Radioimmunodetection in Cancer Identification

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Radiolabeled antibodies are a new class of imaging agents for the detection of sites of disease. The procedure of external imaging to disclose foci of increased radioactivity after injection of radioactive antibodies has been termed radioimmunodetection (RAID) (*1*), or radioimmunoscintigraphy (RIS). Although RAID was first developed to identify malignant tissue (*1*), other applications have resulted, such as for imaging myocardial infarction (*2*), thrombi (*3*), inflammation (*4*), and atherosclerotic plaques (*5*). The same principle of targeting radionuclides to cancers by specific antibodies is being investigated as radioimmunotherapy (*6,7*).

The procedure of RAID not only involves the disciplines of nuclear medicine and immunology, but also such areas as image processing, enhancement, and instrumentation; radiochemistry; immunochemistry; antibody development, purification, and reengineering; antibody pharmacology and pharmacokinetics; and host-tumor interactions (*8,9*). The requirements for successful RAID involve an understanding of the nature, location, and distribution of the antigen to be targeted, the class, character, and form of the antibody developed, the properties of the radiolabel and the nature and effects of the labeling method, the administration and metabolic processing of the antibody and its label, and the imaging system used to disclose both the targeted and nontargeted radioactivity. The sensitivity, specificity, and resolution of RAID are the ultimate considerations, which must translate clinically to this modality contributing new or important confirmatory information to other available cancer detection methods.

TUMOR TARGET

The ideal tumor antigen has not been identified, since this should be truly tumor-specific, uniformly distributed among all tumor cells in a high density, and accessible to the antibody. Preferably, it should not be released into the circulation or lymphatics, where it could complex with the injected antibody. However, the tumor antigens used as targets in RAID have been markers which are quantitatively increased with malignancy, and often circulating in the blood. The first group included the oncofetal antigens, such as carcinoembryonic antigen (CEA) (*10*) in gastrointestinal and diverse carcinomas (*1,11,12*) and alpha-fetoprotein (AFP) in germ cell and hepatocellular carcinomas (*13,14*), followed by human chorionic gonadotropin in germ cell and trophoblastic tumors (*15,16*), prostatic acid phosphatase in prostatic carcinoma (*17*), and alkaline phosphatase in seminoma (*18*). Circulating antigen complexed with the injected antibody in some of these systems, but the reduced avidity for the circulating antigen, or the abundance of antigen at the tumor as compared to that in the blood, favored antibody targeting and RAID (*11,19*). The advent of monoclonal antibodies (Mabs) permitted the identification of target epitopes of certain tumor-associated antigens (*20*), such as in melanoma (*21*), in the human milk fat globule (HMFG) antigen (*22*), also described as epithelial membrane antigen (EMA) (*23*) found in the lining of mammary duct cells and in breast milk (*24*), in CEA (*25,26*), and growth factor receptors (*27*). Antibodies against hormones of endocrine tumors have also been used in RAID (*28,29*). The list of Mabs targeting cancer-associated antigens is long and expanding (Table 1), and it is apparent that: (1) truly cancer-distinct antigens are not required for RAID, (2) many are pan-carcinoma antibodies, and can therefore be used to image many different tumor types, (3) a particular antibody-antigen system can be used in many different patients with the same tumor type, indicating that an individual or "private" specificity is not required, and (4) shed-tumor antigens do not neutralize the injected antibody, and thus do not preclude successful imaging, even when very high titers of circulating antigen are present (*8,9,12*). Targeting has been achieved even when only 15% of the cells express the target antigen (*30*). Thus, antigens of different cellular location, density, and molecular composition and size have all been found to be suitable targets for RAID, indicating the general applicability of this approach. For example, that a single antibody against CEA can target tumors in over 80%-90% of colorectal cancer patients and sites, as well as a number of other tumor types (*8,12*), indicates that despite antigen and cell heterogeneity within

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a tumor, sufficient binding occurs for deposition of the required radioactivity for RAID. This is remarkable in view of all the potential obstacles confronting the deposition of antibody in tumor, as listed in Table 2 and reviewed elsewhere (*8,9*).

ANTIBODY

The first studies of RAID with defined antibodies involved polyclonal IgG antibodies against CEA, which were affinity-purified (*8,9,11*), but could also succeed if not affinity-purified (*31*). As indicated in Table 1, antibodies against a diverse array of tumor antigens have been used successfully for targeting tumors in humans. It is generally believed that antibodies of high affinity and avidity are desirable for tumor targeting (*32*), but this may differ for different antigen targets. For example, an antibody of high affinity may bind more readily to a shed-tumor antigen than one of lower affinity. Indeed, this has been found with a CEA Mab antibody, which at lower affinity does not show a high rate of complexing to circulating CEA, without compromising tumor targeting (*33*). Lower-affinity antibodies may show better tumor penetration because of reduced binding to membrane or extracellular antigen, which would appear to play a more important role for therapy than for imaging (*34*). However, the minimum affinity to achieve targeting is not known (*35*).

