Drugs of the Future 2004, 29(2): 127-135
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CCC: 0377-8282/2004

^{99m}Tc-ior C5

Diagnostic for Colorectal Cancer

Technetium-99m (^{99m}Tc)-labeled murine IgG_1 monoclonal antibody ior C5 that recognizes the tumor-associated antigen (TAA) ior C2

EN: 359933

Abstract

Monoclonal antibody (MAb) ior C5 is a murine IgG_1 antibody which recognizes the tumor-associated antigen (TAA) ior C2 homogeneously expressed in the cytoplasm of normal colon epithelium and heterogeneously expressed in more than 83% of primary colorectal carcinomas. The antigen ior C2 appears distinct in molecular structure and tissue expression compared to other colorectal antigens in that the latter are more strongly expressed in colon cancers compared to corresponding normal tissues and the expression in both normal colonic mucosa and colorectal carcinomas is homogeneous. *In vivo* biodistribution studies have shown no uptake in normal organs and tissues, and studies in human colon cancer xenograft-bearing nude mice have shown good tumor uptake after antibody administration. Biodistribution studies in patients indicated that ior C2 was highly expressed in primary and metastatic colorectal carcinomas and showed very limited expression in normal adult tissues. Based on data from pharmacokinetics and biodistribution studies, ^{99m}Tc-ior C5 appears to be useful as a target for the immunodiagnosis of colorectal tumors. Furthermore, feasibility studies indicate that ^{99m}Tc-ior C5 can be used safely and that adequate tumor-localizing doses can be administered for diagnostic imaging without exposing the patient to excessive radiation.

Introduction

The concept of tumor-associated antigens (TAAs) has been introduced into routine medical practice over the last several decades. This TAAs are good targets for the delivery of anticancer agents (1).

The development of radiolabeled monoclonal antibodies (MAbs) has initiated an expansion of research, development and clinical studies in nuclear medicine. The use of radiolabeled MAbs in the diagnosis of different types of cancer and other malignant diseases is becoming routine clinical practice (1-6).

Colorectal carcinoma is the second leading cause of cancer death in the United States, with an estimated 131,200 new cases in 2003 and an estimated 54,900 deaths. In Europe, according to Cancer Facts and Figures in Europe-2002, colon cancer afflicts 117,000 people annually, with deaths from colon cancer estimated at 77,400 per year. Whereas the 5-year survival rates for localized colon and rectal cancers are 93% and 87%, respectively, regional spread of disease to adjacent organs or lymph nodes results in a considerable decrease in 5-year survival rates to 63% and 53% for colon cancer and rectal cancer, respectively. Survival rates for patients with distant metastases of colon and rectal cancers are less than 7% (7).

According to WHO estimates, there were approximately 192,000 cases of ovarian cancer and 114,000 deaths from the disease worldwide in 2002. The lifetime risk of ovarian cancer in American women with no family history of the disease has been estimated at around 1 in 70. Mortality is high because the majority of women with ovarian cancer are not diagnosed until the disease has spread beyond the ovary (7).

Thus, the identification of a drug or biopharmaceutical with utility as a cancer-specific and functional imaging agent, including diagnosis, detection, staging and evaluation of response to therapy, would be of great interest.

The MAb ior C5 recognizes a novel *O*-linked glycoprotein carbohydrate chain TAA, C2, expressed preferentially on the surface and in the cytoplasm of normal and malignant colorectal cells (1). This MAb has undergone extensive preclinical and clinical evaluation, localization

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studies and tumor targeting in phase I/II studies in colorectal and ovarian cancer patients (1).

The *O*-linked glycoprotein carbohydrate chain TAA C2 epitope is repeatedly expressed on a single molecule, because it is easily detected by an enzyme-linked immunosorbent assay (ELISA) method using the MAb for both capture and detection.

This monograph describes the physicochemical and pharmacological properties of the radiopharmaceutical ^{99m}Tc-ior C5 for the detection of colorectal and ovarian tumors, their metastases and recurrences.

