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Radioimmunodetection with ^{111}In-labeled Monoclonal Antibody Nd2 in Patients with Pancreatic Cancer

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This report summarizes results from an initial clinical evaluation of radioimmunodetection (RAID) in patients with pancreatic cancer using murine monoclonal antibody Nd2, directed against mucins from pancreatic cancer. Nd2 (2 mg) was labeled with ^{111}In (2 mCi) and injected into 19 patients suspected of having pancreatic cancer. Planar scintigrams were taken 3 days post-infusion. As for final diagnoses after surgery, 14 cases were pancreatic cancer, and one case each was chronic pancreatitis, neurilemmoma, islet cell carcinoma, cholangioma, and apparent absence of suspected recurrent lesion of pancreatic cancer. Of 14 patients with pancreatic cancer, RAID was positive in 10 cases (71.4%). Cases other than pancreatic cancer were all negative, so the specificity was 100%. These results demonstrate that RAID using ^{111}In-Nd2 can be useful in differentiating exocrine pancreatic cancer from benign conditions and other types of carcinomas in the pancreatoduodenal regions.

Key words: Radioimmunodetection — Pancreatic cancer — Nd2 — Indium

Although the incidence of pancreatic cancer has increased steadily in recent decades, the prognosis has not improved significantly. Early diagnosis provides some hope for a better prognosis.[1)] Differentiating between malignant and benign tumors of the pancreas is also important for a better prognosis. It is often difficult to make a precise diagnosis before surgery, even with conventional imaging techniques such as computerized tomography and ultrasonography. Monoclonal antibodies (MoAbs) have been useful in identifying and quantifying pancreatic cancer antigens, but the usefulness of serological detection by antibodies such as CA19-9,[2)] SPan-1,[3,4)] and DU-PAN-2[5)] is limited, in part, by the problem of false-positives.[6)] Another application of MoAbs to cancer diagnosis is in radiolocalization or radioimmunodetection (RAID). Animal[7–13)] and clinical[14–18)] studies have established the usefulness of RAID in the management of cancer, including many solid tumors. In gastro-intestinal malignancies, colon cancer[19–22)] has been the main focus of such studies. ^{111}Indium (In)-labeled B72.3 has been extensively studied for the imaging of colorectal cancer.[23)] However, only a few MoAbs have been described for pancreatic cancer.[24,25)] One of these, Nd2,[26)] was generated against mucins purified from xenografts of the human pancreatic cancer cell line SW1990. It reacts with over 80% of pancreatic cancers, 58% of gastric cancers, and 24% of colorectal cancers, but does not react with normal tissues. Moreover, the epitope recognized by Nd2 is not detectable in sera of patients with pancreatic cancer, and radiolabeled Nd2 localized well to xenograft pancreatic tumors in athymic nude mice.[27)] These promising results in animal studies indicated that Nd2 should be evaluated clinically. Here we describe an initial evaluation of the application of Nd2 radiolabeled with ^{111}In to the RAID of pancreatic cancer tumors in patients.

PATIENTS AND METHODS

Patients Nineteen patients, 8 females and 11 males with ages of 35 to 72 years, were studied. Mean age was 56.9 years. All the patients were initially diagnosed to have primary pancreatic cancer, local recurrence or distant metastases. The diagnoses were based on computerized tomography, ultrasound, magnetic resonance imaging, endoscopic retrograde pancreatography, or the blood level of tumor markers. All were scheduled to undergo surgery. The final diagnosis was made histologically on tissues taken at the time of surgery, except for one case (patient 12) who was suspected to have a local recurrence. This patient has been followed for over two years after RAID and has shown no indication of a recurrence. This diagnosis was based on results from serial determinations of tumor markers and conventional imaging techniques. Informed consent was obtained from each patient. The protocol was approved by the Human Ethics Committee of the Osaka City University Medical School. No patient had had prior exposure to murine MoAbs.

