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Radioimmunodetection of lung cancer with IMACIS-1, I-131 labeled monoclonal antibodies to CEA and CA19-9 Comparison of accumulations in irradiated and non-irradiated site

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IMACIS-1 is a radiopharmaceutical containing a mixture of Iodine-131 labeled monoclonal antibodies to CEA and CA19-9. IMACIS-1 immunoscintigraphy was evaluated for tumor detection in 7 primary lung cancer and 2 metastatic lung cancer patients who received radiotherapy. No adverse side effects due to IMACIS-1 were observed in this study. Positive detection was achieved in 5 of 9 patients (55.6%). It was less, but nearly the same as the detection rate obtained with Gallium-67 citrate (^{67}Ga-citrate) in these patients. There was no clear correlation between IMACIS-1 accumulation and the CEA or CA19-9 serum levels. The IMACIS-1 positive detection rate decreased in many of the irradiated lesions. We considered that the decreased number of tumor cells and changes in blood perfusion are some of the factors controlling accumulation in tumors.

Key words: immunoscintigraphy, IMACIS-1, lung cancer, radiotherapy, Ga-67 citrate

INTRODUCTION

RADIOLABELED MONOCLONAL ANTIBODIES have recently been widely used clinically for the radioimmunodetection and radioimmunotherapy of malignant tumors.

We participated in the clinical trial for radioimmunodetection of malignant tumors with IMACIS-1, which is a radiopharmaceutical containing a mixture of F (ab')$_2$ fragments of mouse monoclonal antibodies to CEA and CA19-9 labeled with Iodine-131.[1] The most frequently used radiopharmaceutical for detecting malignant tumors is Gallium-67 citrate (^{67}Ga-citrate). Radioisotopic accumulations of ^{67}Ga-citrate in malignant lesions are known to decrease after effective irradiation or chemotherapy.[2,3] We clinically evaluated the diagnostic efficacy of IMACIS-1 scintigraphy in cases of lung cancer in comparison with gallium scintigraphy. The influence of irradiation on IMACIS-1 accumulation in malignant lesions was also investigated.

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MATERIALS AND METHODS

Ten patients with lung cancer admitted to the Department of Radiology, Gunma University School of Medicine for in-patient radiotherapy were included in this study. Eight patients had primary lung cancer and the others had metastatic lung tumor from rectal cancer. Six of the ten patients had multiple malignant lesions detected by other imaging methods. Histological diagnoses were obtained by bronchoscopic biopsy, percutaneous needle biopsy, pleural effusion cytology, operation, or autopsy. These were 6 adenocarcinomata, 2 squamous cell carcinomata, and 2 anaplastic carcinomata. Table 1 shows the histological types and stages for all patients.

111MBq (3 mCi) of IMACIS-1 was diluted in 100 m*l* of isotonic saline and infused intravenously over 30 minutes. Potassium iodide (600 mg/day) was administered orally for ten days to block the uptake of free I-131 into the thyroid gland. Scintigraphy with a gamma camera (Siemens ZLC7500) and a data

Table 1 Clinical characteristics of lung cancer patients receiving IMACIS-1

Case	Age/Sex	Histological type	TNM
Primary Lung ca.			
1.	59, M	Adenocarcinoma	$T_4N_1M_0$
2.	79, M	Poorly differenciated adenocarcinoma	$T_3N_0M_0$
3.	66, F	Moderately differenciated adenocarcinoma	$T_2N_2M_1$
4.	79, M	Anaplastic carcinoma	$T_2N_0M_1$
5.	79, M	Anaplastic carcinoma	$T_3N_1M_1$
6.	78, F	Poorly differenciated adenocarcinoma	$T_2N_0M_0$
7.	74, M	Squamous cell carcinoma	$T_2N_1M_0$
8.	82, M	Squamous cell carcinoma	$T_4N_0M_0$
Metastatic Lung Tumor			
9.	60, M	Well differenciated adenocarcinoma (Rectum)	
10.	72, F	Mucinous adenocarcinoma (Rectum)	