TABLE 1
Selected Cancers Targeted Clinically with Radiolabeled Antibodies

Cancer Type	Abs	Cancer Type	Abs
Colorectal		Melanoma	
	CEA		p97
	17-1A		96.5
	19-9		9.2.27
	791T/36		225.28S
	CSAp (Mu-9)		ZME018
	B72.3	Breast	
	PR1A3		CEA
Ovarian			B72.3
	CEA		HMFG/MA5
	HMFG-2		3E1.2
	791T/36		3G6F9
	SM3		RCC-1
	OC-125	Lung	
	OV-TL3		CEA
	B72.3		NR-LU-10
	MOV18		Po66
	PLAP	Liver	
Prostate			AFP
	PAP		Ferritin
	PSA	Trophoblast and Germ Cell	
Lymphomas			AFP
	Ferritin		HCG
	Lym-1	Neuroblastoma	
	LL2 (EPB-2)		3F8
	T-101		BW595/9
	MB-1		

TABLE 2
Factors Affecting Cancer Radioimmunodetection

1. Character of the antibody
   a. Specificity/epitope
   b. Purity
   c. Affinity
   d. Whole or fragment
   e. Isotype
   f. Dose
   g. Species
   h. Clearance and pharmacokinetics
2. Nature of radiolabel
   a. Physical properties; half-life
   b. Chemical properties; conjugate stability
   c. Imaging properties
   d. Specific activity
   e. Dose
   f. Effect on Ab immunoreactivity
   g. Clearance; excretion
3. Tumor target
   a. Size and location of tumor(s)
   b. Location and distribution of antigen in tumor
   c. Antigen/epitope density
   d. Antigen modulation
   e. Target/nontarget ratio
   f. Vascularization and vascular permeability
   g. Distribution of target antigen in other body sites or fluids
4. Imaging system and method of interpretation
   a. Planar
   b. Emission tomography (SPECT)
   c. Computer-assisted subtraction or other manipulations
   d. Time of reading after administration
5. Other factors
   a. Route of administration
   b. Presence or absence of anti-Mab in host
   c. Presence or absence of circulating target antigen and complexes

The form of antibody that appears to be ideal for imaging is related to the highest tumor-to-background ratio achieved at the earliest time after antibody injection. When radioactive intact IgG antibodies are injected, blood-pool radioactivity is high during the first 48–72 hr, which accounts for the low tumor-to-background ratios usually experienced. Various methods have been devised, including dual-isotope subtraction (*11,36*), anti-antibody clearance (*37*), use of antibody fragments (*38–40*), and two- and three-step post-labeling techniques (*41,42*), to overcome this problem. The smaller the antibody molecule, the more rapid its tissue and blood clearance, especially where no binding to circulating antigen occurs. It has been found that monovalent or bivalent fragments will show the most rapid achievement of high tumor-to-background ratios, thus enabling early tumor imaging despite the reduced tumor retention compared with whole IgG (*39,40,43,44*). This property permits the use of short-lived isotopes, such as ^{123}I (13 hr) and ^{99m}Tc (6 hr), with bivalent or monovalent fragments (*44–49*). Recently, even smaller antibody units, single-chain peptides (25 kD), have shown promising targeting results in animal models, without the kidney radioactivity seen for the larger fragments (>50

kD) that undergo renal clearance (*50*). Figure 1 presents a schematic of the different forms of antibodies which have shown successful tumor targeting. It also shows how the antibody can be re-engineered to replace murine components with their human counterparts (*51,52*), thus reducing antibody immunogenicity, which is responsible for the development of human anti-mouse antibodies (HAMA) in about 50% of patients receiving a single injection of intact IgG (*53*). The smaller the amount of murine protein injected, such as with "humanized" antibodies or with monovalent antibody fragments, the lower the frequency of HAMA invoked in patients (*53–55*).

When purified polyclonal anti-CEA antibodies initially were used to image cancers, doses of 0.25 mg labeled with ^{131}I were sufficient (*11,12*). With certain Mabs, such as the NP-4 (Immu-4) anti-CEA IgG-F(ab')$_2$ or Fab' (*47,48,55*), or B72.3 (*56*), one to a few milligrams of radiolabeled antibody can image tumors successfully. However, the amount injected can have a profound effect on the distribution of radioactivity, especially for antibodies that cross-react with well-perfused normal organs. In such cases, it has been observed that more favorable tumor uptake in comparison to normal tissues is achieved when the anti-cancer antibody is injected in the tens of milligrams, such as in melanoma (*57*). Antibodies which target certain biologically-active molecules that are widely expressed on normal tissues, such as the epidermal growth factor receptor (EGFR), must be used at very high mass amounts of antibody. For example, in a recent study of ^{111}In and EGFR antibody, high sensitivity for lung cancer detection was observed only at doses above 40 mg of intact antibody (*58*). Thus, for certain antibody systems, the amount of Mab used may be important.

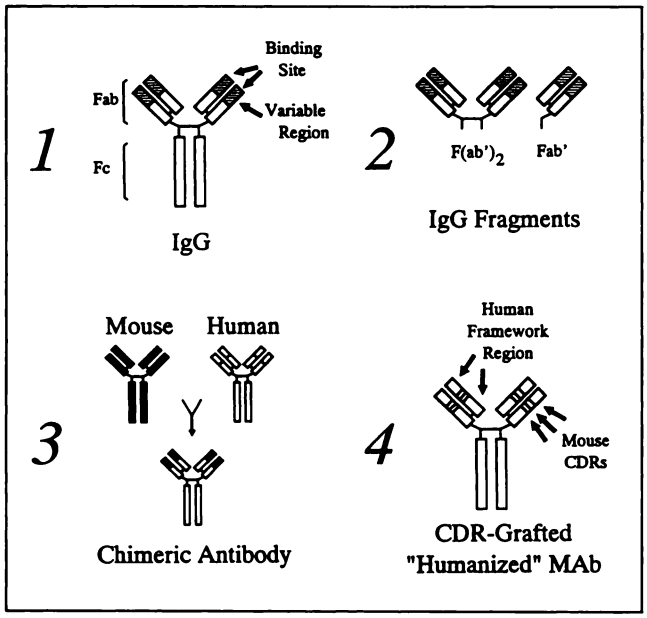

FIGURE 1. Schematic representation of different antibody forms. (1) Whole IgG; (2) IgG fragments; (3) mouse/human chimera; (4) CDR-grafted Mab.

