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Preoperative Clinical Radioimmunodetection of Pancreatic Cancer by ^{111}In-labeled Chimeric Monoclonal Antibody Nd2

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The present study was carried out with the purpose of evaluating the clinical usefulness of radioimmunodetection (RAID) with ^{111}In-labeled murine/human chimeric monoclonal antibody, Nd2 (c-Nd2) in patients with pancreatic cancer. Nineteen patients suspected to have pancreatic cancer were administered intravenously 74 MBq/2 mg ^{111}In-labeled c-Nd2 in 100 ml of saline containing 2% albumin over 30 min. A scintigram was obtained on the 3rd day after infusion by using single photon emission computed tomography (SPECT) imaging. Of the 14 patients finally diagnosed as having pancreatic cancer on the basis of surgical specimens or progress of disease, specific focal uptake at the site of the tumor was detected in 12 (true positive cases), representing a sensitivity of 85.7% (12/14), and liver metastasis was found in one case with metastasis. Of the 5 patients diagnosed with tumor-forming pancreatitis (TFP), 4 patients demonstrated true negative imaging, but one patient whose tumor demonstrated interesting findings in histology and immunostaining, showed false positive imaging. Of patients investigated for human anti-chimeric antibody (HACA) response, none showed HACA response, and no allergic reaction was seen in any of the patients administered c-Nd2. These results suggest that RAID with ^{111}In-labeled c-Nd2 is useful for differential preoperative diagnosis between invasive pancreatic cancer and TFP.

Key words: Radioimmunodetection — Pancreatic cancer — Chimeric monoclonal antibody — Nd2

Pancreatic cancer is one of the most intractable cancers and its prognosis is extremely poor because of its aggressive malignant potential. Earlier precise detection and differential diagnosis by new diagnostic procedures may provide benefits in the management of pancreatic cancer and improvement of its prognosis. Monoclonal antibodies (MoAbs) such as CA19-9, SPan-1[1)] and DU-PAN-2 have been applied for the serological detection of pancreatic cancer. Another application of MoAbs for cancer diagnosis is radioimmunodetection (RAID). Recently, immunoscintigraphy with radiolabeled MoAbs has been shown to be clinically useful in various malignant diseases, particularly colorectal cancer.[2–4)]

Nd2, a murine IgG1 MoAb was produced against mucin fraction purified from xenografts of human pancreatic cancer cell line SW1990[5)] and has been reported to have potential for diagnostic and therapeutic applications.[6–9)] In clinical applications, administration of murine MoAbs can induce human anti-mouse antibody (HAMA), which affects the pharmacokinetics of MoAbs. Thus, we prepared mouse/human chimeric Nd2 (c-Nd2), which retains the high affinity and specificity to pancreatic cancer of murine Nd2 (m-Nd2).[10, 11)] The present study was carried out to investigate the clinical usefulness of c-Nd2 for preoperative diagnosis after labeling with ^{111}In, and its therapeutic potential for pancreatic cancer.

PATIENTS AND METHODS

Patients Nineteen patients (8 females and 11 males, with a mean age of 63.7 years, range 47–75 years), admitted to Osaka City University Hospital with suspected pancreatic cancer, were entered in this study; their characteristics are summarized in Table I. All the patients were initially or tentatively diagnosed as having primary pancreatic cancer, as suggested by computed tomography (CT), ultrasonography (US), magnetic resonance imaging (MRI), endoscopic retrograde pancreatography (ERP), or the serum level of tumor markers (CA19-9, SPan-1 and CEA), and they were scheduled to undergo surgery. Informed consent was obtained from all patients before participation in the study; the study and consent forms were approved by the Human Ethics Committee of the Osaka City University Medical School.