Table 2 Changes in HAMA serum level before and after IMACIS-1 injection

HAMA	before mean±S.D.	6–10 days after injection		more than 18 days after injection	
		mean±S.D.	P value	mean±S.D.	P value
IgG (O.D.)	0.096±0.010 (n=8)	0.092±0.015 (n=6)	N.S.	0.093±0.016 (n=4)	N.S.
IgM (O.D.)	0.095±0.042 (n=5)	0.120±0.038 (n=3)	N.S.	0.085±0.016 (n=3)	N.S.
IgE (U/m*l*)	77.4±104.4 (n=5)	108.0±171.7 (n=3)	N.S.	47.6±72.8 (h=3)	N.S.

O.D.: Optical density at 492 nm
N.S.: Difference is not statistically significant.

processing system (Shimazu Scintipack 700) was carried out 96–120 hours after IMACIS-1 injection. In a few patients, we subtracted the early blood pooling image from the delayed phase (96 hour after injection) image with a Shimazu Scintipack 700.

In all the patients, ^{67}Ga-citrate scintigraphy was also carried out. Intravenous injection of 74 MBq of ^{67}Ga-citrate was followed by scanning 72 hours later with a gamma camera (Siemens ZLC7500). The time interval between administrating IMACIS-1 and Ga-67 citrate was from 7 days to 1.5 months.

A positive detection was defined, with reference to the original imagings, as increased radioactive accumulation in at least one lesion compared with the background level.

We also examined the serum levels of CEA and CA19–9 in all patients before and 3–11 days after IMACIS-1 injection, by CEA RIA Beads (Dinabot) and Centcore CA19–9 RIA Kit, respectively, and the serum levels of HAMA (human anti-mouse antibody: IgG, IgM) by enzyme-linked immunosorbent assay (ELISA). The serum levels of IgE were examined by EIA with a Mitsui II-Kit.

In 2 autopsy patients tissue specimens from irradiated and non-irradiated lesions were studied by immunohistochemical techniques (Cases 3 and 5). Tumor tissues were fixed in formaldehyde, embedded in paraffin, and 3 μm slices were prepared. Slides were deparaffinized and tested by the peroxidase-antiperoxidase (PAP) technique with anti-CEA rabbit polyclonal antibody (DAKO Corporation) and by the avidin-biotin-peroxidase (ABC) technique with anti-CA19–9 mouse monoclonal antibody (International-CIS). Slides were then treated with diaminobenzidine, counter-stained with hematoxylin, dehydrated, and mounted.

RESULTS

Immunoscintigraphy was performed in 9 patients. One patient died due to DIC two days after IMACIS-1 administration. An autopsy found no evidence of adverse histological effects due to IMACIS-1.

Serum HAMA levels were similar before and after IMACIS-1 injection in all patients (Table 2). No adverse effects due to IMACIS-1 were observed in this series.

The positive detection rate in IMACIS-1 scintigraphy was 4 of 7 patients (57.1%) with primary lung cancer, and 1 of 2 (50.0%) with metastatic lung

cancer. The overall positive detection rate was 55.6% (5/9). ^{67}Ga-citrate scintigraphy achieved an overall positive detection rate of 66.7% (6/9) in the same series patients (Table 3).

Immunoscintigraphy showed no clear correlation between IMACIS-1 accumulation and the CEA or CA19-9 serum levels measured before IMACIS-1 injection (Fig. 1). The nine patients had sixteen malignant lesions more than 15 mm in diameter as measured by other imaging methods (Table 4). Four lesions in three patients had received more than 10 Gy Linac X-ray irradiation before immunoscintigraphy. Fig. 2 shows the positive IMACIS-1 accumulation in irradiated and non-irradiated lesions. In the field irradiated with more than 10 Gy,