Radiolabeled antibodies have been administered successfully by various injection routes in addition to intravenous, such as subcutaneously (*59*) and intraperitoneally (*60*). This regional delivery may avoid the dilutional effect of intravenous administration, resulting in direct access of the Mab to the sites of tumor that express the antigen target.

The antibodies in current clinical use have undergone stringent regulatory control to assure safety, particularly the absence of endotoxin and murine viral DNA and RNA (*61*). Different manufacturing methods have been used for large-scale production of antibodies, such as mouse peritoneal ascites or one of many tissue culture procedures, but these do not appear to affect the targeting properties of the antibody. However, reproducibility of the antibody and of the radiolabeled product is very important, thereby usually requiring a commercial source for supply.

RADIOLABEL

The two principal determinants in the choice of radiolabel are photon energy and half-life. The half-life of decay should be consistent with the antibody's kinetics (uptake by the tumor and clearance from background tissues). The highest count rate possible should be delivered to the tumor site selectively, which in turn depends on the amount that is injected and the percentage accreted by the target. This is affected by the character of the antibody, as discussed, as well as the amount of radiolabel bound to antibody. Thus, the number of radiolabel atoms attached to the antibody while retaining immunotargeting, the efficiency and abundance of the gamma rays emitted by the radionuclide, the gamma-ray energy and its interaction with the crystal of the imaging camera's collimator are important factors in determining the choice of radiolabel. The ideal energy of gamma emissions that are detected efficiently with current gamma camera crystals is between 100 and 200 keV; the lower the energy, the more the tissue absorption. Thus, of the four principal radiolabels employed to date for RAID (^{131}I, ^{123}I, ^{111}In, and ^{99m}Tc), ^{123}I and ^{99m}Tc are detected more efficiently than ^{131}I and ^{111}In. The higher the count rate ratio achieved in the tumor rapidly, the earlier is the detection. The physical half-life of ^{99m}Tc (6 hr) requires a rapid targeting antibody, such as the Fab or Fab' fragment, while the half-life of ^{123}I (13.2 hr) permits a somewhat longer imaging time, such as with a F(ab')$_2$ form. Indeed, the optimal imaging time for ^{123}I conjugated to monovalent or bivalent fragments of CEA Mab was found to be 24 hr (*47,48*), whereas ^{99m}Tc-labeled Fab' of the same antibody showed optimal imaging between 4 and 6 hr (*47,48,55*). Based upon signal-to-noise ratios among the different radionuclides, it appears that the highest permitted administered dose is for ^{99m}Tc, resulting in the best imaging properties among the four candidate RAID isotopes (*62*). Indeed, a multicenter study showed that ^{111}In-labeled F(ab')$_2$ was inferior in melanoma detection to the same antibody labeled with ^{99m}Tc (*44*). The short half-life of ^{99m}Tc does not permit recordings

much later than 24 hr, but it does allow the use of high activity, from 740 MBq (20 mCi) to 1,110 MBq (30 mCi), at a reduced radiation exposure. It has been estimated that the radiation exposure from ^{99m}Tc is 1/30 that of ^{111}In labeled to a F(ab')$_2$ Mab (*63*). Where uptake is slower, such as in relatively avascular tumors, a longer-lived radiolabel, such as ^{111}In (67.4 hr), may be required. The use of ^{111}In-labeled antibodies appears to be more suitable for extrahepatic sites of tumor (*64*), since there is a high retention of the label in normal liver, resulting in "cold" foci of tumor instead of "hot" areas of radioactivity (*64–66*). However, there appears to be exceptional antibodies with predominant hot spots in hepatic metastases discussed below.

How the antibody is labeled is also important for the success of RAID and for its clinical acceptance and use. The most important requirement is that the procedure not alter the immunoreactivity of the antibody (*32*). Also, there should be complete uptake of the radiolabel, so that free isotope is not injected. Finally, the labeled antibody should be stable after conjugation and after injection into the body. Rapid and simple commercial kits for stable-labeling antibodies with ^{99m}Tc have now become available (*47,48,55,63,67,68*), making imaging with this radiolabel the method of choice in RAID. Although ^{123}I has similar imaging qualities, high cost and limited availability have restricted its use.

SCANNING PROCEDURES AND SYSTEMS

With the increased availability and use of single-photon emission computed tomography (SPECT), improved resolution of RAID has been achieved, with higher rates of detection reported (*62,69,70*). SPECT offers improved contrast of the target in the section, especially differentiating it from structures lying near the target, which may be overlapping in a planar view. Whereas planar imaging usually has shown the detection of tumors as small as 2 cm, SPECT with RAID can reveal lesions below 1 cm, even at 0.5 cm (*47,48,62,69,71*). Thus, SPECT has proven to be a major advance in RAID (*72,73*).

In principle, diagnostic methods such as SPECT, CT, and MRI imaging can be obtained from the same body region of a patient and can be displayed on the same CRT screens. With the appropriate marker systems, images can be superimposed and the resulting "fused" images will contain both anatomic detail from the CT scan and functional images from the RAID-SPECT study. An example of this fusion imaging is shown in Figure 2 (*74*). This technique is still laborious, requiring more than one hour per patient, but improvements in computing speed will soon decrease the processing time to a point where it can be readily applied on a more routine basis for image manipulation.