The tumor sites of the 19 patients were 9 in the head, 9 in the body and one in the tail (mean size 4.0 cm, range

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Table I. Patient Characteristics and Clinical Data

Patient no.	Age and sex	Tumor site by CT scan	Tumor size (cm)	Tumor marker		
				CA19-9 (U/ml)	SPan-1 (U/ml)	CEA (ng/ml)
01	62F	head	2.0	665	253	3.3
02	59M	head	4.9	25	11	3.3
03	63M	head	3.0	745	261	5.7
04	47M	body+CA inv.+liver meta[a)]	8.0	177	414	3.5
05	71F	body+SMA inv.[b)]	4.0	145	82	4.3
06	58M	body	2.0	122	48	12.4
07	67F	head+CA inv.	5.2	232	101	11.2
08	53M	body+CA inv.+liver meta	7.0	1,105	382	3.7
09	67M	head	4.6	484	47	8.2
10	75M	head	3.0	613	286	9.6
11	59M	head+SMA inv.+liver meta	3.6	1,552	605	5.2
12	66M	body+CA inv.	3.0	439	150	5.1
13	62F	head	3.0	5	1	3.3
14	55F	body+CA inv.	6.5	301	110	6.0
15	75F	tail+liver meta	5.0	10,383	4,845	23.6
16	70F	body	2.0	20	11	1.4
17	66M	body	2.0	6,084	2,124	4.4
18	70M	head+SMA inv.	3.7	88	44	7.0
19	65F	body	3.8	2,674	810	21.9

a) CA inv.+liver meta: celiac artery involvement and liver metastasis.
b) SMA inv.: superior mesenteric artery involvement.

2.0–8.0 cm). Tumor marker levels of CA19-9, SPan-1 and CEA in sera were measured using CA19-9 RIA Kit (Centocor, Malvern, PA), SPan-1 RIABEAD (Dainabot, Tokyo), and CEA RIABEAD (Dainabot). 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.

Preparation and administration of radiolabeled MoAb Nd2 is a murine MoAb of the IgG1 isotype, directed against purified mucins from human pancreatic cancer cell line SW1990.[5)] Rearranged *VH* and *Vκ* genes, which encode variable regions of m-Nd2, were cloned from m-Nd2 hybridoma cells by the standard procedures.[10)] The subcloned VH and Vκ DNAs were combined with human *Cγ1* and *Cκ* genes in the expression vectors pSV2-HG1-Nd2 and pSV2-HCκ-Nd2. Both expression vectors were cotransfected into SP2/0 cells, a non-Ig-secreting mouse myeloma (CRL1581; American Type Culture Collection). The transfectoma cells were cultured in E-RDF medium (Kyokuto Pharmaceutical, Tokyo) containing 2 ng/ml recombinant human interleukin-6, but no fetal calf serum. c-Nd2 was purified from the culture supernatant by affinity chromatography using an Immuno Pure IgG Purification Kit (Pierce, Rockford, IL). A 8:1 molar ratio of cyclic anhydride diethylenetriaminoepentaacetic acid (DTPA) to c-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 the DTPA-conjugated c-Nd2 was labeled with 37 MBq of ^{111}In-chloride by incubating for 30 min at room temperature. The labeling efficiency was greater than 90% without further purification and the final specific activity was nearly 37 MBq/mg.

^{111}In-labeled c-Nd2 (74 MBq/2 mg protein in 100 ml of normal saline solution with 2% human albumin) was infused over 30 min in patients who were confirmed not to be sensitive to c-Nd2 by means of a skin test.

Imaging (RAID) The patients were imaged on the 3rd day after injection according to the data of the previous study,[6)] taking into account the biodistribution of Nd2 and the half life of ^{111}In. SPECT of ^{111}In-labeled c-Nd2 was obtained using a triple-head rotating γ camera; Toshiba GMS-5500 with the photo window in 120 projections with an acquisition time of 30 s each and 360° rotation (128×128 matrix). The raw projections were stored on magnetic disk and after preprocessing of the data by nine-point weighted smoothing, the filtered back-projection method was used for SPECT image reconstruction. Transverse, coronal and sagittal sections were reconstructed and assessed in a nonblinded fashion by two nuclear medicine physicians.

Immunohistochemical antigen expression Surgical specimens from all patients undergoing operation were

fixed in formalin and embedded in paraffin. Immunoperoxidase staining with c-Nd2 was performed using the avidin-biotin-peroxidase complex method.[11] A specimen was considered positive if at least 10% of an optimal field (×20 objective) was stained.