Antibodies and labeling Nd2 is a murine MoAb of the IgG1 isotype, directed against purified mucins from the

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human pancreatic cancer cell line SW1990. Nd2 was purified from ascitic fluid by affinity chromatography on protein A columns (MAPS II kit, Bio-Rad, Hercules, CA). The purity of the IgG was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing and nonreducing conditions. Nd2 was concentrated to 2–3 mg/ml in phosphate-buffered saline. A 10 : 1 molar ratio of cyclic anhydride diethylenetriaminepentaacetic acid (DTPA) to Nd2 was added and the mixture was stirred for 60 min at room temperature. Unconjugated DTPA was removed by gel filtration on a Sephadex G-25 column. One milligram of DTPA-conjugated MoAb in 0.1 *M* citrate buffer, pH 6.0, was aliquoted into vials and stored at −26°C before labeling. Finally, 1 mCi of ^{111}In-chloride was added to each vial, and the vial was agitated for 30 min at room temperature. The labeling efficiency was greater than 90% without further purification. The specific activity was 1 mCi/mg.

RAID The patients were initially tested for sensitivity to Nd2 by an intradermal injection of 20 μg of unlabeled Nd2 antibody in 0.02 ml of normal saline. None of the patients had a positive skin test. ^{111}In-DTPA-Nd2 (2 mCi/2 mg protein in 100 ml of normal saline solution with 2% human albumin) was infused over 30 min. The patients were imaged on a large-field view camera (Bodyscan; Siemens, Germany) interfaced to a dedicated nuclear medicine computer (ICON; Siemens). Overlapping images were obtained from the neck to the pelvis in both anterior and posterior projections. Lateral views were obtained as required. In some patients, we performed liver scintigraphy with 2 mCi of ^{99m}Tc-phytic acid (^{99m}Tc-inositol-6-phosphate) and the liver shape thus determined was subtracted from the images obtained with ^{111}In-labeled Nd2 using a computer to distinguish clearly the specific accumulation in the putative pancreatic tumor from non-specific accumulation in the liver. Images were obtained on the 3rd day after injection, and were assessed in a nonblinded fashion by two nuclear medicine physicians.

Immunohistochemical studies Surgical samples were fixed in formalin and embedded in paraffin. Immunoperoxidase staining was performed using the avidin-biotin-peroxidase complex method.[28] Grading of degree of reactivity was based on the percent of an optical field that was stained (×20 objective): up to 10% (+); 10–50% (++), and >50% (+++).

Immunoassays for human anti-murine antibodies (HAMA) and tumor markers Patients' sera were collected prior to, and at various intervals after, ^{111}In-Nd2 infusion. Ninety-six-well microtiter plates were coated with 100 μl of Nd2 antibody (10 μg/ml) at 4°C overnight. The plates were blocked with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) and then washed. Patients' sera were diluted in 20-fold serial dilutions with 0.1% BSA in PBS and 100 μl samples were added to the wells. The plates were incubated at 37°C for 2 h and washed. One hundred microliters of biotinylated Nd2 (1 μg/ml) was added. After incubation for 2 h at 37°C and washing, 100 μl of streptavidin-peroxidase complex were added to each well and the plates were incubated at room temperature for 30 min and washed. Forty minutes after addition of 2,2′-azino-di-3-ethylbenzthiazolinesulfonic acid, the plate was read at 405 nm on an EAR 400 plate reader (SLT-LAB Instruments Co.). The patient was considered to be HAMA-positive if the measured absorbance was at least twice that of the corresponding pre-dose serum.

Levels of CA19-9, SPan-1 and carcinoembryonic antigen (CEA) in sera of patients were measured using CA19-9 RIA Kits (Centocor, Malvern, PA), SPan-1 RIABEAD (Dainabot, Tokyo), and CEA RIABEAD (Dainabot), respectively. The cut-off values were 37 U/ml of CA19-9, 30 U/ml of SPan-1, and 6.5 ng/ml of CEA, respectively.