Description

O-Linked glycoprotein carbohydrate chain tumor-associated antigen C2

The antigen ior C2 is expressed preferentially on the surface of malignant colorectal cells and the cytoplasm of normal colorectal cells. The antigen ior C2 appears distinct in molecular structure and in tissue expression to the previously described colorectal antigens CEA (8), 17-1A (9), TAG-72 (10), CA 19-9 (11, 12) and A33 (12) in that: 1) the latter antigens are more strongly expressed in colon cancers compared to the corresponding normal tissues; and 2) the expression in both normal colonic mucosa and colorectal carcinomas is homogeneous (13-16).

Immunohistochemical analysis of a large panel of normal and malignant tissues has shown the antigen to be homogeneously expressed in normal colon epithelium and transitional mucosa (adjacent to tumor), and heterogeneously expressed in more than 83% of colon carcinomas, but is not detected in a wide range of other normal tissues (17).

The antigen is a neuraminidase-resistant and periodate-, pronase- and alkali-sensitive epitope present on a major 145-kDa and a minor 190-kDa glycoprotein complex (14-16). The expression of the antigen is essentially organ-specific (colon and gastrointestinal tract) and is quite heterogeneous in tumors. Free antigen has not been detected in the blood, as determined by the ELISA method.

Monoclonal antibody ior C5

The MAb ior C5 is a murine IgG_1 that was generated by immunizing BALB/c mice with the human colorectal adenocarcinoma cell line SW1116 and fusing splenocytes from seleced BALB/c mice with non-SP 2/O Ag 14-secreting myeloma cells (14). It recognizes a TAA expressed preferentially on the surface of malignant colorectal cells and in the cytoplasm of normal colorectal cells, but does not recognize the antigens CEA, Lewis^a, Lewis^b or sialyl Lewis^a, nor antigens from the peripheral blood mononuclear cell (PBMC) membrane or red blood cells. Immunocytochemical staining showed strong surface and cytoplasmic reactivity with the colorectal cancer cell lines SW1116 and SW948, but not with various other cell lines, including breast carcinoma (MDA-MB-134, MDA-MB-157, MDA-MB-435), melanoma (A-375, M-14, FEM-X), lung carcinoma (U-1752, U-2020), B-cell lymphoma (Raji) and T-cell leukemia (CEM) cells, and PBMCs including granulocytes and erythrocytes (15, 16).

^{99m}Tc-ior C5

^{99m}Tc-ior C5 is a technetium-99m (^{99m}Tc)-labeled compound based on a murine IgG_1 antibody that binds specifically to an abnormal form of mucin *O*-linked glycoprotein carbohydrate chain TAA C2 overexpressed in an abnormally glycosylated form on the cell surface of over 95% of colon adenocarcinoma cells.

The antibody is attached to the radioisotope ^{99m}Tc via sulfhydryl groups. Once injected into the body, the antibody acts as a tumor-targeting vehicle, specifically delivering radioactivity to C2-expressing cancer cells. The tumor can then be visualized using a γ camera. ^{99m}Tc-ior C5 is intended for the diagnosis of colorectal and ovarian tumors (1, 18, 19).

The purified MAb ior C5 is labeled with a specific activity of 50 mCi/mg protein. A mean of 97.8 ± 0.6% of ^{99m}Tc binds to IgG_1, as determined by paper chromatography. Instant paper chromatography of labeled MAb in acetone showed about 1.2% or less free pertechnetate at the solvent front (R_f = 1.0). This indicates that pertechnetate was reduced almost quantitatively. When the chromatogram was developed in saline, more than 97.8% of the activity remained at the origin, indicating that the ^{99m}Tc was transchelated from MDP to the MAb ior C5. As a rule, colloid formation, determined by albumin-impregnated ITLC was < 1.2% in all preparations (1, 18).

