Comparison of Iodine-123-Vasoactive Intestinal Peptide Receptor Scintigraphy and Indium-111-CYT-103 Immunoscintigraphy

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Recently, we have shown that the expression of receptors for vasoactive intestinal peptide (VIP) on intestinal adenocarcinomas can be used for in vivo targeting of primary or metastatic tumor sites using ^{123}I-labeled VIP. Several other receptors and antigens including the TAG-72 protein have also been implemented for in vivo localization purposes. In this study, we have compared the in vitro and in vivo binding of ^{123}I-VIP and of the ^{111}In-labeled monoclonal antibody (MAb) directed against TAG-72 (OncoScint®; ^{111}In-CR-103) in patients with intestinal adenocarcinomas in a single-blinded, prospectively randomized trial. **Methods:** Twenty patients were administered either ^{123}I-VIP (150–200 MBq; 1 μg) or ^{111}In-CYT-103 (150 MBq; 1 mg) for one imaging study. After interim analysis demonstrated superior imaging with ^{123}I-VIP, the next 10 patients (accounting for a total of 50 patients) enrolled in this trial underwent both studies in random order to allow for a direct comparison. **Results:** In total, ^{123}I-VIP scans were true-positive in 28 of 30 patients (93%) versus 17 of 30 patients administered ^{111}In-CYT-103 (56%). In the subgroup of 10 patients enrolled in the second part of the study, primary intestinal adenocarcinomas were imaged in five of five patients with ^{123}I-VIP and in only two of these patients with ^{111}In-CYT-103. Liver metastases were visualized in five of six patients by ^{123}I-VIP receptor scanning and in four of these patients with ^{111}In-CYT-103. The in vitro results indicated significant binding of ^{123}I-VIP to primary colorectal tumors as well as to HT29 and COLO320 adenocarcinoma cells. In vitro, adenocarcinoma cells also expressed abundant numbers of the TAG-72 antigen. **Conclusion:** Intestinal adenocarcinomas co-express VIP receptors and the TAG-72 antigen. Despite significant in vitro binding of both agents, however, the VIP receptor scan is more sensitive in localizing intestinal adenocarcinomas and metastatic spread.

Key Words: vasoactive intestinal peptide; tumor-associated glycoprotein 72; gastrointestinal adenocarcinomas; imaging

J Nucl Med 1996; 37:1480–1487

Gastrointestinal malignancies are among the leading causes of morbidity and mortality worldwide (*1*). Because of the strong association between early detection of primary and/or relapsing cancers and prognosis, such patients present a special challenge to clinicians. Apart from endoscopy, noninvasive conventional radiologic imaging by CT or ultrasound measurements, especially in patients suffering from liver metastases, remain the methods most widely applied. Despite their clinical relevance, these techniques still suffer from major shortcomings, as small-sized tumors or peritoneal carcinosis may escape detection by conventional imaging. The use of radiolabeled antibodies directed against certain tumor epitopes seems to be an appealing concept to further enhance the diagnostic armamentarium. Though the experimental results of tumor imaging using radiolabeled antibodies date back to the 1950s (*2*), the first data obtained in patients were reported by Goldenberg in 1978 (*3*). Improvement of immunoscintigraphy was achieved since then by the development of monoclonal antibodies (MAbs) for clinical use. Especially in colorectal cancer, antibodies directed against CEA have given promising results. Also, the B72.3 antibody (^{111}In-CYT-103; OncoScint®) directed against the tumor-associated glycoprotein-72 has gained widespread clinical interest (*4*). This murine monoclonal antibody was first described in 1981 (*5*) and has been used in various studies both in animals (*6*) and man (*7–11*) since then because of its reactivity with a variety of mucin producing adenocarcinomas (*12,13*) including colorectal, gastric, pancreatic and ovarian cancer. Sensitivity rates of up to 100% for radioimaging of colorectal carcinomas in patients with rising serum CEA-levels, but otherwise negative workup (*10*) have been reported. The exact effect of ^{111}In-CYT-103 for the detection and follow-up of gastrointestinal cancers, however, has yet to be established. The exploration of alternative methods is also warranted.

Recently, we demonstrated that ^{123}I-labeled vasoactive intestinal peptide (VIP) has potential for localizing even small gastrointestinal tumors expressing receptors for VIP (*14*). VIP consists of 28 amino acids and acts as a neuroendocrine mediator under physiologic conditions, with an important role in water and electrolyte secretion in the gut (*15*). Recent findings suggest that VIP also promotes growth and proliferation of normal as well as malignant cells (*16–18*). These biologic effects are mediated via receptors located on the cell surface membrane of normal and various neoplastic tissues, including carcinoids (*19*), colorectal cancer (*19–21*) and pancreatic cancer (*20,22*). The high specificity of ^{123}I-VIP receptor scanning for human adenocarcinomas is most probably due to the very high number of VIP binding sites, while normal cells express much lower amounts of receptors (*19*).

The promising results obtained by ^{123}I-VIP receptor imaging in patients with intestinal adenocarcinomas (*14,23*) prompted us to initiate a single-blinded, prospectively randomized trial to allow for a direct comparison between VIP receptor scanning and immunoscintigraphy using ^{111}In-CYT-103.

METHODS

Patients and Study Design

Forty patients with histologically verified adenocarcinoma of the colon, pancreas and stomach were randomized between scanning with either ^{123}I-VIP or ^{111}In-CYT-103. All patients had advanced disease and underwent palliative chemotherapy. For patient characteristics see Table 1. A treatment-free interval of at least 2 wk

Received Sept. 18, 1995; revision accepted Jan. 24, 1996.

