 hours. The elimination half-life is 13.08 ± 3.55 hours, with 27% of the radioactivity excreted via the urine in the first 24 hours after infusion. Human dosimetry studies indicate that radiation doses to all organs are within acceptable limits. Adverse events have been very few to date (e.g., single cases of bursitis in an elbow, nausea, eosinophilia, as well as one case of seizure eight hours post-injection which was not believed to be due to the administration of the antibody) (40). As with any administered murine-derived monoclonal antibody, the potential for allergic reaction can be manifested as bronchospasm or hypotension, and less severe symptoms such as itching, erythema, and hives. In considering Tc-99m IMMU-4, other potential side effects that could be encountered following administration to patients are chills, fever, and malaise. Though none of these outcomes have been reported in patients to date receiving this agent, it is advisable to have an emergency cart (specifically, items such as epinephrine, antihistamine, corticosteroid, and pressor agents) available. The incidence of human anti-mouse antibody responses has also been reported to be less than 1.0% (40).

SUMMARY

A number of biotechnology companies have been conducting clinical investigations in the U.S. to assess the efficacy of radioimmunodetection and radioimmunotherapy. Radioimmunopharmaceuticals using indium-111 and technetium-99m radionuclides as the signal generator are undergoing clinical trials which so far have led to the approval of an antibody-based imaging agent, OncoScint®CR/OV-In. Other products being considered by the FDA for approval include those for imaging various cancers and other non-malignant disease states. In addition, targeted radioimmunotherapy is under clinical investigation using iodine-131, rhenium-186 and yttrium-90 labeled MAb. The safety and efficacy of these preparations have proven acceptable; however, final approval of a radioimmunotherapeutic pharmaceutical by the FDA is still pending.
REFERENCES


**QUESTIONS**

1. Which of the following radiopharmaceuticals has not been used for the localization of various cancers?

   A. Ga-67 citrate
   B. I-131 iodomethylcholesterol
   C. Tc-99m MDP
   D. Rb-82 chloride

2. Radioimmunodetection of cancer is indicated for which of the following reasons?

   A. detection of occult disease in those patients with high clinical suspicion
   B. the staging of the extent and degree of disease pre- and post-therapy and the assessment of tissue viability
   C. as an adjunct to other diagnostic tests that are indeterminate
   D. all of the above
Radioimmunotherapy of solid tumors is progressing and current investigative efforts focus attention on all of the following tumors except:

A. brain  
B. prostate  
C. melanoma  
D. ovarian  

For the radioimmunodetection of cancer, radionuclides with gamma photon emissions in the range of _____ keV are ideal:

A. 0 to 100  
B. 100 to 200  
C. 200 to 300  
D. 300 to 400  

Which of the following radionuclides has not been widely investigated for the radioimmunodetection of cancer?

A. In-111  
B. I-123  
C. In-113m  
D. Tc-99m  

Which of the following is the tumor-associated glycoprotein recognized by the MAb B72.3?

A. CEA  
B. NP-4  
C. SCLC  
D. TAG-72  

The monoclonal antibody ZCE-025 recognizes the antigen known as:

A. CEA  
B. NP-4  
C. SCLC  
D. TAG-72  

ZCE-025 is a monoclonal antibody of which isotype?

A. IgG1  
B. IgG2  
C. IgG3  
D. IgG4  

MAb B72.3 has a sensitivity and specificity of:

A. 65% and 80%, respectively  
B. 72% and 85%, respectively  
C. 70% and 90%, respectively  
D. 85% and 90%, respectively  

The positive predictive value for MAb B72.3 is:

A. 72%  
B. 85%  
C. 90%  
D. 97%  

Satumomab pendetide is an immunoconjugate produced from a __________ derived monoclonal antibody:

A. bovine  
B. equine  
C. murine  
D. porcine  

The recommended adult dosage of the antibody conjugate B72.3 is:

A. 0.1 mg  
B. 1.0 mg  
C. 5.0 mg  
D. 20.0 mg  

Which of the following forms of In-111 is required to radiolabel satumomab pendetide?

A. In-111 bleomycin  
B. In-111 chloride  
C. In-111 oxine  
D. In-111 pentetate (DTPA)  

Following radiolabeling procedures, OncoScint®CR/OV-In should be used within ______ hours because it contains no preservative.

A. 2  
B. 5  
C. 6  
D. 8
15. The optimal imaging time following patient administration of In-111 satumomab pendetide is usually at:

A. 6-12 hours  
B. 12-24 hours  
C. 24-48 hours  
D. 48-72 hours

16. Which of the following organs is not part of the normal biodistribution of In-111 satumomab pendetide?

A. bone marrow  
B. liver  
C. lungs  
D. spleen

17. Which of the following is not an advantage of using the "linker-chelate" technique of preparing antibodies for eventual radiolabeling?

A. helps to preserve the immuno-reactivity of the antibody  
B. provides a means by which a large number of radioactive atoms can be bound to the Fc portion of the antibody, thereby increasing the specific activity of product when radiolabeling occurs  
C. facilitates the formulation of a reagent kit to which a radiolabel may be added  
D. increases the shelf life of the product

18. When Hybri-CEAker® is used in combination with computed tomography for the detection of cancer, the overall sensitivity for the two modalities has been reported to be:

A. 75%  
B. 82%  
C. 86%  
D. 92%

19. When determined by various immunoassay methods, the CEA content of normal colon is approximately:

A. 0.1 μg/g  
B. 1.0 μg/g  
C. 2.5 μg/g  
D. 5.0 μg/g

20. Serum CEA levels that are above ______ ng/ml are usually considered to be abnormal.

A. 1.0  
B. 2.5  
C. 5.0  
D. 10.0

21. IMMU-4 has been formulated into a reagent kit for labeling with Tc-99m by which of the following companies?

A. RhoMed  
B. Immunomedics  
C. Hybritech  
D. Cytogen

22. IMMU-4 shows reactivity with which antigen?

A. AFP  
B. CEA  
C. PLAP  
D. SCLC

23. Following radiolabeling procedures, ImmuRAID™ should be used within ______ hours:

A. 2  
B. 5  
C. 6  
D. 8

24. When performing radiochemical purity chromatographic analysis of the final In-111 satumomab pendetide product, the developing solvent used is:

A. acetone  
B. n-butanol (30%)  
C. methyl ethyl ketone (50%)  
D. sodium chloride (0.9%)

25. When performing radiochemical purity chromatographic analysis of the final radiolabeled IMMU-4 product, the developing solvent used is:

A. acetone  
B. n-butanol (30%)  
C. methyl ethyl ketone (50%)  
D. sodium chloride (0.9%)