Immunoreactivity and biological activity of the reduced antibody were assessed by a competitive ELISA measuring its ability to compete with the antigen ior C2. Reduced was compared with nonreduced (native) antibody for the ability to compete with the antigen. Data from 3-5 independent experiments were averaged and plotted (Fig. 1).

Pharmacological Actions

A study of the normal tissue recognition of MAb ior C5 was carried out using immmunohistochemical methodology. These studies have shown wide distribution and a variable staining intensity (15-17). In normal adult tissues, the antibody stained epithelial antigenic determinants of restricted distribution, predominantly expressed in the gastrointestinal tract. Positive staining of the intestinal villi (3/3) and a homogeneous staining pattern in the colonic mucosa (3/3) were observed. The bronchial epithelial cell lining was also intensively stained (2/3). No reaction was seen in the other normal tissues studied (skin, kidneys,

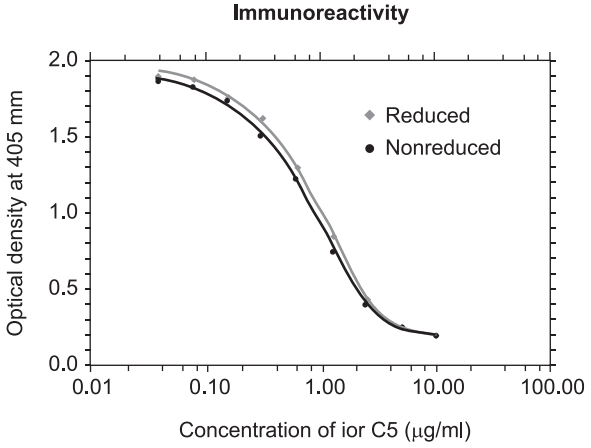

Fig. 1. The immunoreactivity and biological activity of the reduced and nonreduced (native) MAb ior C5 tested by a competitive ELISA method.

brain, thyroid glands, heart, adrenal glands, hypophysis, stomach, spleen, pancreas, liver) (14).

Studies on the recognition of tumor tissues by ior C5 using immunohistochemistry showed differential staining intensity and distribution of the MAb in different types of tumors, depending mostly on the levels of expression of the antigen in that tumor type. Results can be seen in Table I.

Immunohistochemical studies performed on paraffin-embedded tissues using a biotin-streptavidin peroxidase complex method (17) demonstrated a heterogeneous staining pattern for colon adenocarcinoma cells (10/12 tumors), with predominantly cytoplasmic and apical staining of the cells. Also, positive staining of a variable number of epithelial cells of malignant tumors of the breast (4/8), lung (2/4) and endometrium (3/3), and of both mucinous and serous components of ovarian tumors (6/8) and their luminal secretions, was observed for ior C5.

Table II: Pharmacokinetic parameters following ^{99m}Tc-ior C5 i.v. bolus injection in rabbits.

Pharmacokinetic parameter	Mean ± SD (n = 4)
$t_{1/2\alpha}$ (h)	2.91 ± 1.26
$t_{1/2\beta}$ (h)	29.82 ± 0.54
AUC (% ID/ml·h)	7.78 ± 1.93
C_o (% ID/ml)	2.05 ± 0.59
V_c (ml/kg)	26.74 ± 6.32
V_{ss} (ml/kg)	26.83 ± 6.76
Cl_D (ml/kg)	6.74 ± 1.45

ID = injected dose.

Pharmacokinetics

In preclinical pharmacokinetic studies, rabbits were administered an i.v. bolus of a dose of 250 μg/1.5 mCi of ^{99m}Tc-ior C5. Serum samples from the animals were collected at 10, 20, 30 and 45 min, and then at 1, 3, 5, 8, 16, 24, 36, 48 and 72 h postadministration. Serum disappearance curves for ^{99m}Tc-ior C5 were best described by a biexponential equation with a distribution half-life ($t_{1/2\alpha}$) of 2.91 ± 1.26 h and an elimination half-life ($t_{1/2\beta}$) of 29.82 ± 0.54 h (Table II). The area under the concentration-time curve (AUC) was 7.78 ± 1.93% injected dose (ID)/ml·h and the maximal activity concentration (C_o) was 2.05 ± 0.59% ID/ml·h.