Immune response The human anti-chimeric antibody (HACA) response was determined in serum samples collected prior to infusion and at 2–3 weeks after infusion, by using a sandwich enzyme immunoassay.[7] Briefly, 96-well plates were coated with c-Nd2. Serum samples were added followed by biotinylated c-Nd2. After incubation and washing, streptavidin-peroxidase complex was added, followed by the substrate, 2,2′-azino-di-3-ethylbenzthiazolinesulfonic acid. The optical density was measured at 405 nm, and a HACA response was considered positive when the mean absorbance of the post-infusion sample was at least twice the mean absorbance of the pre-infusion sample.

RESULTS

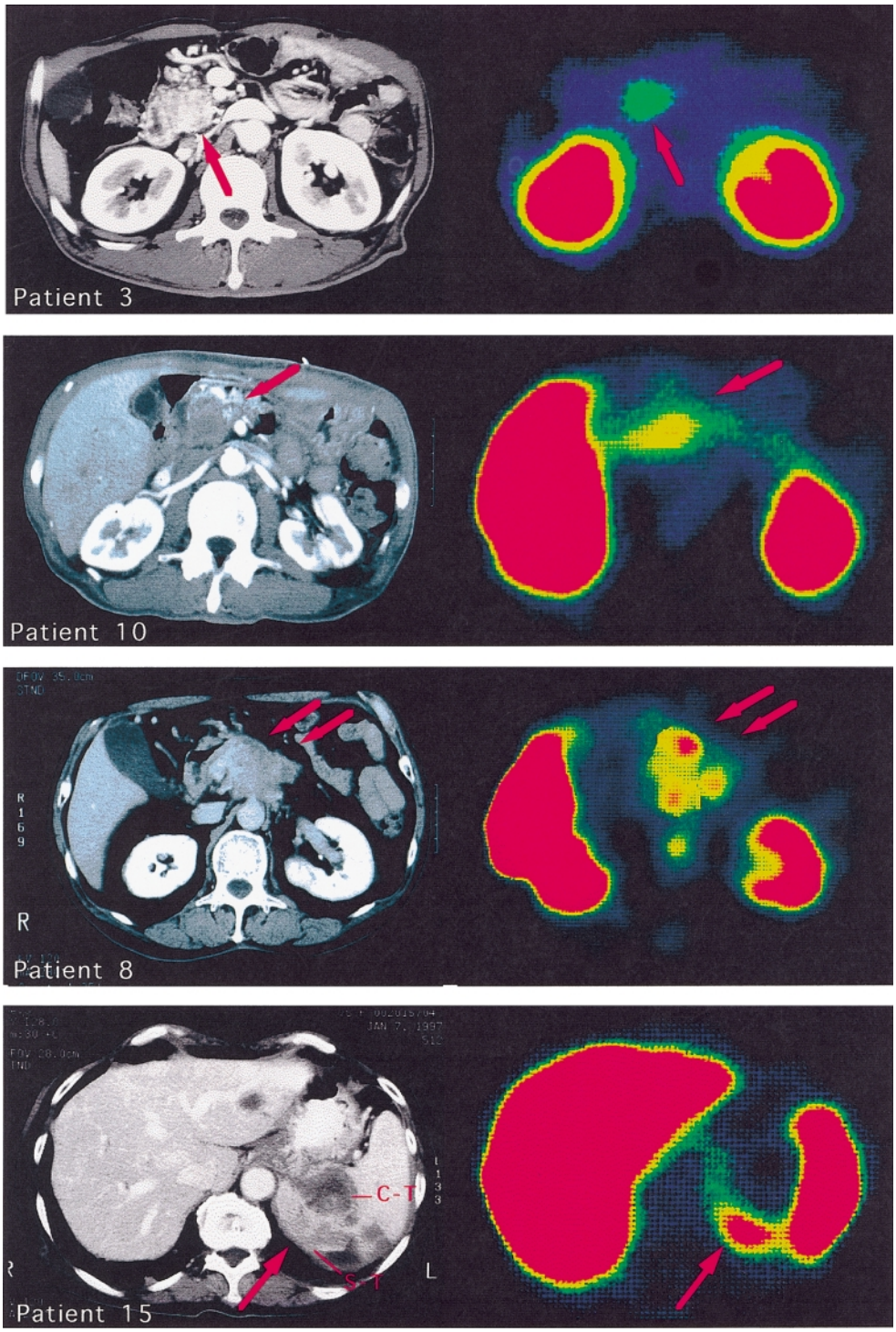
Patients and outcome of RAID The final diagnosis, treatment of patients and outcome of RAID are summarized in Table II. Of the 19 patients, 14 were finally diagnosed as primary pancreatic cancer and 5 as chronic pancreatitis (TFP).