RESULTS

Patients Patients' characteristics and clinical data are summarized in Table I. All patients received ^{111}In-Nd2. The preoperative diagnoses for 19 patients were: primary pancreatic cancer (16), local recurrence of pancreatic cancer (2), distant lymph node metastasis of supraclavicular region (1). Patients with primary pancreatic cancer had tumors localized in the head of the pancreas (12 cases), pancreatic body (2 cases), and pancreatic tail (2 cases). Fifteen of 19 patients showed elevated levels of pancreatic cancer-associated antigens (CA19-9 or SPan-1). Of 19 patients studied, 18 went to surgery. On final diagnosis, 13 of the 19 patients had primary or metastatic pancreatic cancer. There was one recurrence (patient 9). In the remaining 5 cases, there was one case each of islet cell carcinoma of the pancreas, cholangioma, chronic pancreatitis (tumor forming), and retroperitoneal tumor (neurilemmoma), and the fifth patient was found to have no evidence of local recurrence on further clinical follow-up (patient 13). Primary tumors ranged in size from T2 (2.1–4 cm) to T4 (over 6.1 cm).

RAID On the third day post-injection, primary and metastatic tumor sites were often clearly detectable in a ^{111}In-Nd2 scintigram. Most of the positive cases were visualized by planar imaging. However, in one case (tumor located at the pancreatic head, patient 12), an image obtained by subtraction of the liver image was used. Scintigraphic results are summarized in Table II. There were 10 true positives (Figs. 1 and 2), including the case of recurrence. There were 5 true negatives, 4 false negatives, and no false positives. True negatives were one cholangioma, one chronic pancreatitis (tumor

Table I. Patients' Characteristics and Clinical Data

Patient	Age	Sex	Tumor site by CT scan	Preoperative diagnosis	Tumor size	Tumor marker		
						CA19-9 (U/ml)	SPan-1 (U/ml)	CEA (ng/ml)
1	50	M	body	primary PC	T3	1124	530	2.3
2	69	M	head	primary PC	T2	614	230	2.9
3	59	M	virchow	lymph node's meta	2.5 cm	15	136	3.3
4	68	M	head	primary PC	T3	250	240	0.9
5	68	M	tail	primary PC	T4	3555	950	194.0
6	67	M	head	primary PC	T2	183	90	9.9
7	52	F	head	primary PC	T3	121	68	63.0
8	60	M	head	primary PC	T3	565	23	7.8
9	60	M	body	local recurrence	T3	17	160	3.2
10	61	M	body	primary PC	T3	2967	920	7.7
11	47	F	head	primary PC	T3	30	23	1.3
12	72	F	head	primary PC	T3	3	230	3.2
13	61	M	body	primary PC	T2	105	75	2.3
14	47	F	head	primary PC	T3	5080	1700	8.7
15	62	F	body	primary PC	T3	15	<10	1.6
16	39	F	tail	primary PC	T2	17	120	2.1
17	45	M	body	primary PC	T2	5	<10	1.0
18	35	F	body	local recurrence	—	1232	190	3.2
19	60	F	head	primary PC	T3	22	15	1.3

PC, pancreatic cancer.

Table II. Final Diagnosis and RAID of Pancreatic Cancer with ^{111}In-Nd2

Patient	Final diagnosis	Histological findings	RAID	Immunostaining
1	pancreatic cancer	well	true positive	+++
2	pancreatic cancer	well	true positive	+++
3	lymph node's metastasis	well	true positive	+++
4	pancreatic cancer	moderately	true positive	+++
5	pancreatic cancer	well	true positive	+++
6	pancreatic cancer	well	true positive	++
7	pancreatic cancer	moderately	true positive	++
8	pancreatic cancer	moderately	true positive	++
9	local recurrence	well	true positive	++
10	pancreatic cancer	poorly	true positive	++
11	pancreatic cancer	well	false negative	++
12	pancreatic cancer	poorly	false negative	+
13	pancreatic cancer	moderately	false negative	+
14	pancreatic cancer	poorly	false negative	−
15	retroperitoneal tumor	neurilemmoma	true negative	−
16	tumor forming pancreatitis	chronic pancreatitis	true negative	−
17	islet cell carcinoma	non-functioning	true negative	−
18	no disease	(no evidence of recurrence)	true negative	NE
19	cholangioma	cholangiocell carcinoma	true negative	−

well, well differentiated tubular adenocarcinoma; moderately, moderately differentiated tubular adenocarcinoma; poorly, poorly differentiated tubular adenocarcinoma; NE: not examined.

forming), one retroperitoneal tumor, one with no evidence of disease and one islet cell tumor. Diagnostic accuracy can be summarized as follows: sensitivity 71.4% (10/14), specificity 100% (5/5) and accuracy 78.9% (15/19).