In a typical scanning protocol with the newer ^{99m}Tc-Mab kits (*47,48,55,68,75,76*), the patient is given potassium perchlorate (400 mg p.o.) just before the intravenous injection of 1 mg Fab' Mab labeled with 25 mCi ^{99m}Tc. The radioimmunoconjugate is prepared by simply adding the desired quantity of technetium pertechnetate to a single vial of lyophilized Fab', shaking the vial, and then injection into the patient within 5 min. The labeled Fab' is stable in vivo, since there is no thyroid activity of ^{99m}Tc in patients who have not been given any thyroid-blocking agent. The patient is then scanned while positioned supine under the gamma camera equipped with a low-energy collimator. Images are made of the whole body and of each body region in different views by planar scans, beginning 2–4 hr after injection of the radioimmunoconjugate, and then by SPECT. A total of 500,000 to 1 million counts per view are made for the early planar images, followed by 300,000 to 500,000 counts per view for delayed (24 hr) scans. Early images show blood-pool activity, especially in the region of the great vessels in the chest, which should not be mistaken for abnormal uptake in the mediastinum. The patients should void prior to pelvic imaging, in order to minimize activity in the urinary bladder. SPECT imaging is obtained at 3 to 6 hr following Mab administration. A 64 × 64 matrix and 360° rotation with 128 stops are used for acquisition. About 100,000 counts are accumulated at each stop. The tomographic data are reconstructed by using a Butterworth filter with a critical frequency of 0.25. Over-smoothing of SPECT images results in a loss of resolution and, hence, low-intensity lesions may not be recognized. To reduce streak artifacts from the kidney and bladder in the pelvic SPECT images, acquisition should begin just after the patient has urinated. An example of a ^{99m}Tc-CEA-RAID study is given in Figure 3. It is important that before injection of the Mab, it should be determined if the patient has a history of allergy, particularly to foreign (animal) proteins. An allergy to animal proteins, especially mouse proteins, is a contraindication to the injection of a murine Mab, unless the patient is suitably premedicated with corticosteroids and antihistamines.

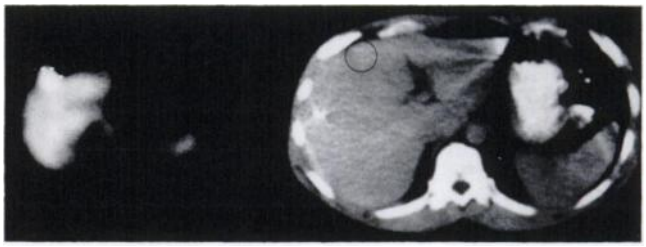

FIGURE 2. Transaxial SPECT image of ^{111}In-C110 anti-CEA Mab (left), showing an area of high uptake in a surgically confirmed metastatic colorectal cancer (12 o'clock), in an area that is completely without evidence of tumor on the transaxial CT scan (right). The circle on the CT scan corresponds to the region in question. Reproduced from Kramer and Noz (74) with permission of the publisher.

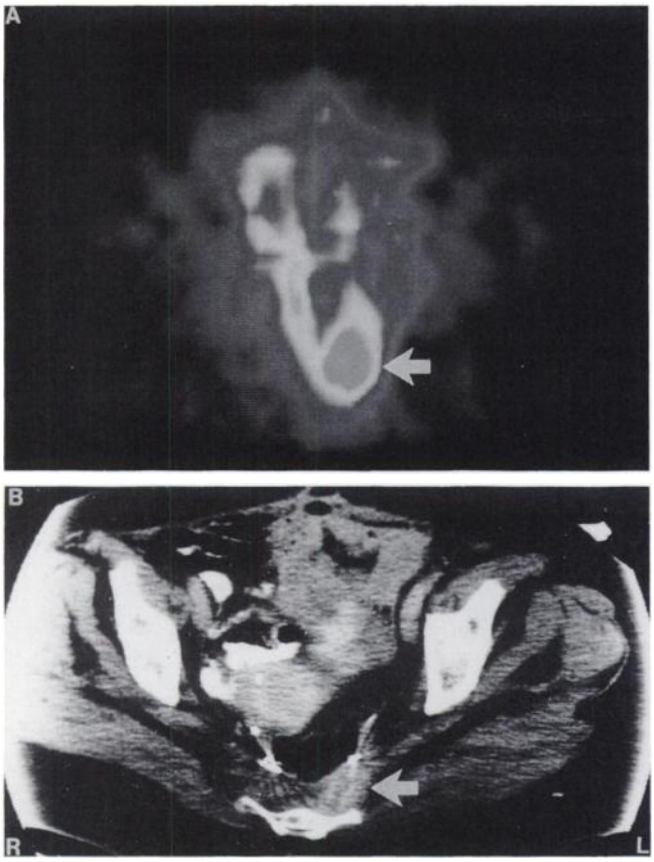

FIGURE 3. (A) Transverse SPECT image of the pelvis of a 69-yr-old female injected intravenously 3 hr earlier with 1 mg of ImmuRAID-CEA (Immunomedics, Warren, NJ) labeled with 25 mCi ^{99m}Tc. The patient underwent a resection of a rectal adenocarcinoma in February 1988, followed by chemotherapy. In 1990, surgery for recurrence to the pelvic wall, followed by chemotherapy, were instituted. A rising plasma CEA 2–4 mo later resulted in a CEA-RAID study, which shows a focal area of radioactivity in the posterior pelvis (sacral region), as indicated by the arrow. (B) CT scan shows thickening in the presacral region (arrow), which could be tumor or postoperative fibrosis. The RAID study clearly supports the interpretation of tumor recurrence.