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was required for application of the radiotracers, and no prior therapeutic or diagnostic antibody injections were allowed. After histologic verification of target lesions, informed consent according to institutional guidelines was obtained. Randomization was performed by the oncologist; nuclear physicians were blinded to the exact nature, stage and location of disease. All patients had CT scans, ultrasound or endoscopic evaluations not older than 6 wk at the time of tracer application. Patients randomized to receive ^{123}I-VIP were given sodium perchlorate and potassium iodide for thyroid gland blockade. Patients were followed by means of CT, sonography and x-ray, respectively, until death or for at least 6 mo to document the exact course of the disease, i.e., development of new metastatic lesions or progression of known lesions.

Endoscopic investigations were performed only initially for biopsy, but were not routinely repeated during the trial. Endpoints of the first part of the trial were the number of true positive and negative scan results as compared with conventional radiologic and endoscopic diagnosis, and results of surgical exploration. These repeated measurements were aimed to indirectly assess the scan results, and to exclude possibly false-positive scan results (defined as tracer accumulation without documented radiological counterpart at the time of the scan). According to the study protocol, a false-positive scan result in the final evaluation would have to be changed to a true-positive result if radiological or surgical proof was provided. Furthermore, autopsy results, if available, were taken into account for patients who died during the 6-mo follow-up period.

Of 20 patients (9 women, 11 men) randomized to receive ^{123}I-VIP, 14 had colorectal cancer and 6 had pancreatic adenocarcinomas. Five patients with colorectal cancer presented with local recurrence of their disease; in nine patients the primary tumor had been resected and metastases were known at the time of scintigraphy. Whereas lesions were confined to the liver in five patients and to the lung in one, two patients suffered from disease metastatic to both liver and lymph nodes, and one patient to liver and lung, respectively. Locally inoperable pancreatic cancer as the only manifestation of disease was present in two patients. In the other four patients with pancreatic cancer, the primary tumor had been resected and liver metastases were known at the scanning time.

Primary tumor types in the 20 patients (11 women, 9 men) randomized to application of ^{111}In-CYT-103 were as follows: pancreatic cancer (3 patients), gastric cancer (4 patients), and colorectal cancer (13 patients). The three patients with pancreatic cancer and three of the four patients with gastric cancer had inoperable disease confined to the primary tumor site, whereas liver metastases were present in the fourth patient with gastric cancer. In all patients with colorectal adenocarcinomas, the primary tumor was resected and liver (all 13 patients), lung (3 patients) and lymph node (2 patients) metastases were known at the scanning time.

Three women and seven men with histologically verified adenocarcinomas of the gastrointestinal tract were enrolled in the second part of the trial. Eight patients suffered from colorectal cancer, one had gastric cancer and one pancreatic cancer. In all colorectal cancer patients, resection of the primary tumor had been performed. Of this group, four patients had liver metastases as the only site of recurrence at the time of study entry, one was diagnosed with tumor recurrence in the lung, two patients suffered from locally recurrent disease and one patient presented both with locally recurrent cancer and liver lesions at the time of study entry. The remaining two patients (one patient with gastric and one with pancreatic adenocarcinoma) were deemed unresectable at the time of diagnosis. While local tumor spread without evidence of metastases was present in the patient with gastric cancer, the other patient also had liver metastases along with the primary pancreatic tumor. Gastroenteroanastomosis had been performed in both patients for symptomatic palliation. All patients had advanced disease and were undergoing 5-fluorouracil-based palliative chemotherapy. A treatment-free interval of at least 2 wk was required for application of the VIP- or CYT-103-scan in random order with a time of 4 to 6 wk between these two modes of imaging. No previous diagnostic or therapeutic antibody injections were allowed, and all patients gave informed consent according to institutional guidelines. All patients had CT scans, ultrasound or endoscopic investigations not older than 6 wk at the time of study entry.

Endpoints of the trial were the evaluation of the number of true-positive and true-negative scan results with regards to primary tumor site, liver metastases and further radiologically verified sites of tumor spread. Again, nuclear physicians were blinded not only to the nature of the primary malignancy (colorectal, pancreatic or gastric cancer), but also to the extent of tumor manifestations until the final evaluation of all scans performed during the trial. Patients were randomized by the medical oncologist by tossing a coin between application of the VIP-scan or CYT-103 scan as the first imaging modality. Follow-up procedures, including repeated radiologic assessment of tumor growth, were identical to those performed in patients randomized to only one scanning modality (vide supra).

According to the study protocol, HAMA testing was to be performed in this trial only in patients developing side effects and/or in whom repeated ^{111}In-CYT-103 scintigraphy would be repeated.

Preparation of Radioiodinated VIP

The preparation of radioiodinated VIP has been described previously in detail (*12,21*). In brief, VIP was generated by a peptide synthesizing machine and labeled with ^{123}I using a modified lodogen method. Iodine-123-VIP was purified by preparative HPLC (column: RP C18, 5 μm, 4 × 250 mm, eluent: 74% (volume/volume) aqueous 0.25 *M* triethylammoniumformiate, pH 3, 26% (volume/volume) acetonitrile at 1 ml/min) to obtain a high specific activity. The column eluent passed through a scintillation radioactivity detector and UV (280 nm) detector in a series. The system was calibrated with unlabeled VIP and enabled collection of pure radioiodinated VIP, separated from unlabeled VIP, reagents and inorganic iodine species. The eluent was evaporated at reduced pressure. The product was dissolved in phosphate-buffered saline containing 0.1% (weight/volume) Tween 80 (Koch-Light Lab. Ltd., Colnbroke, UK). The labeled product was analyzed by analytical HPLC (corresponding to the preparative system, however, using a dedicated analytical column) and zone electrophoresis (Whatman 3 MM paper, 0.1 *M* barbital buffer, pH 8.6, using a field of 300 V for 10 min). The percentage of unbound iodine (<3% in all preparations) remained stable over at least 24 hr. The biological activity of labeled and unlabeled VIP was identical as determined by its ability to enhance cAMP-formation, ^{32}P-ATP-incorporation as well as ^{3}H-thymidine-uptake (*12,18*). Before injection, ^{123}I-VIP was filtered through sterile Millex GV 0.2 μm membranes (Millipore, Milford, MA). Iodine-123-VIP was administered as a single intravenous bolus injection in 3 ml 0.9% NaCl-solution (150–200 MBq; 1 μg VIP). During application of labeled VIP, all patients were monitored for blood pressure (*12*).