Immunostaining Nd2 stained surgically excised specimens to varying degrees. Out of 18 patients the expression of Nd2 was found to be negative in 5 patients, mildly positive (+) in 2 patients, moderately positive (++) in 6 patients, and markedly positive (+++) in 5 patients.

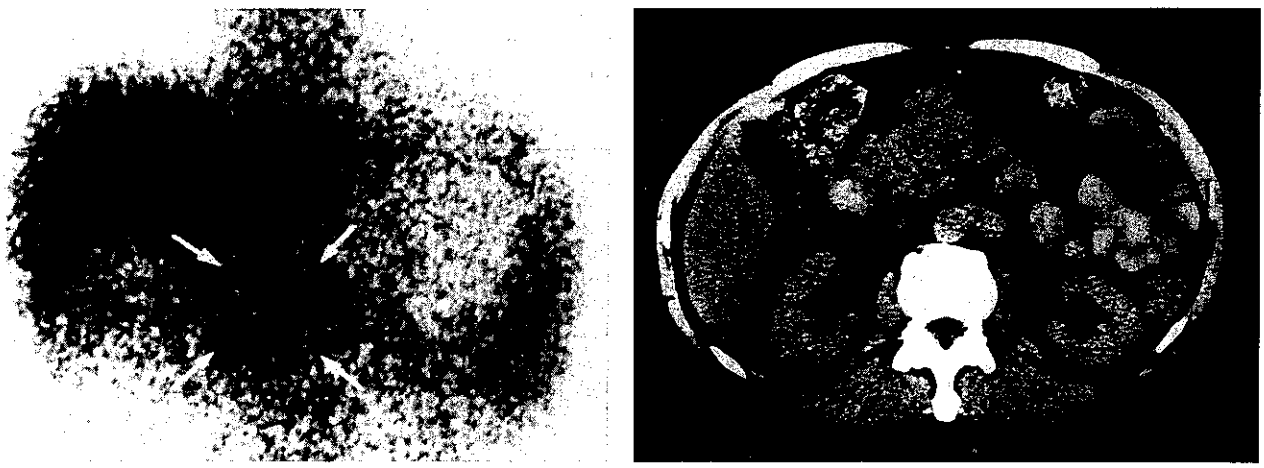

Fig. 1. Anterior planar scintigram with ^{111}In-Nd2 and plain abdominal CT scan in a case of true-positive RAID (patient 1 in Table I). The planar scintigram reveals specific accumulation of ^{111}In-Nd2 at a site corresponding to the tumor lesion (arrow), which can be detected in the head and body of the pancreas in the CT scan, in addition to non-specific accumulation in the liver.