Of the 14 patients with pancreatic cancer, 10 patients underwent laparotomy and final diagnosis was made histologically with surgical specimens, but the other 4 patients (patients; 12, 15, 18 and 19) were found to have inoperable advanced cancer accompanied with involvement of the celiac or superior mesenteric artery, or multiple liver metastasis by diagnostic procedures such as CT, US or MRI. These patients were finally diagnosed as pancreatic cancer on the basis of progress of disease and underwent only palliative therapy (radiation and/or medication) mainly for controlling pain. Of the 10 patients who underwent surgery, 4 underwent resection of cancer (3 pancreaticoduodenectomy and one distal pancreatectomy) with intraoperative radiation therapy (IOR; 20–25 Gy), two underwent probe laparotomy only with tumor biopsy, and 4 underwent a by-pass surgical procedure (4 gastrojejunostomy, in one with choledochojejunostomy). These patients were histologically confirmed to have pancreatic cancer (3 well differentiated, 6 moderately differentiated and one poorly differentiated ductal adenocarcinomas).

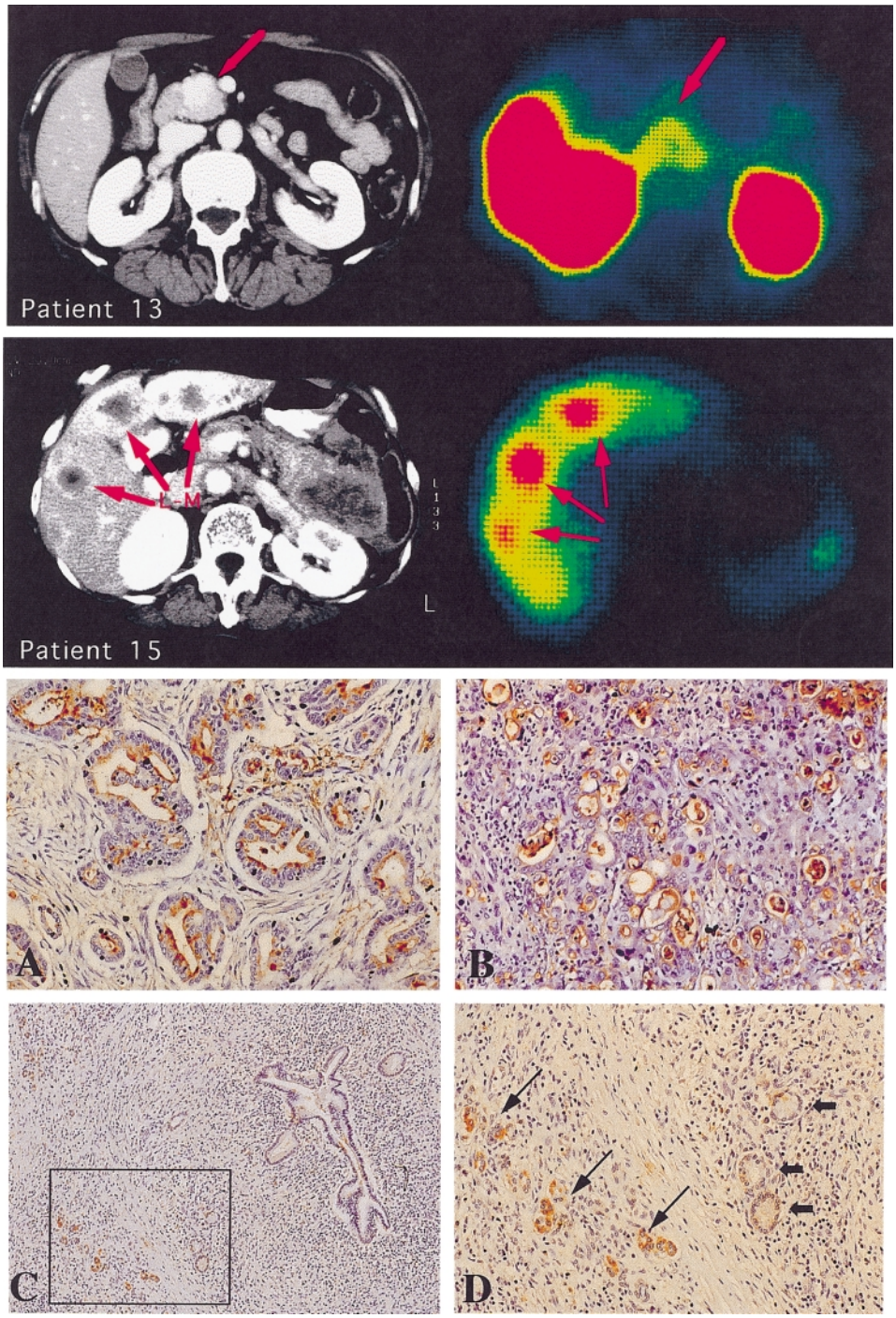
Table II. Treatment, Final Diagnosis and Outcome of RAID by ^{111}In-cNd2