Preparation of Indium-111-CYT-103

The commercially available antibody (OncoScint®, ^{111}In-CR-103) was purchased from Cytogen (Princeton, NJ) and labeled with $^{111}InCl_3$ according to the manufacturer's recommendations. The final specific activity amounted to approximately 150 MBq/mg MAb per patient. Radiochemical purity was assessed by paper chromatography and yielded more than 96% in each preparation.

TABLE 1
Patient Characteristics

Number of patients	50 (23 women, 27 men)
Mean age (yr)	64 (range 32–80)
Median performance status	1 (0–2)
Diameter of primary tumors*:	(2–10 cm, median 4.5 cm)
Colorectal cancer	4–10 cm median 5.3 cm
Pancreatic cancer	2–8 cm median 4.2 cm
Gastric cancer	4–8 cm median 5.1 cm
Diameter of metastases*:	
Liver	1.5–8 cm median 3.2 cm
Lung	1–4 cm median 2.2 cm
Lymph-nodes	2–3.5 cm median 2.5 cm

*As measured by conventional radiologic imaging (i.e., CT, US).

In Vitro Binding Studies

Binding studies were performed with primary tumor cell membrane fractions, tumor cell lines (COLO320, HT29) and normal blood cells (platelets and peripheral mononuclear cells, PMNCs). Assays were performed under essentially the same conditions reported earlier for other tumor tissues (*24*) and cells (*25*). Primary tumor specimens were obtained at surgery and kept at −70°C until used in receptor assays. Cell membrane fractions were prepared according to previously reported methods (*18,23*). The final protein content of the membrane suspension amounted to 100 μg protein/ml. Saturation studies were performed with intact cells or membrane fractions by incubating increasing concentrations of ^{123}I-VIP or ^{111}In-CYT-103 (0.01 to 100 n*M*) in the absence (total binding) and the presence of the same unlabeled ligand (100 n*M*, nonspecific binding). After incubation, the reaction mixture was diluted 1:10 with assay buffer (4°C) and rapidly centrifuged (5000 g, 10 min, 4°C) to separate membrane-bound from free ligand. The resulting pellet was washed twice with buffer and counted in a gamma counter for 1 min. Binding data were calculated according to Scatchard (*26*).

Gamma Camera Imaging and Analysis

Planar and SPECT acquisitions were performed with a large field of view gamma camera equipped with a low-energy (^{123}I-VIP receptor scintigraphy) or medium-energy (^{111}In-CYT-103 immunoscintigraphy) parallel-hole collimator.

For VIP-receptor scanning, sequential images (abdominal view) were recorded every minute for 30 min (matrix 128 × 128 pixels). Thereafter, planar images in anterior, posterior and lateral views of at least two regions covering the thorax and abdomen were acquired at 30 min, 2–4 hr and 18–24 hr after injection (matrix 128 × 128 pixels; 150–300 kcs were acquired). SPECT imaging of the abdomen was performed at 2–4 hr after tracer application in 21 of 30 patients.

For ^{111}In-CYT-103 scanning, planar imaging was performed at 48 and 72 hr after injection in anterior, posterior and lateral views of at least two regions covering the thorax and abdomen (matrix 128 × 128, 500 kcounts were required). SPECT imaging of the same regions was performed after completion of the planar study at 48 hr as well as 72 hr postinjection. For ^{111}In-CYT-103 scanning, both energy peaks (set at 173 keV and 247 keV) were used, both with a 20% window. All 30 patients underwent the 48-hr SPECT study, while 4 missed the 72-hr SPECT study.

SPECT was performed in a 360° circle in 6° steps, 30 sec per step. After processing by a dedicated computer (prefiltering with a Wiener filter or low-pass filter 4th order with a multiplier of 1.00, 10% cutoff frequency, postfiltering with a ramp filter), the data were reconstructed in three planes with slice with thicknesses of 7 mm. All scans were reconstructed and viewed by at least two, and in case of disagreement, by a third nuclear medicine physician. A simple yes-or-no evaluation system was used to evaluate the tumors or metastases as visualized by gamma camera imaging.

RESULTS

In Vitro Binding of Iodine-VIP and Indium-CYT-103 to Adenocarcinomas

Primary tumor specimens derived from patients with adenocarcinomas as well as the HT29 and COLO320 adenocarcinoma cells expressed significant amounts of VIP receptors as well as the TAG-72 antigen. The respective binding data are listed in Table 2, which indicates significantly ($p < 0.001$) higher numbers of ^{123}I-VIP receptors as compared with normal peripheral blood cells (platelets and PMNCs). In all experiments, the affinity constants were in the lower nanomolar range for both ligands. On adenocarcinoma cells, high numbers of antibody recognition sites were also demonstrated, whereas antibody surface binding to platelets and PMNCs was not found.