Biodistribution studies were performed in normal male Sprague-Dawley rats injected with 25 μg/150 μCi of ^{99m}Tc-ior C5. Rats were sacrificed at 10 min and then at 1, 3, 5 and 24 h after i.v. injection. Time courses of radioactivity in heart, liver, lungs, kidneys, stomach and intestine showed similarities among individual animals (data not shown). The results of the uptake measurements are given in Table III. Among the various organs examined, significant accumulation of the radiolabel was found only in kidneys at 5 and 24 h after administration of the radiopharmaceutical (Table III), *i.e.*, 1.05 ± 0.11% and 2.05 ± 0.14% ID/g tissue at 5 and 24 h, respectively. Localization of radioactivity in kidneys following injection

Table I: Tumor tissue recognition of ior C5.

			Intensity			
Tumor localization	Histology	Total no. of cases	–	+	++	+++
Colon	Adenocarcinoma	24	0	0	3	21
Lung	Large cell carcinoma	4	2	0	0	2
Breast	Canalicular	5	2	0	0	3
	Lobular	3	2	0	1	0
Ovarian	Serous cystoadenocarcinoma	3	1	0	0	2
	Mucinous cystoadenocarcinoma	3	1	0	0	2
	Seromucinous cystoadenocarcinoma	2	0	0	0	2
Endometrial	Adenocarcinoma	3	0	0	0	3
Skin	Basal cell carcinoma	1	0	0	0	1
	Epidermoid carcinoma	1	0	0	0	1

– Negative; + weak; ++ moderate; +++ intense.

Table III: Normal tissue uptake of ^{99m}Tc-ior C5 in Sprague-Dawley rats at 10 min and 1, 3, 5 and 24 h after injection of the radiopharmaceutical.

Organ/tissue	Time postinjection (h)	% Injected dose (ID)/g tissue (mean ± SD)
Heart	0.16	0.03 ± 0.00
	1.0	0.06 ± 0.00
	3.0	0.07 ± 0.00
	5.0	0.13 ± 0.02
	24.0	0.16 ± 0.03
Liver	0.16	0.04 ± 0.00
	1.0	0.05 ± 0.00
	3.0	0.16 ± 0.05
	5.0	0.04 ± 0.00
	24.0	0.11 ± 0.03
Lungs	0.16	0.02 ± 0.00
	1.0	0.03 ± 0.00
	3.0	0.02 ± 0.00
	5.0	0.04 ± 0.00
	24.0	0.12 ± 0.01
Kidneys	0.16	0.08 ± 0.01
	1.0	0.19 ± 0.05
	3.0	0.66 ± 0.09
	5.0	1.05 ± 0.11
	24.0	2.05 ± 0.14
Stomach	0.16	0.03 ± 0.00
	1.0	0.01 ± 0.00
	3.0	0.01 ± 0.00
	5.0	0.03 ± 0.00
	24.0	0.04 ± 0.00
Intestine	0.16	0.02 ± 0.00
	1.0	0.02 ± 0.00
	3.0	0.03 ± 0.00
	5.0	0.05 ± 0.00
	24.0	0.05 ± 0.00

of ^{99m}Tc-ior C5 was rapid, with a retention time of up to 24 h postinjection, consistent with the fact that radiolabeled MAbs and their degradation products are excreted via the kidneys.