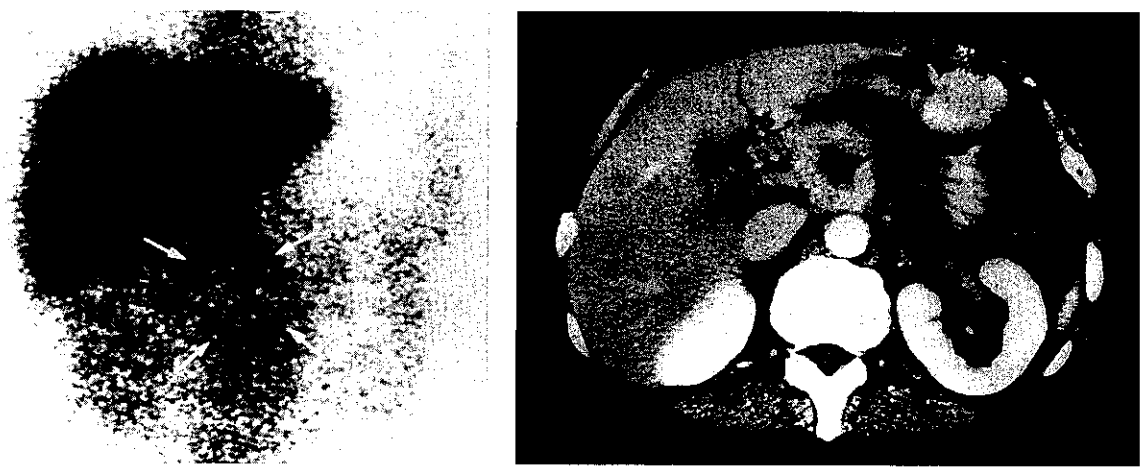

Fig. 2. Anterior planar scintigram with ^{111}In-Nd2 and enhanced CT scan in a patient with primary pancreatic cancer (patient 4). The scintigram reveals positive RAID corresponding to the pancreatic tumor (arrow) detected in the CT scan, accompanied with central necrosis.

All those that were successfully imaged with ^{111}In-Nd2 had staining grades of ++ or +++. The 4 false-negative cases had immunohistochemical staining grades of + or ++. Of these cases, 3 had tumors localized to the head of the pancreas. Two tumors were poorly differentiated (head), one was moderately differentiated (body) and one was well differentiated (head). None of the true-negative cases expressed the Nd2 antigen immunohistochemically (Fig. 3). The correlation between RAID and immunostaining is summarized in Table III. When Nd2 antigen was present in at least 50% of the cells (+++) the RAID rate was 100%.

HAMA induction and side effects No immediate or delayed allergic reaction was seen, and there were no abnormal laboratory results relating to Nd2 infusion. The HAMA levels were determined for 18 patients. Thirteen of 18 patients were HAMA-positive (72.2%). Samples from 2 patients were obtained at week 1 and showed no response to Nd2. The other 16 patients' sera were obtained at week 2 or later. Thirteen of these 16