CLINICAL RESULTS

Different isotopes, antibodies, antibody forms, and imaging protocols have yielded different imaging results. Therefore, it is not surprising that there has been some controversy and disagreement regarding the usefulness of RAID, particularly when studying patients with diverse tumors and stages of disease. Results with ^{131}I-labeled intact IgG have shown a general sensitivity of 80% to 90% (*12,14,31,48,62,77–81*). Studies with ^{123}I-labeled Fab′ and F(ab′)$_2$ fragments have yielded similar results (*44–49*), but with earlier imaging and without the need of methods to compensate for high background radioactivity, such as dual-isotope subtraction (*36*). Most of these studies were performed with antibodies against CEA (*48,77–81*). Indium-111-labeled whole antibodies against CEA or TAG-72 generally have shown lower detection rates if "cold" lesions in the liver are counted as false-negatives, since it has been found that almost all such preparations evaluated to date have a high propensity of accretion of the label by normal liver, spleen, and bone marrow (*30,48,49,58,62, 64–66,77–84*). Therefore, tumor detection rates of 50% to 70% are more common with these ^{111}In-labeled antibodies, except in extrahepatic lymph node sites, where higher rates are achieved. Indeed, ^{111}In-labeled Mabs have had relatively poor results in presurgical staging of colorectal cancer because of the poor targeting of metastases in the liver (*82*). For example, sensitivity of RAID with an ^{111}In-labeled CEA Mab was 17.7% in the liver, and 41.7% for extrahepatic abdominal metastases (*83*). In this series, CT showed better results for hepatic metastases and was inferior, in comparison to RAID, for disclosing extrahepatic abdominal metastases. When considering abdominopelvic or thoracic tumor sites, ^{111}In-labeled anti-CEA or 19.9 Mabs were more sensitive than CT and ultrasound (82% for RAID, 52% for CT, and 59% for ultrasound) (*84*). However, improvements in ^{111}In-labeled Mabs are being made. A recent report by Griffin and associates noted a greater than 90% sensitivity (20/21 lesions verified occult lesions detected), with metastatic lesions to liver verified as "hot spots" seen with an ^{111}In-labeled anti-CEA Mab (*85*). This excellent imaging may be due to an improved chelate for ^{111}In binding to an anti-CEA Mab. As discussed, the most recent data with the rapid ^{99m}Tc-antibody kits indicate detection rates, using CEA Mabs, of 80% to over 90%, with imaging performed within 24 hr (*47,48,63,75, 76*), and the highest sensitivity being achieved with SPECT methods combined with these technetium agents.

A large number of pilot, multicenter, and prospective trials have been conducted in patients with various tumors, using different antibody preparations and imaging protocols. Initially, the performance of the radioimmunoconjugate has been evaluated in patients with tumors whose site and size were known, which permitted the determination of patient and lesion sensitivity, specificity, and accuracy. Thereafter, prospective studies in patients with suspected tumors have been conducted in comparison to other imaging methods, preferably in patients who are candidates for surgery or biopsy. These results permit an evaluation of the clinical role of the new imaging modality. In a multicenter trial in Italy using ^{131}I- or ^{111}In-labeled CEA F(ab′)$_2$, Siccardi et al. (*86*) found that in 35 patients, RAID contributed to the early detection of tumor recurrences. In this trial of 509 patients, an overall sensitivity of 79.0%, a specificity of 96.7%, an accuracy of 82.2%, and a positive predictive value of 99.0%, on a patient-basis, were reported. Baum et al. (*87*) reviewed the European experience and found RAID to be helpful, mainly by complementing other diagnostic methods, in 46% of the patients studied. In 20%, RAID disclosed occult lesions, and was found to alter patient management in 13%. In a recent multicenter study of ^{99m}Tc-labeled anti-CEA Fab′, a clinical benefit of up to 42% was observed in colorectal cancer patients (*55*). Thus, a number of indications have

emerged for RAID on the basis of these studies. The two most prominent are: (1) the confirmation of cancer sites first revealed by conventional radiological methods and (2) the disclosure of occult tumors. The major use of RAID has been in the evaluation of cancer patients who have been treated and are being followed after surgery, radiotherapy, or chemotherapy. The presence of a functional marker of a neoplasm, such as the tumor-associated antigens produced by the neoplastic cells, allows RAID to distinguish between a viable recurrence and anatomic lesions revealed by diagnostic x-ray, ultrasound, CT scans, MRI, and the like. For example, post-therapy fibrotic lesions are detected by these conventional radiological methods as density changes consistent with a mass, but not as a functional neoplasm that can be identified by RAID. In certain circumstances where CT, MRI, ultrasound, and x-ray are unreliable, such as in abdominal and retroperitoneal sites, particularly in the interpretation of normal-sized lymph nodes, RAID can detect subradiological (subclinical, or occult) recurrent or metastatic tumors (*8,9,11–18,31,43–49,55,60,62–64,71,75–84,86–92*). This is of major importance, since nodal metastases are found usually in normal-sized lymph nodes (*91*), and at the time of their presentation, about half of the patients with colorectal cancer have undetected micrometastases and will die of their disease (*93*). In the liver, which is the principal distant site of metastasis for gastrointestinal tumors, RAID has been shown to disclose tumor spread more accurately, particularly with the use of SPECT imaging (*81*). It has been estimated that about 20% of colorectal cancer patients have liver metastases (*94*), so it is not unreasonable to expect that RAID may demonstrate a much higher rate than with conventional methods. In patients whose serum markers have shown elevations without other evidence of recurrence, RAID has revealed the sites of marker production in almost half the cases (*86, 90*).