Imaging of Gastrointestinal Adenocarcinomas

Results of the First Study Phase (Randomized Study Phase). All 40 patients, randomized between imaging with ^{123}I-VIP and ^{111}In-CYT-103 as well as the 10 patients receiving both tracers randomly, were evaluated (for results see Tables 3–5). As assessed by CT, endoscopy and/or surgery, the size of the primary tumor masses ranged between 2 and 10 cm (median 4.5 cm) and the size of liver metastases ranged between 1.5 and 8 cm (median 3.2 cm).

Positive ^{123}I-VIP scans were obtained in 13 of 14 patients with colorectal and 5 of 6 patients with pancreatic cancer (Table 3). Primary tumors were imaged in five of five patients with locally recurrent colorectal and in one of two patients with pancreatic cancer. Visualization of liver metastases was successful in all eight patients with colorectal and in four patients with pancreatic cancer. Lymph node metastases were imaged in two of two patients and lung metastases in two of two patients. In total, only one scan was false-negative (one patient with localized adenocarcinoma of the pancreatic head).

Imaging of primary tumors with ^{111}In-CYT-103 was successful in two of six patients (one patient with pancreatic, one with gastric cancer. Liver metastases were imaged in 8 of 13 patients with colorectal cancer and in one patient with gastric cancer. The primary tumor could not be visualized in 4 of 6 patients and liver metastases were not detected in 5 of 14 patients. Lung metastases (three patients) and abdominal lymph node metastases (two patients) were not visualized on ^{111}In-CYT-103 images (Table 4).

In both studies, no side effects were seen, with the exception of a mild drop in blood pressure in patients in the ^{123}I-VIP group as reported previously (*14*).

Results of the Second Study Phase (Direct Comparative Study). Of the 10 patients receiving radiotracers randomly, 6 were given ^{123}I-VIP before ^{111}In-CYT-103 and 4 received the latter in their first study. VIP receptor scanning gave positive results in five of five patients with primary or recurrent tumor sites (colorectal (Figs. 1, 2), one pancreatic and one gastric adenocarcinoma) and was true-negative in five of five patients who had undergone resection of their primary tumor before tracer application (Table 5). Comparative ^{111}In-CYT-103-scanning (Fig. 2) was true-positive in two of five of these patients and true-negative in two of five patients, respectively. Imaging of metastatic liver lesions (Fig. 3) was true-positive in five of six and true-negative in four of four patients with ^{123}I-VIP

TABLE 2
In Vitro Binding of Iodine-123-VIP and Indium-111-CYT-103 to Adenocarcinoma Cells and to Normal Peripheral Blood Cells

		^{123}I-VIP		^{111}In-CYT-103	
		Sites/mg protein	K_d	Sites/mg protein	K_d
Prim. adenocarcinomas	H	$0.3 \pm 0.1 \times 10^{14}$	1.0 ± 0.4	$2.5 \pm 0.9 \times 10^{13}$	3.2 ± 1.5
(n = 8)	L	$1.0 \pm 0.3 \times 10^{14}$	3.1 ± 0.7		
HT29 cells (n = 6)	H	$0.8 \pm 0.4 \times 10^{14}$	0.5 ± 0.2	$5.6 \pm 2.0 \times 10^{13}$	5.4 ± 2.5
	L	$5.2 \pm 1.2 \times 10^{14}$	2.0 ± 0.9		
COLO320 cells (n = 6)	H	$1.0 \pm 0.6 \times 10^{14}$	0.4 ± 0.2	$6.8 \pm 2.5 \times 10^{14}$	3.5 ± 1.5
	L	$6.3 \pm 1.3 \times 10^{14}$	1.8 ± 0.8		
Platelets (n = 6)	H	$0.6 \pm 0.2 \times 10^{11}$	1.3 ± 0.3	No binding	
PMNCs (n = 6)	H	$0.7 \pm 0.3 \times 10^{11}$	0.2 ± 0.1	No binding	
	L	$2.1 \pm 1.2 \times 10^{11}$	1.1 ± 0.3		

K_d in nmol/liter. H = high-affinity sites, L = low-affinity sites.

versus four of six and one of four patients with ^{111}In-CYT-103, respectively. The lung metastases documented in one patient were not imaged with either ^{123}I-VIP or ^{111}In-CYT-103.

Overall Scan Evaluation. Iodine-123-VIP scans were positive in 28 of 30 patients (93%). Indium-111-CYT-103 scans were positive in 17 of 30 patients (56%), providing a statistical significance of $p < 0.01$ (χ^2 test). In total, 32 of 35 lesion sites (91%) were depicted with ^{123}I-VIP in 30 patients, whereas ^{111}In-CYT-103 depicted 21 of 37 lesion sites (57%) in 30 patients (χ^2 test: $p < 0.01$).

When compared with the overall scan results, a similar difference also was found in patients investigated in the second study phase alone in which 10 of 12 lesion sites (83%) were imaged with ^{123}I-VIP and only 6 of the same 12 lesion sites (50%) were detected by ^{111}In-CYT-103 (χ^2 test: $p < 0.01$).

Follow-up Period

During the 6-mo follow-up period, repeat CT and ultrasound investigations, routinely performed at least every 8 wk or in shorter intervals at the physician's discretion, were used to monitor disease course as shown in Tables 3–5.