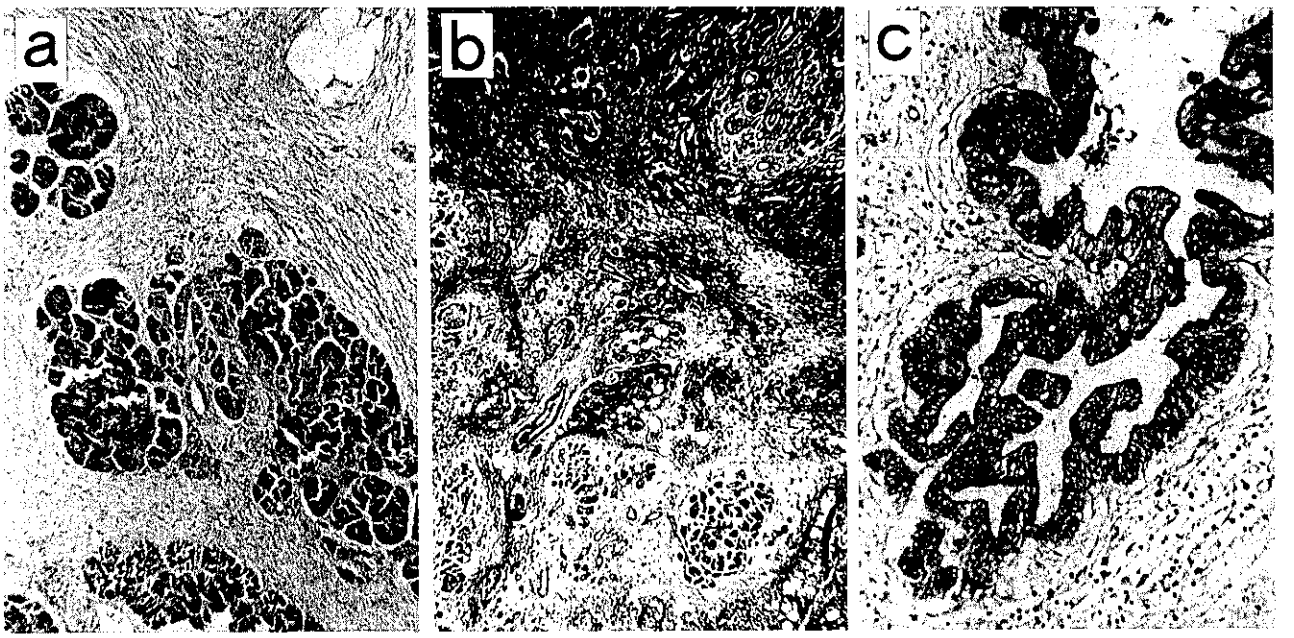

Fig. 3. Immunohistological expression of Nd2 in surgical specimens. A tumor resulting from pancreatitis showed no expression of Nd2 (a) and was not imaged with ^{111}In-Nd2. Markedly positive expression of Nd2 antigen was present in primary pancreatic tumors that gave positive radioimages with ^{111}In-Nd2 (b and c).

Table III. Correlation between RAID and Immunostaining

Degree of positivity	(N)	RAID by ^{111}In-Nd2	
		Positive	Negative
negative	5	0%	100% (5/5)
+	2	0%	100% (2/2)
++	6	83.3% (5/6)	16.7% (1/6)
+++	5	100% (5/5)	0%

Table IV. Human Anti-Mouse Antibody Response

Patient no.	Weeks post-infusion					
	0	1	2	3–4	5–6	7–8
1	19			**218**		
2	36		**121**			
3	24			**164**		
4	35			**647**	**406**	
5	38	63	**189**			
6	19	**65**	**133**	**714**	**835**	**625**
7	82		51	**731**	**902**	
8	61		**431**	**611**		
9	39		**100**			**467**
10	6			**285**		
11	113			**231**		
12	102			**218**		
13	159	120				
14	31		40	60		
15	31	43				
16	88	51		68		
17	12		**91**			
18	176	76		160	142	

All values are expressed as absorbance $\times 10^3$.
Responder values are shown in bold type.

patients were HAMA-positive. There was a tendency for serum samples obtained at week 2 or after to be HAMA-positive. Serum samples obtained after week 5 (patients 4, 6, 7 and 9) still had high levels at that time (Table IV).

DISCUSSION

Although RAID has been successfully applied to several kinds of solid tumors such as colon, lung, liver, ovarian cancer, there have been few studies on pancreatic cancer.[24, 25, 29] Klapdor and Montz have described RAID studies using several MoAbs (anti CA19-9+anti CEA, and BW 494/32).[24] They did not think that RAID was effective in the detection of early pancreatic cancer, but considered that it could be superior to other imaging methods for the detection of recurrence. It also has potential clinical value to assess the feasibility of subsequent therapy with MoAbs. On the other hand, Goldenberg[29] reported a high sensitivity rate (80%) to CEA antibodies labeled with ^{131}I, and suggested that

much better results could be achieved with a combination of isotopes such as ^{99m}Tc and single photon emission computed tomography (SPECT) imaging. Several investigators[15, 20, 24, 29] have suggested that RAID with SPECT improves the detection of lesions in the colon and pancreas. Non-specific accumulation of ^{111}In-Nd2 in the reticuloendothelial system of the liver occurs, so that lesions of the head of the pancreas are difficult to image by planar scintigraphy. SPECT may solve this problem.