Table 3 Positive tumor detection by scintigraphy

	IMACIS-1	Ga-67
Lung cancer	4/7 (57.1%)	5/7 (71.4%)
Rectal cancer		
Lung metastases	1/2 (50.0)	1/2 (50.0)
Total	5/9 (55.6)	6/9 (66.7)

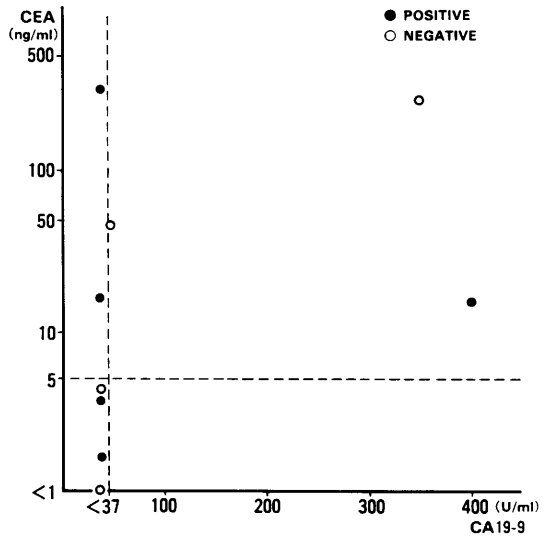


Fig. 1 Correlation between positive IMACIS-1 accumulation and tumor marker serum levels.

Table 4 IMACIS-1 and Ga-67 scintigraphy for malignant lesions†

Case	Tumor site	Size mm × mm	IMACIS-1 Scintigraphy	Ga-67 Scintigraphy
1	Lung	15 × 18 30 × 30	N♯	P*
	Pleural effusion (malignant)		P	N
2	Lung	40 × 50	N	N
3	Lung	55 × 60	P	P
	Th-spine	40 × 45	P	P
	Scapula	40 × 75	P	P
4	Lung	100 × 115	N	N
5	Lung	55 × 60	P	N
	Brain	30 × 30	P	N
6	Lung	35 × 35	P	P
7	Lung	25 × 60	N	P
9	Pelvis	20 × 35	N	N
	Liver	35 × 35	N	N
	Lung	30 × 30	N	N
10	Pelvis	40 × 40	P	P
	Lung	40 × 50	P	P

†: They were limited to lesions more than 15 mm in diameter. P*: Positive accumulation; N♯: Negative accumulation

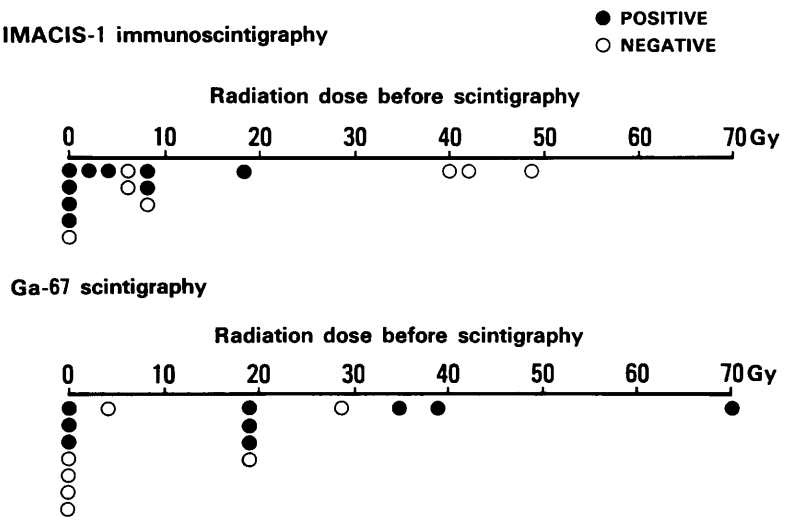


Fig. 2 Radiation dose to malignant lesions and positiveness of immunoscintigraphy detection.