The role of RAID in initial diagnosis and in tumor staging is still under investigation and has been studied to different degrees for different tumor types. The most extensively studied tumor type has been colorectal cancer (*48,80,81*), followed by ovarian carcinoma and then some of the other major cancer forms (*62,80*). More studies need to be undertaken to define the clinical indications for RAID for each agent and in each tumor type and stage, since there is a diversity of problems in each instance. If performed before surgery, a RAID study can alter management if distant metastasis or more extensive disease is found. In the post-surgical situation, RAID can complement other diagnostic modalities in defining extent of disease when there is a known recurrence or metastasis, or it can aid in the disclosure of occult tumor sites in patients with suspected recurrence.

Different antibodies, labels and image processing methods may show various areas of increased radioactivity unrelated to true antigen targeting. As mentioned, ^{111}In-Mabs have a proclivity for liver, spleen, bone marrow, and intestine, often showing "cold" lesions in the liver. Radioiodine preparations can dehalogenate, resulting in increased activity in the thyroid and stomach, as well as the urinary bladder. Depending upon the ^{99m}Tc-Mab preparation used, activity in the gall bladder and intestines can occur (*93*). With conjugation of any radiolabel to univalent Fab fragments, particularly ^{99m}Tc, increased renal and urinary bladder activity can be seen (*47,96*). In general, very large tumors often appear as cold lesions, sometimes with a rim of increased radioactivity, presumably because of poor blood flow and penetration. Accordingly, familiarity with physiological and nontumor Mab and radionuclide distribution is important in order to maximize the specificity of RAID studies.

The question of RAID specificity has been studied in different ways. Most studies record the true-negative rate, from which specificity can be derived, and this can be very high, over 90% (*12,48,77,78,80,86,87*). Reports of positive localization in the absence of the appropriate antigen (*97*) have led to a challenge of the immunological specificity of antibody targeting (*98*). At the very beginning of studies with polyclonal anti-CEA antibodies labeled with ^{131}I, control studies with normal goat IgG were also performed, and it was found that very large tumors could be imaged with the irrelevant IgG, while a few benign lesions, such as empyema and diverticulosis, were imaged with the specific antibody IgG (*12*). More recently, Abdel-Nabi et al. (*99*) found that ^{111}In-labeled CEA or B72.3 Mabs localized in tumor-free benign lesions, most commonly degenerative joint disease, abdominal aneurysms, postoperative bowel adhesions, and local inflammatory changes, in 10%–13% of the patients. The increase of nonspecific macromolecules in neoplasms has been known for over 50 yr (*100*), so large, highly vascular tumors or other lesions accreting immunoglobulins should not be surprising, and may include increased vascularization and vascular permeability as partial explanations. This could explain the targeting of tumors and of inflammatory lesions with normal human IgG (*101*), but it is likely to be less useful for visualizing very small tumors (*12*). In another situation, DeLand et al. (*59,102*) and, later, Beatty et al. (*82*) and Granowska et al. (*103*) discussed the imaging by CEA Mabs of tumor-free lymph nodes draining a tumor area. These studies interpreted this finding as due to soluble antigen draining into the nodes. Although this is considered a false-positive finding for tumor cells, it may be of biological importance in demonstrating pending lymph node tumor spread in such patients and could certainly be truly positive from the immunological perspective.

COLORECTAL CARCINOMA

Colorectal cancer has received the most attention of all tumor types in RAID (*48,78,80,81*). The majority of studies evaluated patients with recurrent or metastatic disease,

and with the best results, in that detection rates in over 80% of known tumor sites have been reported, mostly with CEA antibodies (*12,48,77,80,81*). Tumors as small as 0.5 cm have been disclosed with SPECT imaging (*47, 48*), but usually tumors above 2 cm (*86*) or 3 cm (*64*) have had the highest detection rates. The role of RAID in the diagnosis of primary colorectal cancer is more difficult to determine, since fewer prospective studies have focused on this issue. However, evidence to date suggests that RAID can contribute to the conventional methods used to diagnose tumors of the colon and rectum, depending upon the expression of the target antigen by the tumor, as well as other factors. In this regard, when a target antigen, such as CEA, is shed and circulates in the blood, the higher the level of antigen, the higher the tumor detection rate by RAID (*12,77,86*). However, normal plasma CEA titers do not preclude the presence of tumor that can be disclosed by RAID (*48,77,87*). Even the formation of antibody-antigen complexes does not affect RAID, supporting earlier observations with CEA polyclonal antibodies (*12,19, 77,104*). Thus, in presurgical studies, RAID has not only confirmed known sites of cancer, but has identified occult lesions that were not revealed previously by other methods (*30,70,82,86,88,105,106*).

The majority of RAID studies in colorectal cancer have been performed with various antibodies against CEA and labeled with ^{131}I, ^{123}I, ^{111}In or ^{99m}Tc. Results with other Mabs, such as B72.3 and 791T/36, have been encouraging and basically similar to the findings with CEA Mabs (*30, 36,60,107–110*). However, colorectal tumor detection rates with Mab B72.3 appear to be less than with CEA Mabs (*30,56,107,108*). Both totally human (*111*) and human/mouse chimeric (*112*) antibodies have been introduced as colorectal cancer imaging agents. Yet, the tumor detection rates in these initial RAID studies do not appear to be superior to the best murine-based agents. Although it would appear that these human or humanized forms are less immunogenic than whole murine IgG Mabs, a comparison to murine antibody Fab fragments, especially with repeated applications, is needed. Unfortunately, direct comparisons of these many different agents, either in the same patients or in different patients in the same study, have rarely been reported. Since the methods used by different investigators can have considerable effects on the results, it is difficult to compare the results of different groups using different antibodies, labels, and imaging procedures.