The results of the present study demonstrate the ability of ^{111}In-Nd2 successfully to image pancreatic cancer in approximately 70% of patients with planar imaging. This initial evaluation suggests that ^{111}In-Nd2 provides diagnostic information that complements results from standard radiographic imaging modalities and improves the accuracy of differential diagnosis between pancreatic cancer and diseases other than pancreatic cancer, including benign conditions. It is noteworthy that there were no false-positive cases and all positive cases were true positives. Thus, the specificity was 100%. This is consistent with results from immunohistochemical studies[27] in which Nd2 antigen was frequently expressed in tissues of pancreatic cancer, but was not expressed in normal acinar cells, ductal cells, endocrine cells or inflammatory conditions such as chronic pancreatitis. In this study, there was one case of islet cell carcinoma and one case of a tumor resulting from chronic pancreatitis. Both had negative radioimages and negative immunohistochemical staining for the Nd2 antigen.

In this study we had no cases of T1 tumor (<2 cm in diameter). However, patients with T2 were detectable by ^{111}In-Nd2. Most T2-sized pancreatic tumors are resectable.[30] Therefore, RAID with Nd2 may provide information that will assist in determining whether surgical intervention is warranted. Goldenberg[29] pointed out that tumors as small as 0.5 cm can be detected by RAID. Further studies are in progress to determine if such tumors are detectable with ^{111}In-Nd2.

There have been reports in animal[31] and clinical[32] studies that antigen-antibody complexes have altered the blood clearance and normal organ distribution of a radiolabeled antibody and produced adverse effects such as poor tumor targeting and a reduction in antibody level. Beatty *et al.*[33] have shown in animal models that complexation with circulating CEA results in an increased hepatic uptake of ^{111}In-anti CEA-CEA complexes. Pretreatment with unlabeled anti-CEA antibody reduced the liver uptake of radiolabeled anti-CEA. On the other hand, there have been several other studies[34–36] in which no correlation was found between circulating CEA and tumor targeting. Carrasquillo *et al.*[37] and Colcher *et al.*[38] have reported a positive correlation between circulating TAG.72 and tumor detectability, suggesting that circulating antigen did not interfere with tumor targeting. Thus, the situation is not clear-cut. Nd2 may be advantageous in tumor targeting, because its antigen is not present in the circulation.

The present study confirms the safety of a single 2 mg intravenous dose of ^{111}In-Nd2. There were no significant abnormal laboratory results secondary to MoAb infusion. On the other hand, 13 of 18 patients developed a HAMA response after Nd2 infusion. This was not unexpected. The development of HAMA has previously been reported in clinical studies with several MoAbs (B72.3, CC49, CC83, MN-14 and Mu-9). Sharkey *et al.*[19] have reported that the incidence of HAMA development was markedly higher in patients who received high doses of MoAb compared with those who received a lower dose. Patients who received intact MoAbs showed higher levels of HAMA compared to those who received the $F(ab')_2$ fragments. The generation of HAMA may affect the pharmacokinetics of the MoAb. There may be a faster whole-body elimination of radioactivity, decreased tumor uptake, and lack of therapeutic response in patients treated with radiolabeled MoAbs.[39] Moreover, when radioisotope-labeled antibodies or drug conjugates of antibodies are used in therapy, the patient would receive multiple doses of immunoconjugates rather than a single infusion. Chimeric human-mouse MoAbs elicit a lower level of HAMA[40–42] than the original murine MoAb.

A mouse/human chimera of Nd2 has been prepared that has the same specificity for human pancreatic cancer tissues and the same biodistribution kinetics in the mouse model as the original murine MoAb.[43] The fact that ^{111}In-Nd2 accumulates in pancreatic tumors indicates that radioisotope-labeled Nd2 or drug conjugates of Nd2 may be useful in therapy. ^{131}I-Nd2 produced tumor regression in nude mouse models.[44] Therefore, RAID with ^{111}In-Nd2 provides not only diagnostic information, but can also be used to indicate whether targeting therapy with Nd2 is feasible.

In conclusion, this initial clinical evaluation showed that ^{111}In-Nd2 is highly specific in the identification of pancreatic exocrine tumors. It differentiated exocrine pancreatic tumors from tumor masses formed in benign conditions and in endocrine pancreatic cancer. Moreover, it had a 100% sensitivity in the radiolocalization of exocrine tumors in those cases where Nd2 antigen was expressed by at least 50% of the cell population.

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