For *in vivo* biodistribution studies, female athymic nude mice (*nu/nu*) were injected s.c. in the right flank with human colorectal carcinoma SW948 cells (5 x 10^6 in 1 ml of saline). Two weeks after injection when tumors had grown to an average weight of 0.38 g and an average volume of 1.07 cm^3, biodistribution studies were performed by injection of ^{99m}Tc-ior C5 (80 μg/150 μCi). Eight mice were injected via the lateral tail vein with a total volume of 0.15 ml. Among the various organs, significant accumulation of radiolabel was found in the blood (11.38 ± 2.90%), heart (2.84 ± 0.46%), kidneys (3.63 ± 1.16%), lungs (2.49 ± 0.63%) and spleen (2.16 ± 0.89%) at 4 h (Table IV). These values were reduced to 7.79 ± 2.02%, 2.37 ± 0.74%, 3.60 ± 0.12%, 2.21 ± 0.32% and 1.21 ± 0.09%, respectively, at 24 h. The significant amount of radioactivity in kidneys suggests radiopharmaceutical clearance via the urinary bladder, and the low accumulation of radioactivity in the liver and small intestine suggests that the clearance of this radiopharmaceutical is not

Table IV: Biodistribution of ^{99m}Tc-ior C5 in nu/nu mice at 4 and 24 h postinjection.

Organ/tissue	Time postinjection (h)	% Injected dose (ID)/g tissue (mean ± SD)
Blood	4	11.38 ± 2.90
	24	7.79 ± 2.02
Tumor	4	1.75 ± 0.89
	24	7.79 ± 4.66
Heart	4	2.84 ± 0.46
	24	2.37 ± 0.74
Liver	4	2.47 ± 0.86
	24	1.92 ± 0.29
Kidneys	4	3.63 ± 1.16
	24	3.60 ± 0.12
Lungs	4	2.49 ± 0.63
	24	2.21 ± 0.32
Stomach	4	1.01 ± 0.44
	24	0.87 ± 0.12
Spleen	4	2.16 ± 0.89
	24	1.21 ± 0.09
Small intestine	4	0.93 ± 0.14
	24	1.13 ± 0.34
Large intestine	4	1.21 ± 0.31
	24	1.89 ± 0.05

via the hepatobiliary-intestinal route. The uptake of ^{99m}Tc-ior C5 in tumors was considerably higher at both time points and the radiopharmaceutical showed strongly positive tumor/nontumor ratios at the later time point.

Ten patients were administered 3 mg of ior C5 radiolabeled with ^{99m}Tc activity of 1435.0 ± 123 MBq by i.v. bolus injection. Blood and urine samples were collected from 4 of 10 patients at intervals from 10 min up to 24 h after injection of ^{99m}Tc-ior C5 for pharmacokinetic studies. The mean plasma clearance curve of the ^{99m}Tc-labeled MAb in patients bearing colorectal carcinomas is shown in Figure 2. The best-fitting plasma time-activity curves in all patients had a distribution half-life ($t_{1/2\alpha}$) of 4.31 ±

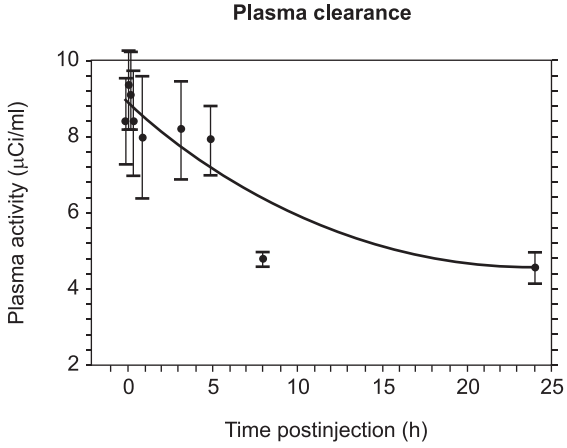

Fig. 2. Mean plasma clearance curve following an i.v. bolus injection of ^{99m}Tc-ior C5 in 4 patients. There were no statistically significant differences between individuals ($p < 0.05$).