Initial scan results indicated an abdominal mass in two patients by ^{123}I-VIP (Table 3, Fig. 1) not visualized by conventional methods at the time of tracer application. During the follow-up period, local tumor recurrence was strongly suggested by appearance of corresponding lesions on CT (6 and 8 wk after tracer injection, respectively). Surgical exploration was not performed in both individuals due to the patients' refusal of interventional measures. Both patients requested conservative treatment and started radiochemotherapy immediately after the CT results were available. They died from progressive disease 5.5 and 7 mo after initiation of therapy, respectively, and autopsy confirmed the presence of malignant tissue in both. The initial evaluation of a false-positive VIP scan therefore was turned into a true-positive VIP scan in the final evaluation. As determined by follow-up procedures, no ^{111}In-CYT-103 results were changed retrospectively.

DISCUSSION

Comparative Receptor and Antibody Scanning

The results of this prospective, randomized and single-blinded study indicate that the targeting of VIP receptors has promising aspects for further clinical evaluation. As previously reported (*14,23*), ^{123}I-VIP can be safely administered to patients. When using ^{123}I-VIP no adverse reactions, apart from a transient mild decrease in blood pressure, were observed. Our findings confirm previous results showing that even relatively small tumor masses, such as 2 cm in diameter, can be imaged with ^{123}I-VIP (*14,23*).

In this study, VIP compared favorably with ^{111}In-CYT-103. The overall rate of positive scans obtained with ^{123}I-VIP was as high as 93% (28 of 30 scans; positive in 11 of 12 patients with primary/recurrent tumors and in 17 of 18 patients with liver metastases), whereas (false) negative VIP scan results were obtained in only 2 patients. Correspondingly, only 55% of all ^{111}In-CYT-103 scans were positive.

In previous reports, the percentage of positive scan results obtained with ^{111}In-CYT-103 antibody in primary (*5,8,10*) or recurrent colorectal cancer patients (*7,9,11*) was somewhat higher. For instance, in a multicenter trial performed in 155 patients with colorectal cancer (*11*), the sensitivity and specificity of immunoscintigraphy with ^{111}In-CYT-103 was 69% and 77%, respectively, which compares well with the patients' CT results. In contrast to previous studies with ^{111}In-CYT-103, the lower proportion of positive scans found in the present study may be deduced from the fact that nuclear medicine physicians were blinded to the exact nature and localization of the malignancy. Furthermore, comparison with conventional radiologic imaging results was performed after completion of analysis of all scans obtained during the trial. Therefore, bias in favor of ^{123}I-VIP resulting from beforehand knowledge of tumor localization can be excluded.

We cannot, however, offer a definitive explanation for the differences between our findings with ^{111}In-CYT-103 and the results mentioned above. Quality assessment was similar to other trials reported, as histologic verification of all target lesions had been performed before randomization and accurate follow-up was done until death or for at least 6 mo (see also Tables 3–5). Given the fact that histologic confirmation of malignant disease by biopsy/surgery was one of the entry criteria, and that all patients had advanced-stage disease, suspected (new) lesions on VIP/OncoScint® scans were not subjected to repeated biopsy or surgery for histological proof. Instead, patients were followed until death, or for at least 6 mo by conventional radiologic methods to allow for an exact documentation of sites of disease progression or appearance of new lesions, thus allowing for delayed verification of false-positive results. According to the study protocol, appearance of a lesion on conventional images in a site where the scan had been false-positive changed the results into true-positive in our final evaluation. Given these facts, it seems very unlikely that the presence of tumor disease was underestimated, even if the radiographic method of proof has the tendency to miss small extrahepatic intestinal tumors. Therefore, in turn, it seems also

TABLE 3
Results of Iodine-123-VIP Receptor Scintigraphy

Patient no.	Sex	Age (yr)	Localization	Scan result	CT (initial)	CT at 6 mo
Colorectal cancer						
1	M	64	Locoregional recurrence	Pos	Pos	—
2	M	72	Locoregional recurrence	Pos	Pos	Pos, P
3	F	60	Locoregional recurrence	Pos	Neg	Pos, P
4	M	65	Locoregional recurrence	Pos	Pos	—*
5	F	60	Locoregional recurrence	Pos	Pos	Pos, NC
6	M	62	Liver metastases	Pos	Pos	Pos, P
7	M	64	Liver metastases	Pos	Pos	—*
8	F	71	Liver metastases	Pos	Pos	Pos, P
9	F	63	Liver metastases	Pos	Pos	—*
10	M	72	Liver metastases	Pos	Pos	—*
11	F	73	Lung metastases	Pos	Pos	Pos, P
12	M	73	Liver metastases	Pos	Pos	Pos, NC
			Abdominal lymph nodes	Pos	Pos	Pos, P
13	M	64	Liver metastases	Pos	Pos	—*
			Abdominal lymph nodes	Pos	Pos	—*
14	F	73	Liver metastases	Pos	Pos	—*
			Lung metastases	Pos	Pos	—*
Pancreatic Cancer						
15	F	32	Locally inoperable	Pos	Neg	Pos, P
16	F	65	Locally inoperable	Neg	Pos	—*
17	M	54	Liver metastases	Pos	Pos	—*
18	F	62	Liver metastases	Pos	Pos	—*
19	M	59	Liver metastases	Pos	Pos	—*
20	M	61	Liver metastases	Pos	Pos	—*

*Patient died.
P = disease progression on CT; NC = no change on CT.

unlikely that the sensitivity to image the tumors was overestimated for ^{123}I-VIP and underestimated for ^{111}In-CYT-103. Furthermore, as opposed to other trials we used a state-of-the-art multidetector SPECT system in our study that allowed us to perform several SPECT studies in one patient.