Table 5 Changes in tumor marker serum levels before and after IMACIS-1 injection

Case	Before injection		After injection	
	CEA	CA19-9	CEA	CA19-9
	(ng/m*l*)	(U/m*l*)	(ng/m*l*)	(U/m*l*)
1.	315	<37	173	<37
2.	45.2	39	34.2	<37
3.	15.6	<37	14.6	<37
4.	4.5	<37	1.6	<37
5.	3.7	<37	1.9	<37
6.	1.7	<37	1.0	<37
7.	<1	<37	<1	<37
8.	5.7	<37	—	—
9.	272	354	623*	270
10.	14.9	400	17.2	510

* Three weeks after IMACIS-1 injection

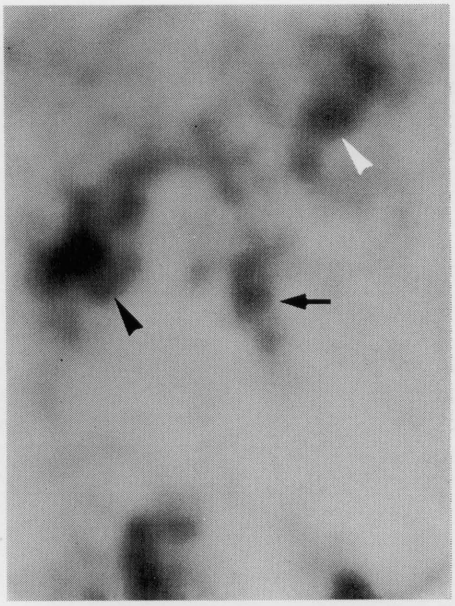
Table 6 Immunohistochemistry of malignant lesions. Correlation with radioimmunodetection and radiation
Case 3

	Immuno-scintigraphy	Immunostaining with anti-CEA antibody
Irradiated site (lung tumor)	(+) 8 Gy	(+)* 46 Gy
Non-irradiated site (adrenal gland)	(−)	(+)*

* positive staining in cytoplasm of tumor cells.
Case 5

	Immuno-scintigraphy	Immunostaining with anti-CEA antibody
Irradiated site (brain tumor)	(+) 2 Gy	(−) 48 Gy
Non-irradiated site		
① (lung tumor)	(+)	(−)~(±)♯
② (adrenal gland)	(−)	(−)

♯ localized staining in some peeling cells.



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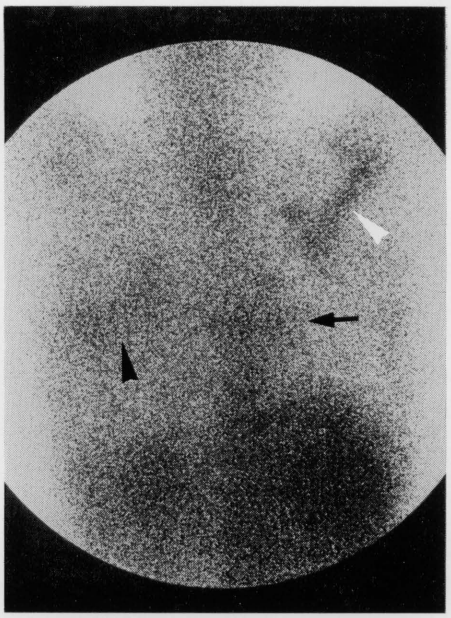

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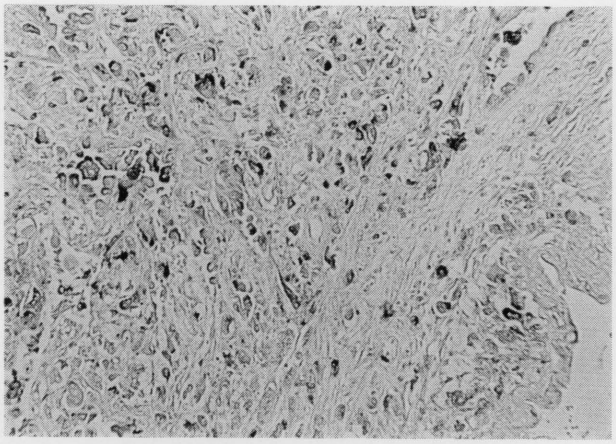
Fig. 3 Case 3. (posterior chest view) a) IMACIS-1 immunoscintigraphy: primary lung tumor (left S6: ▲) and metastatic lesions in right scapula (△) and thoracic vertebrae (▲) are positive. (Subtraction technique is used.) b) ^{67}Ga-citrate scintigraphy.