OVARIAN CARCINOMA

CEA antibodies were the first to be used to image ovarian tumors (*11,12,113*). However, the majority of studies have been performed with other Mabs, particularly HMFG2 (*71,114–120*), OC 125 (*121–124*), B72.3 (*125*), 791T/36 (*126,127*), and, more recently, OV-TL3 (*128, 129*). Antibodies against human milk fat globule (HMFG1 or HMFG2) have been used by several investigators to image ovarian carcinoma. HMFG2 labeled with ^{123}I or ^{131}I has a high detection rate, sometimes disclosing tumors missed by ultrasound or CT. It is not useful for prospective screening of patients because it is not specific for ovarian carcinoma. This antibody is reactive with a large number of metastases, thus, it is useful in patient monitoring and management (*130*). In an important prospective, multicenter French trial of ^{111}In-OC 125, recurrent ovarian cancer in 47 patients could be well excluded with RAID alone, while RAID combined with CT had a high predictive value for recurrence (>80%) (*131*). Also of recent interest are results with OV-TL3 labeled with ^{111}In (*129*). In a study performed with the ^{111}In-labeled $F(ab')_2$ fragment of OV-TL3 (*129*), ovarian tumors were detected in 16 of 17 patients (94%). Of 45 tumor sites found at surgery, 67% were localized by RAID, while CT and ultrasound visualized 53% and 23%, respectively (*129*). However, peritoneal carcinomatosis and liver and lymph node metastasis were excluded from this analysis. Small liver metastases were seen only as cold defects. An advantage of this antibody was the disclosure of omental metastases by SPECT (which was superior to planar imaging), whereas neither CT nor ultrasound could conclusively reveal these tumor sites. Since the antigen target of OV-TL3 is not detected in patient serum, it appears that this reagent has advantages over the others used for ovarian cancer RAID, and the only limitations with OV-TL3 appear to be ^{111}In-related.

The major indications for the use of RAID in ovarian carcinoma appear to be in the disclosure of intra-abdominal and, in particular, peritoneal spread. Omental and lymph node metastases, which are particularly difficult to discern by other methods, may be revealed by RAID. Whether RAID can improve the initial diagnosis of ovarian carcinoma remains to be investigated in prospective trials.

BREAST CANCER

Fewer antibody imaging studies have been reported on breast cancer than with other tumor types, such as colorectal or ovarian carcinoma. However, a number of different antibodies and labels have been used for breast cancer imaging, including CEA, B6.2, B72.3, M8, 791T/36, 3E1.2, 3C6F9, and HMFG1 and HMFG2 (reviewed in *130*). Most of the studies involved either i.v. or s.c. injection of the radiolabeled Mab, and in general variable results have been reported, which are expected when different antibodies, routes of administration, radiolabels, and patient groups are used. The s.c. route has been employed for lymphoscintigraphy, particularly the imaging of axillary lymph node metastasis in these patients. In a study of nine breast cancer patients who received interdigital web injections of ^{131}I-labeled anti-CEA goat IgG, DeLand et al. (*59*) reported increased axillary radioactivity in eight, all of which were confirmed either surgically or clinically; the ninth had a benign lesion. This was the first clinical

demonstration of the feasibility of demonstrating breast cancer metastases in lymph nodes with radiolabeled antibodies. It also raised the important question of whether positive lymph node imaging can be due to sequestered antigen draining from a regional tumor, since some sites showing positive accretion of radiolabeled antibody were free of tumor by histology (*59,132*). Thompson et al. (*133*) injected another ^{131}I-labeled Mab (3E1.2) s.c. in eight breast cancer patients and was able to visualize most of the lymph node metastases, even those of apparently normal size. A patient with non-Hodgkin's lymphoma and palpable nodes failed to show accretion of the antibody in the involved lymph nodes (*133*). In yet another study of antibody lymphoscintigraphy in breast cancer patients, Mandeville et al. (*134*), using ^{123}I-labeled Mab 3C6F9, showed seven of nine axillas positive for tumor, of which six were confirmed for tumor metastasis by histopathology. Lymphoscintigraphy with ^{131}I-labeled RCC-1 Mab was performed in a prospective study of 26 breast cancer patients (*135*). A second, unlabeled, unreactive Mab was given simultaneously to block the uptake by normal lymph nodes. This method resulted in a sensitivity of 86% and a specificity of 92% (*135*). In another lymphoscintigraphy study with a ^{99m}Tc-labeled CEA Mab, a sensitivity and specificity of 90% and 88%, respectively, were found for superclavicular and axillary lymph nodes in 18 breast cancer patients (*136*). In a recent preoperative study of RAID with the same ^{99m}Tc-labeled whole anti-CEA IgG administered intravenous in 45 women with suspected primary, recurrent or metastatic breast cancer, a sensitivity of 83% was reported with SPECT imaging; the smallest tumor identified was 0.7 cm (*137*). In contrast, this investigation found that only 17% of the patients with verified breast cancer had elevated serum CEA levels (*137*). The investigators also reported that planar imaging was unsuitable for RAID of breast cancer (*137*). Additional prospective studies to assess the diagnostic accuracy of this method of preoperative assessment of the primary tumor and of revealing regional lymph node involvement in breast cancer are needed, especially since a number of different antibodies and labels have shown promising results.