It is generally believed that antibodies or peptides of high-binding affinity are desirable for tumor imaging. As indicated by our in vitro studies (Table 2), ^{123}I-VIP as well as ^{111}In-CYT-103 bound to adenocarcinoma cells both in primary tissues and cultured cells (HT29 and COLO320 cells) with affinity constants in the lower nanomolar range suggesting the expression of high-affinity binding sites at the cell surface for both ligands. The high expression of VIP receptors on the cell surface of adenocarcinoma cells as opposed to normal peripheral blood cells provided the basis for the use of radioiodinated VIP for VIP-receptor scanning (*14*) in vivo. VIP-receptor-deficient primary adenocarcinomas have not been observed so far. The negative ^{123}I-VIP-scans obtained in two patients may be explained by loss of affinity for the radioligand, occupation by endogenous ligands or dedifferentiation of receptors. As opposed to peptide receptor scanning, targeting of the TAG-72 antigen by ^{111}In-CYT-103 has been achieved even when only 15% of the cells expressed the target antigen (*9*). We found high level expression of the TAG-72 antigen by adenocarcinoma cells, while normal peripheral blood cells did not express the TAG-72.3 antigen. The possibility of upregulating the expression of TAG-72 antigens on tumor cells by cytokines suggests that immunomodulatory pretreatment may lead to improved ^{111}In-CYT-103 scanning results (*27,28*).

Advantages of Peptide Scanning over Immunoscintigraphy

Accumulation of the MAb in the normal liver makes the visualization of hepatic metastases difficult. This fact is reflected by the relatively low percentage of true-positive and true-negative ^{111}In-CYT-103 scans found in this trial in comparison to ^{123}I-VIP (see also (*14*)) and CT (*11*). A similar low proportion of detectable liver metastases in patients with primary or recurrent colorectal cancer has been reported repeatedly for ^{111}In-CYT-103 (*9,11*). Due to these facts, the use of ^{111}In-CYT-103 can not be advocated for routine visualization of known or suspected hepatic lesions, and potential problems in interpreting scans can also result from appearance of lesions both as cold or hot spots (*29*).

However, we felt justified in including patients with hepatic metastases in our trial not only for scientific reasons, but also because a high percentage of such patients is encountered in general oncologic practice. Since many of these subjects die from hepatic manifestations of gastrointestinal cancer, evaluation of a tracer for potential clinical routine use for the localization of these lesions seems highly important.

In contrast to the these pharmacologic properties of ^{111}In-CYT-103, the lung is the major organ of VIP uptake after injection of ^{123}I-VIP, while uptake in the normal liver is low and radioactivity is excreted through the urinary tract (*14,23*). This biodistribution behavior makes the ^{123}I-VIP receptor scan a valuable means for imaging of VIP receptor-expressing hepatic metastases. Potential problems arising with the interference of background activity in the urinary tract with the imaging of small rectal cancers can be avoided by performing lateral view images and/or SPECT studies. Since gastrointestinal cancers metastasize preferentially to the liver, the high proportion of liver metastases detected by ^{123}I-VIP is especially attractive for further clinical approaches. Especially noteworthy is the high percentage of true-positive ^{123}I-VIP scans in patients with pancreatic adenocarcinomas. Since this tumor entity offers

TABLE 4
Results of Indium-111-CYT-103 scanning

Patient no.	Sex	Age (yr)	Localization	Scan result	CT (initial)	CT at 6 mo
Colorectal cancer						
1	M	58	Liver metastases	Neg	Pos	—*
			Lung metastases	Neg	Pos	—*
2	F	79	Liver metastases	Pos	Pos	Pos, P
			Lung metastases	Neg	Pos	Pos, NC
3	F	62	Liver metastases	Neg	Pos	—*
			Lung metastases	Neg	Pos	—*
4	M	69	Liver metastases	Neg	Pos	Pos, NC
			Abdominal lymph nodes	Neg	Pos	Pos, NC
5	F	78	Liver metastases	Neg	Pos	—*
			Abdominal lymph nodes	Neg	Pos	—*
6	M	80	Liver metastases	Pos	Pos	Pos, P
7	M	48	Liver metastases	Pos	Pos	Pos, NC
8	F	64	Liver metastases	Pos	Pos	—*
9	F	55	Liver metastases	Pos	Pos	Pos, P
10	F	61	Liver metastases	Pos	Pos	Pos, P
11	F	72	Liver metastases	Neg	Pos	—*
12	F	78	Liver metastases	Pos	Pos	Pos, P
13	F	59	Liver metastases	Pos	Pos	—*
Pancreatic cancer						
14	F	66	Locally inoperable primary	Pos	Pos	—*
15	M	63	Locally inoperable primary	Neg	Pos	—*
16	M	67	Locally inoperable primary	Neg	Pos	Pos, NC
Gastric cancer						
17	M	52	Locally inoperable primary	Pos	Pos	—*
18	M	36	Locally inoperable primary	Neg	Pos	Pos, P
19	M	68	Liver metastases	Pos	Pos	—*
20	F	60	Locally inoperable primary	Neg	Pos	—*

*Patient died.
P = progression on CT; NC = no change on CT.

a special diagnostic challenge, further investigations are warranted.