Table V: Pharmacokinetic parameters following ^{99m}Tc-ior C5 i.v. bolus injection in cancer patients.

Pharmacokinetic parameter	Mean ± SD (n = 4)
$t_{1/2\alpha}$	4.31 ± 2.18
$t_{1/2\beta}$	32.64 ± 3.82
AUC (μCi/ml·h)	211.52 ± 107.41
C_o (μCi/ml)	12.43 ± 4.39
V_c (ml/kg)	54.74 ± 20.92
V_{ss} (ml/kg)	129.86 ± 70.01
Cl_D (ml/kg)	3.54 ± 1.71

2.18 h and an elimination half-life ($t_{1/2\beta}$) of 32.64 ± 3.82 h (Table V). The AUC was 211.52 ± 107.41 μCi/ml·h and the C_o was 12.43 ± 4.39 μCi/ml. The mean apparent volume of distribution of the central compartment was 54.74 ± 20.92 ml/kg, the mean apparent steady-state volume of distribution was 129.86 ± 70.01 ml/kg, and the mean systemic clearance was 3.54 ± 1.71 ml/kg. In the 5 patients studied, maximum urine levels were reached at 3-6 h after injection. The mean % ID of ^{99m}Tc-ior C5 excreted in the urine by 24 h under physiological conditions was 15.89 ± 0.23%. There were no significant differences among patients for any pharmacokinetic parameter.

Biodistribution studies were conducted to quantify immunospecific localization and provide a basis for developing dose estimates. ^{99m}Tc-ior C5 was detected in the liver, heart (vascular pool), spleen and kidneys already at 1 h postinjection, and was still visible through 24 h postinjection. Figure 3 presents the anterior and posterior whole-body images obtained at 1, 3, 5 and 24 h postadministration, which illustrates the normal biodistribution of ^{99m}Tc-ior C5. These images are typical of those obtained in patients thought to be disease-free and they showed early, persistent and high activity levels in heart, liver and spleen. Good clearance was seen at 24 h for all organs. In the image at 1 h, some activity was seen in the blood pool, but by 24 h radioactivity in blood vessels was barely discernible. At 24 h, some bowel radioactivity, probably due to hepatobiliary excretion, was seen, indicating that the bowel constitutes one of the major sites of excretion. The uptake in major organs (liver, heart, spleen, kidneys and the urinary bladder) was determined as a function of time. Radioactivity in the urinary bladder was calculated using the measured radioactivity excreted in the urine and assuming a bladder voiding interval of 3 h (20, 21). Radioactivity in the remainder of the body (expressed as percentage of the administered activity) as a function of time was determined as 100% minus the percent uptake in liver, heart, spleen, lungs, kidneys and urinary bladder and excreted radioactivity in urine. Among the main target organs, accumulation of the radiolabeled antibody was found in liver (9.38 ± 0.80%), heart (8.92 ± 0.94%) and spleen (1.37 ± 0.30%) at 5 min postadministration. These values were reduced at 24 h to 5.91 ± 0.73% and 0.62 ± 0.22%, respectively, for the heart and spleen, and increased to 9.78 ± 1.99% for the liver. Localization of the radioactivity in the liver following injection of ^{99m}Tc-ior C5 for up to 24 h indicated that the liver has the greatest number of sites of excretion and/or