Patient no.	Treatment[a] (Surgical procedure)	Final diagnosis[b] (Histological finding)	Stage[c]	Nd2 imaging	Outcome of RAID
01	PD+IOR	PC (well)	III	+	True positive
02	Probe laparotomy	TFP	0	–	True negative
03	PD+IOR	PC (well)	III	+	True positive
04	Gastrojejunostomy	PC (moderately)	IV	+	True positive
05	Probe laparotomy	PC (well)	IV	–	*False negative*
06	DP+IOR	PC (moderately)	II	–	*False negative*
07	Probe laparotomy	PC (poorly)	IV	+	True positive
08	Gastrojejunostomy+IOR	PC (moderately)	IV	+	True positive
09	Choledochojejunostomy	TFP	0	–	True negative
10	PD+IOR	PC (moderately)	IV	+	True positive
11	Gastrojejunostomy+ choledochojejunostomy	PC (moderately)	IV	+	True positive
12	no surgical treatment	PC (–)	IV	+	True positive
13	PpPD	TFP	0	+	*False positive*
14	Gastrojejunostomy+IOR	PC (moderately)	IV	+	True positive
15	no surgical treatment	PC (–)	IV	+	True positive
16	no surgical treatment	TFP	0	–	True negative
17	no surgical treatment	TFP	0	–	True negative
18	no surgical treatment	PC (–)	III	+	True positive
19	no surgical treatment	PC (–)	III	+	True positive

a) PD, pancreaticoduodenectomy; DP, distal pancreatectomy; PpPD, pylorus-preserving pancreaticoduodenectomy; IOR, intraoperative radiation therapy.
b) PC, pancreatic cancer; well, well differentiated ductal adenocarcinoma; moderately, moderately differentiated ductal adenocarcinoma; poorly, poorly differentiated ductal adenocarcinoma; TFP, tumor-forming chronic pancreatitis; (–), no histological diagnosis without surgery.
c) pTNM (UICC) stage; 0, can not be classified because of no malignancy.



ig. 1. Enhanced abdominal CT scan and single photon emission computed tomography (SPECT) with ^{111}In-labeled chimeric Nd2 in atients with primary pancreatic cancer. Patients 3, 10: SPECT reveals specific accumulation of ^{111}In-labeled chimeric Nd2 (arrow; ight) at a site corresponding to the tumor in the head of the pancreas detected in the CT scan (arrow; left). Patient 8: true positive PECT imaging corresponds to tumor location in the body of the pancreas. Patient 15: true positive imaging in the tail of the pancreas S-T, part of solid tumor; C-T, part of cystic tumor).



ig. 2. Enhanced CT scan and SPECT imaging in patients with TFP and liver metastasis, and immunohistochemical stainings in the esected specimens (A–D). Patient 13: positive tumor imaging in SPECT corresponds to tumor location in the head of the pancreas. The esected specimen of this patient revealed benign tumor-forming pancreatitis without malignancy (C: ×100), implying *false positive* maging. Partial ductal hyperplasia on distal pancreatic ducts is recognized (short arrow in D, ×200), and positive staining by chimeric d2 can be seen in these ducts and destroyed acinar cells neighboring these ducts (long arrow in D). Patient 15: focal high uptakes cor-esponding to the metastatic sites in the liver (L-M) are seen by SPECT imaging in patient 15 with multiple liver metastases. Immuno-istochemical staining with chimeric Nd2 is positive in the resected specimens (A, B: ×200); A, well differentiated adenocarcinoma in atient 10; B, moderately differentiated adenocarcinoma in the liver metastasis in patient 8.