Usually, the highest tumor-to-background ratio achieved at the earliest time after antibody injection makes an antibody ideal for imaging. In this study, we imaged the patients at 48 and 72 hr after ^{111}In-CYT-103 injection, similar to the imaging protocols previously used by others (*11*). In contrast to this immunoscintigraphic procedure, ^{123}I-VIP receptor scanning can provide valuable results to the clinician within a few hours (Fig. 2). Although this may not be of major importance for clinical

TABLE 5
Results of Comparative Scanning with Iodine-123-VIP and Indium-111-CYT-103

Patient no.	Sex	Age (yr)	Verified localization	VIP-scan	CYT-103	CT (initial)	CT at 6 mo
Colorectal cancer							
1	F	72	Liver metastases	Pos	Pos	Pos	Pos, P
2	M	80	Liver metastases	Pos	Neg	Pos	Pos, P
3	M	66	Locoregional recurrence	Pos	Pos	Pos	—*
			Liver metastases	Neg	Neg	Pos	—*
4	M	73	Liver metastases	Pos	Pos	Pos	Pos, NC
5	F	77	Lung metastases	Neg	Neg	Pos	Pos, P
6	F	78	Locoregional recurrence	Pos	Neg	Neg	Pos, P
7	M	49	Liver metastases	Pos	Pos	Pos	—*
8	M	48	Locoregional recurrence	Pos	Pos	Pos	Pos, P
Gastric cancer							
9	M	68	Locally inoperable primary	Pos	Neg	Pos	—*
Pancreatic cancer							
10	M	53	Primary tumor	Pos	Neg	Pos	—*
			Liver metastases	Pos	Pos	Pos	—*

*Patient died.
P = progression on CT, NC = no change on CT.

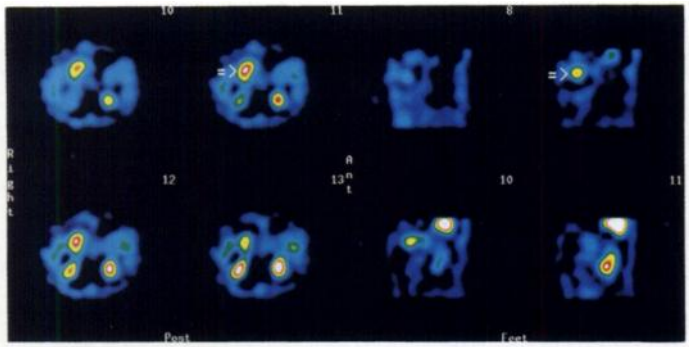

FIGURE 1. Recurrent adenocarcinoma (arrow) visualized by ^{123}I-VIP (Table 5, Patient 6). SPECT reconstruction (four transverse slices, left panel; four sagittal slices, right panel) identified the recurrent tumor at 3 hr postinjection in the transverse colon. In this patient, CT did not indicate tumor recurrence at the time of VIP receptor scanning. However, during the follow-up period, CT scan also indicated tumor mass at 8 wk after ^{123}I-VIP-injection. Indium-111-CYT-103 scintigraphy performed 4 wk after VIP receptor scanning gave negative results.

management, the psychological effect on patients facing an interval of anxiety between radiotracer application and definite results is far from negligible.

Apart from its high sensitivity, ^{123}I-VIP also offers other advantages over the use of MAbs. The estimated costs for ^{123}I-VIP receptor scanning are lower than those for ^{111}In-CYT-103 or MAbs generally. The cost-effectiveness of the receptor scan will even be higher once a ^{99m}Tc-labeled VIP receptor imaging compound will be available. Furthermore, there is ample evidence in the current literature that HAMA-development can be a problem encountered with the clinical use of whole antibodies. In a study applying 1 mg of the TAG-72 antibody, anti-mouse antibodies (HAMA) were developing (*11*) in 40% of patients, while in another trial 17% of all patients injected had a positive HAMA test (*30*). HAMA formation might prevent repeated successful imaging procedures with antibodies of murine origin in such patients, apart from the problem of potential hypersensitivity reactions with repeated administration. Our protocol was structured to investigate the presence of HAMA only in case of side effects, or in patients in whom application of a second dose of ^{111}In-CYT-103 would seem necessary.

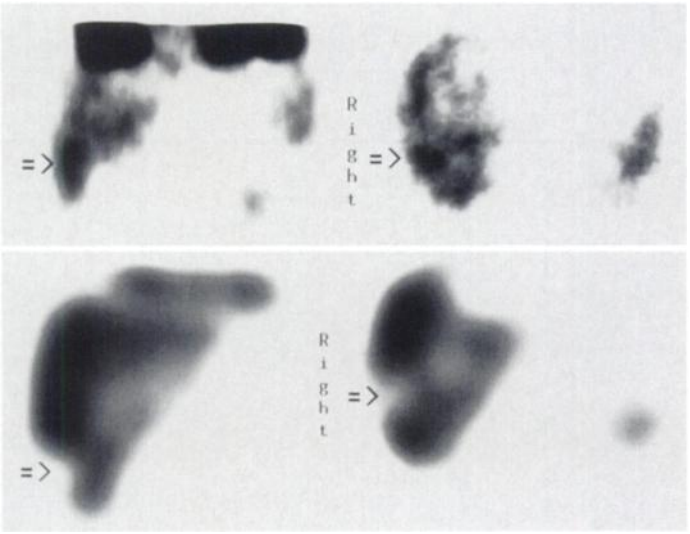

FIGURE 2. Liver metastases (arrow) visualized by ^{123}I-VIP and ^{111}In-CYT-103 (Table 5, Patient 1). Corresponding SPECT-reconstruction indicated the liver metastases in a patient suffering from colonic adenocarcinoma. Upper panel: ^{123}I-VIP SPECT study performed at 2 hr after injection (left: coronal slice, right: transverse slice). Lower panel: ^{111}In-CYT-103 SPECT study performed at 48 hr after injection (left: coronal slice, right: transverse slice).