three of the four lesions had negative IMACIS-1 accumulation, and only two of the eight lesions had negative ^{67}Ga-citrate accumulation. In radiotherapeutic field received less than 10 Gy or in non-irradiated sites, eight of the twelve lesions showed positive IMACIS-1 accumulation. On the other hand, three of the eight lesions showed signs of positive ^{67}Ga-citrate accumulation.

Two lesions appeared positive in ^{67}Ga-citrate, but negative in IMACIS-1 scintigraphy. They were attributable to inflammatory changes, pneumonia and radiation-induced pneumonitis, diagnosed by plain X-P, X-ray CT, and clinical features.

In contrast, a brain metastasis in an anaplastic cancer patient was positive in IMACIS-1 and negative in gallium scintigraphy. He had received only 6 Gy whole brain irradiation before IMACIS-1 immunoscintigraphy, but 18 Gy before gallium scintigraphy.

No irradiated lesions had enhanced accumulation in IMACIS-1 immunoscintigraphy when compared with non-irradiated lesions in the same patient.



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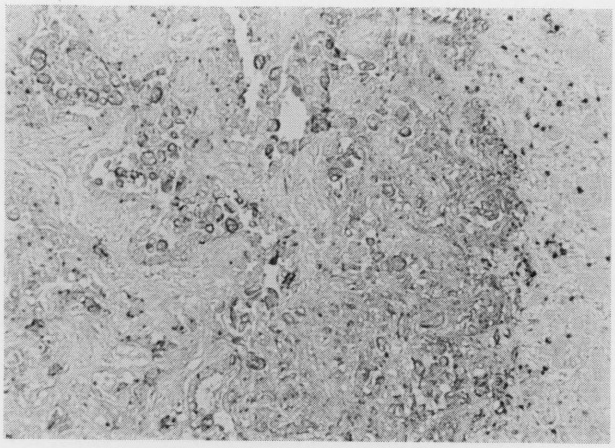

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Fig. 4 Immunohistochemical staining of autopsy tissue specimens with anti-CEA antibody. (Case 3) a) irradiated site: primary lung tumor b) non-irradiated site: tumor in the adrenal gland.

Table 5 shows the difference in CEA and CA19–9 serum levels before and after IMACIS-1 injection. Most patients with extensive diseases (cases 3, 4, 5, 9, 10) showed no change or increased tumor marker values. In contrast, patients with localized primary lung cancer had reduced levels after IMACIS-1 scintigraphy. After scintigraphy, the patients had received external radiotherapy continuously. There were no patients whose serum levels of the tumor markers seemed increased after antibody injection in our quantitative procedure.

Table 6 shows the immunohistochemical and IMACIS-1 immunoscintigraphic findings for malignant tumors in the two autopsy patients. Case 3 with adenocarcinoma had a primary lung lesion irradiated with 46 Gy and a non-irradiated metastatic tumor in the adrenal gland. She had multiple bone metastases, for example in the thoracic vertebrae, scapula, and ribs. Both primary lung tumor and metastatic bony lesions appeared positive in the IMACIS-1 immunoscintigram (Fig. 3). The 6 mm diameter metastatic tumor in the adrenal gland was the only non-irradiated tumor, and was found on autopsy. It was not detected by scintigraphy. Tissue specimens from irradiated and non-irradiated sites had stained to the same degree with anti-CEA antibody (Fig. 4). However, immunostaining with anti-CA19–9 antibody was negative in both.