OTHER TUMORS

Many other malignant tumor types have been targeted and imaged with radiolabeled antibodies, including melanoma (*40,44,49,57,96*), lung carcinomas (*58,138,139*), neuroblastoma (*140*), glioma (*141–143*), cervical carcinoma (*12,18*), lymphomas (*144–146*), hepatocellular carcinoma (*14*), and sarcomas (*147,148*). Both pancarcinoma antibodies (such as CEA, B72.3, and HMFG) and more specific antibody reagents, such as prostatic acid phosphatase antibodies in prostatic carcinoma (*17*), human chorionic gonadotropin antibodies in trophoblast and germ-cell tumors (*15,16*), alpha-fetoprotein antibodies in germ-cell tumors and hepatomas (*13,14*), thyroglobulin antibody in thyroid carcinoma (*28*), insulin antibody in insulinoma (*29*), and placental alkaline phosphatase antibody in seminoma (*18,149*), have been utilized. Studies in these tumors have not been as extensive as those in the cancers discussed above. Opportunities for RAID in almost all tumor types will be afforded as markers and antibodies are identified, even if only quantitatively increased in cancer as compared to their normal organ source.

ADVERSE REACTIONS

The development of human anti-mouse antibodies (HAMA) after single or multiple injections of murine antibodies limits the use of some antibodies, especially intact IgG agents, where the development of HAMA alters biodistribution (*53,150–153*). The use of Fab and Fab' fragments greatly decreases the incidence of HAMA, however, and agents based on fragments may be reinjected without HAMA. Nevertheless, RAID agents that are not as immunogenic as murine protein are being sought, including entirely human (*111*) and substantially human (*51,52*) Mabs.

The presence of HAMA can also affect immunoassay results of certain analytes in the blood specimens of such patients, which can lead to unnecessary clinical interventions. For example, it has been observed that CEA titers can be falsely elevated in the blood of patients with high HAMA levels (*154–156*), presumably due to interference with the murine Mabs used in certain immunoassays.

Adverse reactions to the use of murine Mabs for RAID have been very rare, especially after a single injection. An untoward reaction rate of 1 per 1,100 is claimed to be one-tenth the rate of side-effects to roentgen contrast agents (*62*).

CONCLUSION

This review has emphasized that the technology of RAID continues to advance and is close to becoming a routine modality in the identification of sites of cancer. It is also likely that it will make a contribution to the diagnosis of nonmalignant conditions, but we cannot agree with a recent editorial (*157*) favoring the use of RAID in nonmalignant over malignant conditions, as others have already responded (*158–160*).

The essential question, we believe, is whether RAID provides accurate and specific diagnostic information that influences patient management. This requirement has two important parts. First, how should we measure accuracy and specificity? Most RAID studies have done this by comparing RAID results to the findings of CT and other radiological modalities. As a retrospective study, RAID results can, at best, complement these other findings, which were the basis of undertaking the antibody imaging studies. Thus, sensitivity, specificity, and accuracy rates in these studies are of limited value. On the other hand, prospective trials provide a more reliable comparison, and

in such studies RAID generally has been found to be superior to other imaging methods. Since RAID is a more functional diagnostic test than the conventional, anatomically-oriented radiological modalities, it can be more tumor-specific as cancer-related markers evolve. Undoubtedly, patient selection (i.e., knowing when to use RAID in patient management) will be an important factor in optimizing the usefulness of this test.

The second issue of influencing patient management is best assessed in patients whose tumors are responsive to therapy. Unfortunately, most of the tumor types studied by RAID have been those that are the least amenable to treatment, and therefore most difficult to prove as being affected by antibody imaging. Nevertheless, it is a paradigm that the disclosure of occult tumor sites, especially in patients believed to be free of disease, is of value in their management, even if it merely avoids unnecessary treatment. Studies supporting the value of RAID in revealing these occult lesions are expanding, particularly as the advances in RAID permit the disclosure of tumors in the 0.5-cm to 1.0-cm range.

Despite the progress already made, prospects exist for improving the imaging procedure and targeting molecules even further. Recently, Paganelli et al. (*161*) described a method to post-label biotinylated Mabs pretargeted to tumor when most of the nontargeted Mab was cleared from the body as avidin-bound complexes. However, although this procedure resulted in very improved tumor-to-nontarget ratios, it required three injections over a period of 5 days, and the avidin used was immunogenic. Also, Stickney et al. (*162*) recently showed improved target ratios with a bispecific antibody preparation in 14 colorectal cancer patients. One arm of the $F(ab')_2$ reacted with CEA, and the other with the ^{111}In-linker. The ^{111}In-hapten was then administered after the bifunctional antibody cleared from normal tissues, 4 days later. Whether this method is preferable to the use of Mab fragments needs to be determined.

The targeting molecules have been reducing in size progressively, from whole IgG (160 kD) to single-chain antibody Fv fragments of 25 kD (*163*). The smaller forms retain antigen-binding properties and hold promise for targeting to tumor very rapidly, while not being metabolized by the kidneys (*50,164*), as is the case with larger (50 kD) antibody fragments (*40,47*). Whether these small units bear sufficient immunogenicity when derived from murine antibodies to result in HAMA reactions needs to be determined. Suffice it to say that the next generations of antibody imaging agents are already under investigation even before the first generation has been approved by the FDA for conventional use. This is not surprising, given the different missions of clinical investigators and regulatory agencies. However, there must be a delicate balance tipped to the advantage of the cancer patient, who is in desperate need of innovative approaches for earlier and more accurate diagnosis, as well as methods of more specific therapy, and for whom RAID may serve as a basis. The current record of very high safety for RAID should allow for a liberal use of these new radiopharmaceuticals in the management of cancer, and it is timely to look more to the future, especially in the transition to radioimmunotherapy (*165,166*), than to continue debating the past and present:

"If we open a quarrel between the past and present, we shall find that we have lost the future."
Sir Winston Churchill
Speech, House of Commons, June 18, 1940.

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