Of the 5 patients with TFP, 3 patients (patients; 2, 9 and 13) underwent surgery. One patient underwent pylorus-preserving pancreaticoduodenectomy (PpPD) owing to the obstruction of the duodenum and common bile duct by the tumor, two underwent choledochojejunostomy accompanied with tumor biopsy, without resection of tumors. The other two patients (patients; 15 and 16) were diagnosed as TFP on the basis of various examinations, and did not undergo any surgical treatment. While they have been followed for over two years after RAID, they have shown no finding indicative of malignant tumor.

In all patients administered ^{111}In-labeled c-Nd2, a non-specific normal uptake of radioactivity was found in the liver, spleen, bone marrow, and kidneys. Of the 14 patients with primary pancreatic cancer (UICC stage; II-1, III-9, and IV-4), specific focal uptake at the site of the tumor was found in 12 patients, implying a sensitivity for the detection of tumors of 85.7% (12/14) (Fig. 1; patients 3, 10, 8 and 15). Of the patients with liver metastasis, one patient exhibited higher focal uptakes at metastatic sites, when the window level was raised to reduce non-specific accumulation in normal liver (Fig. 2; patient 15). Of the 5 patients with benign chronic pancreatitis, four patients had true negative scan, and the specificity was 80.0% (4/5), but one patient with TFP who underwent PpPD was false positive (Fig. 2; patient 13). Therefore, the final diagnostic accuracy was 84.2% (16/19).

Immunostaining and HACA response Immunostaining with c-Nd2 was performed on 13 specimens obtained by tumor resection or biopsy; 10 were pancreatic cancer and 3 were TFP. Of the 10 cancer specimens, 8 showed fairly strong positive stainings corresponding to the results of positive RAID (Fig. 2, A and B). Normal pancreatic tissues and regions of chronic pancreatitis accompanying these resected specimens did not show any expression of c-Nd2, and tumor-forming chronic pancreatitis exhibited no reaction to c-Nd2 except for one false positive case (patient 13). Although the TFP specimens revealed destroyed acinar cells, fibrosis and lymphocytic infiltration without malignancy, partial ductal hyperplasia on distal pancreatic ducts was recognized and c-Nd2 revealed mild reaction to these hyperplastic ducts and destroyed acinar cells neighboring these ducts, but not to islet cells (Fig. 2, C and D).

HACA response was investigated in 10 patients, and the ratio of measured post-infusion absorbance against pre-infusion absorbance was in the range of 0.88–1.71 (mean ratio; 1.16). Of the patients investigated for HACA response, all were considered negative, and no immediate or delayed allergic reaction and no abnormal laboratory results were seen in any patient after administration of c-Nd2.

DISCUSSION

The RAID of malignant disease with MoAb can be valuable in terms of detection (location, staging, and recurrence), differential diagnosis, and selection of patients for therapy. Although RAIDs have been successfully applied to several kinds of malignant tumors in organs such as colon,[2–4] lung,[12] and others,[13] there have been few studies on pancreatic cancer.[14, 15]

^{111}In usually accumulates nonspecifically in the reticuloendothelial system, such as liver, spleen and bone marrow. DTPA is usually excreted from kidney. Therefore, in the case of ^{111}In-DTPA conjugated MoAb, nonspecific accumulation in these organs is generally observed, and sometimes prevents distinct imaging of a tumor. In the previous study, we described clinical RAID with ^{111}In-labeled m-Nd2 in patients with pancreatic cancer by using anterior planar scintigraphy without SPECT, and those planar imagings produced 8 true positives in 12 primary pancreatic cancers (sensitivity; 66.7%).[7] While no false positive was observed, SPECT imagings were thought to be necessary to improve the clinical accuracy.