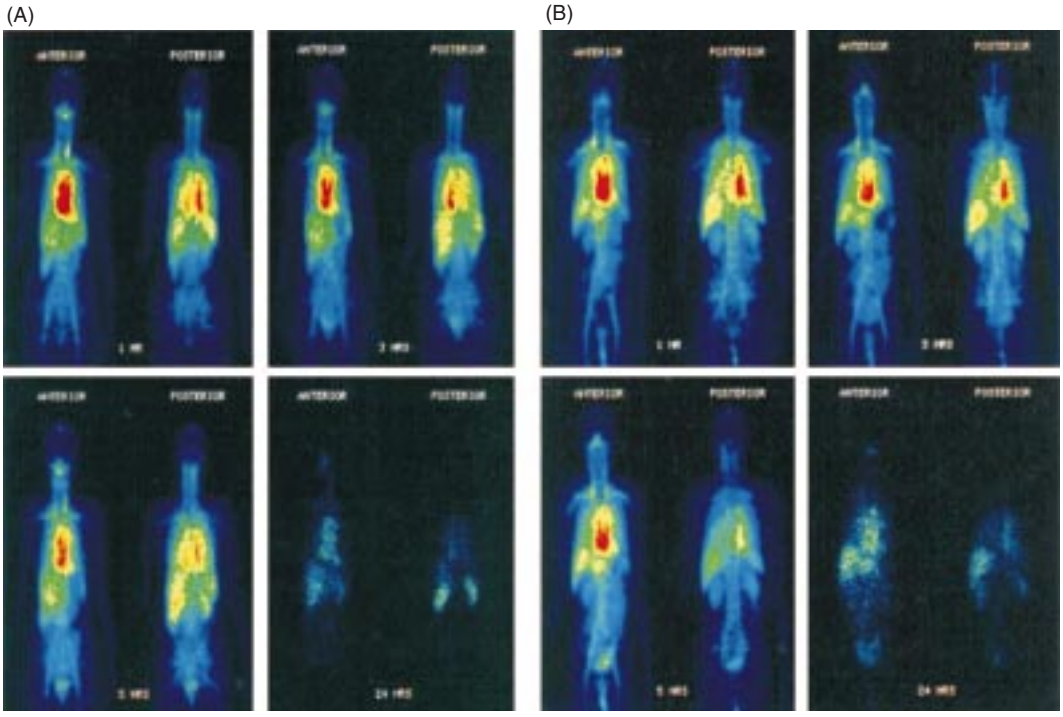

Fig. 3. Normal uptake kinetics of ^{99m}Tc-ior C5 administered as an i.v. bolus of 3 mg. Serial whole-body anterior and posterior scans in patients No. 2 (A) and 3 (B) taken at 1, 3, 5 and 24 h after injection, indicating very low accumulation in liver and lungs.

Table VI: Absorbed radiation dose in normal organs and tissues after administration of ^{99m}Tc-ior C5.

Organ/tissue	Absorbed radiation dose (rad/mCi)
Heart wall	0.0768 ± 0.0090
Kidneys	0.0530 ± 0.0260
Spleen	0.0540 ± 0.0128
Urinary bladder	0.0430 ± 0.0070
Liver	0.0565 ± 0.0109
Whole body	0.0181 ± 0.0017
	rem/mCi
Effective dose equivalent	0.0314 ± 0.0031
Effective dose	0.0249 ± 0.0027

metabolism of radiolabeled MAb and its degradation products. Good clearance was seen from the lungs, spleen and urinary bladder at 24 h postinjection.

In this study, dose calculations for normal organs based on the MIRD scheme (20-23) for ^{99m}Tc-ior C5 were performed in 4 patients using the residence times. Instantaneous uptake, homogeneous distribution and mono- or biexponential clearance of the radioactive substance from the tissues were assumed. Average absorbed dose estimates for normal organs for a 3-mg (38.78 ± 3.32 mCi) dose of ^{99m}Tc-ior C5 expressed in rads/mCi administered (Table VI) were: heart wall, 0.0768 ± 0.0090; kidneys, 0.0530 ± 0.0260; spleen, 0.0540 ± 0.0128; urinary bladder, 0.0430 ± 0.0070; liver, 0.0565 ± 0.0109; and whole body, 0.0181 ± 0.0017. The heart wall received the highest dose. The large accumulation and retention of activity in the vascular pool suggested that a large amount of the radiolabeled antibody does not bind to normal tissues and remains circulating in the body (24). The effective dose equivalent and effective dose estimates for adults (Table VI) were: 0.0314 ± 0.0031 and 0.0249 ± 0.0027 rem/mCi. There were no statistically significant differences in the dose estimates of the various normal organs or in the effective dose equivalent or effective dose in comparison to the direct estimates for each individual (data not shown) (24).