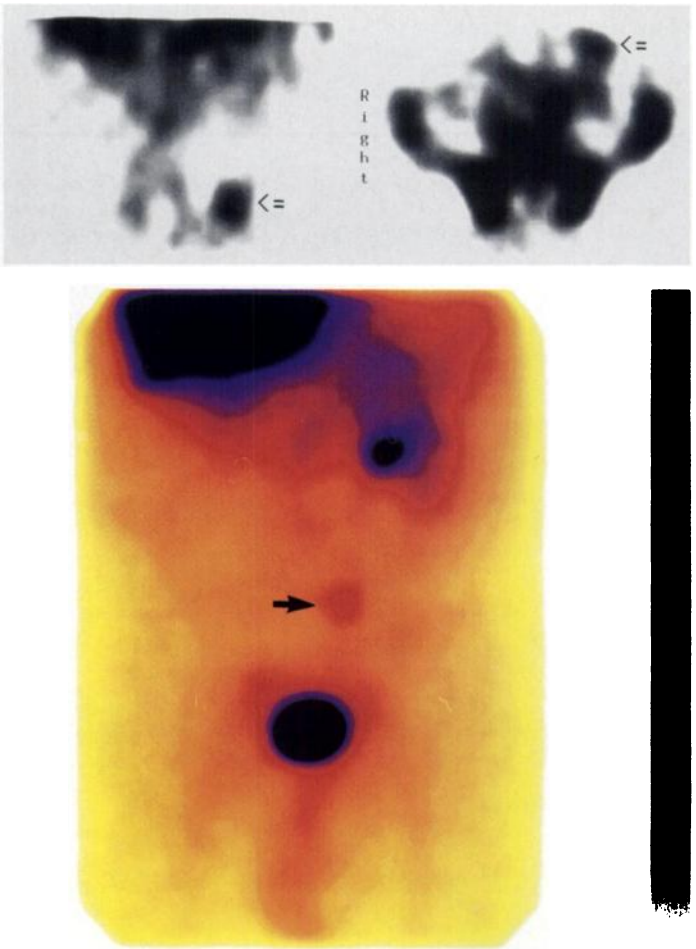

FIGURE 3. Recurrent rectal adenocarcinoma (arrow) visualized by ^{111}In-CYT-103 and ^{123}I-VIP (Table 5, Patient 8). Upper panel: The ^{111}In-CYT-103 scan (SPECT reconstruction; left: coronal slice; right: transverse slice) indicated the tumor recurrence at the resection site at 48 hr (images) and at 72 hr (not shown) after injection. Lower panel: ^{123}I-VIP planar imaging of the abdomen in anterior view showing the corresponding lesion indicating the recurrent tumor.

On the other hand, HAMA is not a problem in patients undergoing peptide receptor scanning. Peptide-based tracers can therefore be considered for repeated administration and thus for possible routine follow-up and tumor assessment in cancer patients undergoing therapy.

CONCLUSION

Iodine-123-VIP receptor scanning was diagnostically superior to ^{111}In-CYT-103 immunoscintigraphy. Our cohort of patients, however, was highly unselected. Its exact effect on localization and early detection has yet to be established and further studies against other antibodies and/or fragments such as ^{99m}Tc-labeled Fab fragments (*31,32*) are warranted. Although it is tempting to speculate that VIP-receptor scanning could be incorporated into routine use, further investigations, especially in presurgical patients, are clearly necessary.

ACKNOWLEDGMENTS

We thank Judith Bednar, Ingrid Blazek, Bettina Mosing and Karoline Krivanek for technical assistance as well as to Brigitte Maca and her team of nursing staff in the Department of Oncology. This study was supported by the Foundation of the Mayor of the City of Vienna (Bürgermeisterfonds), the Austrian National Bank

(Jubilaumsfonds no. 4560 and 5439) and the Hochschuljulnlaums fonds.

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Thallium-201 and Iodine-131 Scintigraphy in Differentiated Thyroid Carcinoma

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The purpose of this study was to determine the concordance and discordance between diagnostic ^{131}I and ^{201}Tl whole-body scintigraphy in patients with differentiated carcinoma of the thyroid. **Methods:** Following thyroidectomy for differentiated thyroid carcinoma, 50 patients underwent whole-body ^{131}I and ^{201}Tl scanning (60 pairs of scans in total). Fifteen pairs of studies were obtained before ablative therapy, 30 pairs after ablative therapy and 15 pairs after ^{131}I therapy for metastatic disease. Serum thyroglobulin levels were concurrently determined by radioimmunoassay. **Results:** Thirty-six ^{131}I whole-body scans (in 34 patients) showed residual uptake in the neck, but only six (17%) of the corresponding whole-body thallium studies had detectable uptake in the neck. Fourteen ^{131}I scans (in nine patients) identified multiple metastatic lesions, whereas the thallium scans were interpreted as either negative, nonspecific or showing fewer lesions. In four study pairs, the thallium scans showed solitary lesions that were not detected by the corresponding radioiodine scans. In 16 scans, the thallium studies gave false-positive results. **Conclusion:** Iodine-131 scintigraphy for differentiated thyroid carcinoma is more sensitive and more specific than ^{201}Tl scintigraphy for detection of distant metastases and residual activity in the neck following thyroidectomy.

Key Words: thyroid carcinoma; thyroidectomy; iodine-131; thallium-201; thyroglobulin

J Nucl Med 1996; 37:1487–1491

Thyroid cancer is the most frequently diagnosed malignant endocrine lesion. The incidence in the United States is approximately 15,600 new cases occur annually, with 1200 deaths each year (*1*). Most of these tumors are well-differentiated and

Received Oct. 26, 1995; revision accepted Mar. 6, 1996.

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