Case 5 had a metastatic brain tumor irradiated with 48 Gy, and non-irradiated primary lung tumor and metastatic tumor in the adrenal gland. No specimen stained with anti-CA19–9 antibody. Neither specimen of metastatic lesion stained with anti-CEA antibody. However, the specimen from the non-irradiated primary lung tumor stained locally in some tumor cells peeling away from the proliferative thickening alveolar wall. Both brain tumor after 2 Gy irradiation and the non-irradiated primary lung tumor were positive in IMACIS-1 immunoscintigraphy.

DISCUSSION

IMACIS-1 administration caused no significant change in the general condition or clinical data in all but one patient, who died of advanced lung cancer. Autopsy proved that no adverse effect due to IMACIS-1 had caused death. The HAMA serum levels in all patients were similar before and after IMACIS-1 injection. IMACIS-1 radioimmunodetection in this study detected malignant lesions in 5 of 9 (55.6%) lung cancer patients. IMACIS-1 scintigraphy was reported to be positive in 2 of 10 (20%) or 6 of 9 (67%) lung cancer patients.[1,4] In our series, IMACIS-1 achieved nearly the same positive detection rate as ^{67}Ga-citrate, which is now the most popular radiopharmaceutical for detecting malignant lesions. ^{67}Ga accumulation in primary lung cancer occurs in 64–100% of patients.[5] However, accumulation in adenocarcinoma is apparently less than in squamous cell carcinoma and undifferentiated carcinoma.[5,6] In metastatic lesions from the urinary and gastro-intestinal tracts, gallium also rarely accumulates.[5] Our study included 6 cases of adenocarcinoma (4 primary lung cancer, 2 metastases from rectal cancer), so we were able to confirm positive results in cases by means of ^{67}Ga-citrate scintigraphy.

^{67}Ga-citrate scintigraphy is known to detect inflammatory changes clearly. A positive indication must therefore differentiate between true neoplasm and other diseases. We found 2 positive lesions with ^{67}Ga-citrate which were negative with IMACIS-1. These were attributable to inflammatory changes.

Unfortunately, it was impossible to get the same anti-CEA antibody as in IMACIS-1. So we used

an anti-CEA polyclonal antibody for the immunohistochemical study. If anti-CEA antibody staining was nonspecific for lung tissue in Case 5, in which negative serum marker levels were detected, the mechanism of radioimmunodetection for lung tumor and brain tumor might be non-specifically dependent on the blood pool; for example, on the destruction of the blood brain barrier or increased permeability of small vessels at the tumor site.

In many irradiated lesions, IMACIS-1 had a less positive detection rate. *In vitro* and *in vivo* studies have demonstrated that external radiation increases radiolabeled antibody uptake.[7–10] Experimental tumor detection with IMACIS-1 for human lung adenocarcinoma xenografts in nude mice showed that the greatest radioisotopic accumulation was in the necrotic part of the tumor.[11] However, our study found no irradiated lesions with enhanced IMACIS-1 accumulation, nor an increase in the level of serum tumor marker from the irradiated patients.

The ^{67}Ga-citrate accumulation in a tumor diminishes after effective irradiation or chemotherapy.[2,3] The mechanism may be related to a decrease in the number of tumor cells, changes in the permeability of tumor cell membranes, or interruption of the blood supply. The accumulation may also be related to lysosomal enzyme activity or lymphocytes in tumor areas stimulated by tumor antigens.[2] The mechanism of IMACIS-1 accumulation may also be related to the number of tumor cells, blood supply and changes in tumor antigens. Immunohistochemical tissue investigation in Case 3 showed no difference between immunostaining density in irradiated and non-irradiated tumors. Although we saw few cases, we consider that this may show that a decrease in the number of tumor cells results in a decrease in IMACIS-1 accumulation in irradiated sites.

The administration of a monoclonal antibody cocktail is reported to increase the chances of tumor detection when compared with the use of a single antibody.[12,13] However, CA19–9 is less effective for the detection of primary lung cancer because of the low production of CA19–9 in primary lung cancer.[14]

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