RAID with SPECT imaging is now offering good results.[2, 3, 12] The present study with ^{111}In-labeled c-Nd2 and SPECT produced a sensitivity of 85.7% (12/14). Two false negative cancers actually revealed no reactivity to c-Nd2 on immunostaining, so it could be said that the real sensitivity was 100%. In this study, we had no case of T1, less than 20-mm-diameter tumor, but Goldenberg[2] has pointed out that tumors as small as 5 mm diameter can be detected. Therefore, it may be possible to detect small pancreatic cancers by RAID with c-Nd2, but further study is needed to confirm this.

Recently, the development of diagnostic techniques has increased the ability to detect pancreatic tumors. But the differential diagnosis of pancreatic cancer remains inadequate even with histo-cytological diagnosis by endoscopic tumor biopsy or ERP because of technical difficulties and stress to patients. Pancreatic cancer should be discriminated from other pancreatic tumors, particularly benign TFP. While our present study included five cases of TFP in patients with suspected pancreatic cancer, two cases (patients; 2 and 9) were confirmed histologically as TFP with no malignancy, and immunostaining also revealed negative reaction to c-Nd2. But one case of TFP (patient; 13) was false positive in RAID with c-Nd2. While Nd2, in general, shows no reaction to tissues of normal pancreas (including pancreatic ducts, acinar cells and islet cells) and chronic pancreatitis,[5, 11] this surgical specimen demonstrated positive reactivity to Nd2 on acinar cells surrounding the branch of the pancreatic duct with epithelial hyperplasia, which is an unusual finding in chronic pancreatitis. Hyperplasia of pancreatic ducts is usually found in cases of mucin-producing pancreatic tumor (particularly

intraductal papillary adenoma) and cystadenoma, which have high malignant potential. The histological and immunohistochemical findings suggest that this tumor may have the potential to transform, so we intend to follow up this patient rigorously. While two cases of TFP showed high values of tumor markers, patient 9 had jaundice induced by the obstruction of bile duct by the tumor and patient 17 was found to have bronchiectasis and adrenal gland swelling besides TFP without malignant disease. These conditions were supposed to be the reason for the high values of tumor markers.

Because of the immunogenicity of murine and other animal antibodies, humanized (chimeric) or human antibodies should be prepared for clinical RAID and therapeutic trials.[16–18] While we used ^{111}In-labeled m-Nd2 for RAID of pancreatic tumors in the previous study, of 18 patients investigated for HAMA response in sera, 13 were positive (72.2%). This result is consistent with other reports on the HAMA response in RAID with murine MoAbs.[19, 20] The present study has confirmed the low immunogenicity of c-Nd2 in HACA assay, and these findings suggest that c-Nd2 would be advantageous in the clinical application of immunotherapy, for which multiple doses of antibodies are required.

It has been reported that chimeric antibodies with the human IgG1 isotype are able to induce antibody-dependent cell-mediated cytotoxicity (ADCC) more efficiently than their parental murine antibodies,[7, 21–23] and several chimeric antibodies have been used for therapy in the clinical setting in patients with lung, colon, ovary, and breast cancer.[18, 24, 25] Recently, we reported that c-Nd2 can also induce *in vitro* ADCC activity against pancreatic cancer cells,[26] and shows *in vivo* inhibitory effect on the growth of pancreatic cancer in nude mice. These results suggest that c-Nd2 may have potential for immunotherapy of pancreatic cancer to prevent recurrence and metastasis after surgical curative resection, and/or to improve the prognosis and QOL of unresectable advanced cancer patients.

While pancreatic cancer incidence is increasing in Europe, United States and Japan, only about 20–30% of patients undergo pancreatic resection (4/14; 28.6% in the present study).[27] Most patients having pancreatic cancer (ductal adenocarcinoma) present with aggressive local invasion to vessels or with liver metastasis, preventing curative resection, and therefore early diagnosis, particularly in T1 or stage I, is required. Even patients treated surgically often show local recurrence or liver metastasis within two years after resection, and the 5-year survival rate is as low as 10%.[28] Therefore, it seems worthwhile to evaluate the clinical utility of c-Nd2 for anti-pancreatic tumor therapy through ADCC.

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