Toxicity

Single-dose toxicity studies with ^{99m}Tc-ior C5 were carried out in male and female Sprague-Dawley rats. Four groups of animals of 10 animals per group (5 female and 5 male) were administered an i.v. bolus of saline control solution and 0.34, 3.4 and 6.8 mg/kg of ior C5, equivalent to 1, 10 and 20 times, respectively, the maximum dose to be used in humans. Daily physical examination of the animals showed that there were no alterations in behavior or significant signs of toxicity attributable to the product at any dose. No notable drug-related systemic or local toxicity at the injection site was seen. The increase in body weight showed a similar trend in all groups. No clinical signs of toxicity or abnormalities were recorded on autopsy. Thus, the toxicological evaluation of ^{99m}Tc-ior C5 as single i.v. doses up to 20 times the maximum dose proposed to be administered to humans showed no evidence of clinical signs of toxicity in rats.

Repeated-dose toxicity studies with ^{99m}Tc-ior C5 were also carried out in male and female Sprague-Dawley rats. The proposed dose in humans of 1 mg corresponds to 0.67 mg/m^2, which is equivalent to 0.11 mg/kg in rats. Four groups of animals of 8 animals per group (4 female and 4 male) were administered an i.v. bolus of saline control solution and 0.11, 1.1 and 2.75 mg/kg of ior C5, doses equivalent to 1, 10 and 25 times, respectively, the maximum dose in humans, every day for 15 days. ^{99m}Tc-ior C5 administration was not associated with alterations that might be considered signs of toxicity in the treated animals. The anatomopathological analysis of the organs and tissues did shown evidence of macroscopic alterations attributable to the product. The hematological and biochemical parameters of the animals did not show intergroup differences that could be interpreted as signs of toxicity. Thus, repeated i.v. doses up to 25 times the proposed human dose showed no evidence of clinical signs of toxicity in rats.

To date, no studies have been performed in experimental animal models to evaluate the effects of the radiopharmaceutical on reproductive function in women and men.

Clinical Studies

A phase I clinical trial was conducted in 10 patients administered a dose of 3 mg (24). Among the patients, 7 had primary tumors and 3 suspected recurrences, including 5 colon adenocarcinomas, 2 rectal carcinomas, 1 anal canal carcinoma, 1 anorectal ring carcinoma and 1 well-differentiated carcinoma of the rectosigmoidal junction. Using a gamma camera, radiolabeled antibody was detected in 8 of 10 patients, whereas 2 patients with suspected recurrence were negative, for an overall sensitivity of 100%. In this study in patients with colorectal adenocarcinomas, 99mTc-ior C5 localized tumors of the colon and rectum and some unknown metastatic tumor sites.

A phase II clinical trial was performed in another 10 patients who were administered 1 mg of MAb labeled with 40 mCi of ^{99m}Tc. This trial included 2 patients with primary tumors and another 8 with suspected recurrence or metastasis. Six of 8 patients were positive after the 1-mg dose of ^{99m}Tc-ior C5 and 2 patients with suspected recurrence were negative, for an overall sensitivity of 100%.

Phase III studies were conducted in 74 patients who were randomized to 2 groups. In Group A (n=35), patients received ^{99m}Tc-ior C5 initially, followed 7 days later by MAb ^{99m}Tc-ior cea1, the gold standard, and in Group B (n=39), patients received an initial injection of ^{99m}Tc-ior cea1, followed 7 days later by ^{99m}Tc-ior C5. The overall sensitivities were 96.3% for ^{99m}Tc-ior C5 and 100% for ^{99m}Tc-ior cea1 in Group A, and 83.9% for ^{99m}Tc-ior cea1

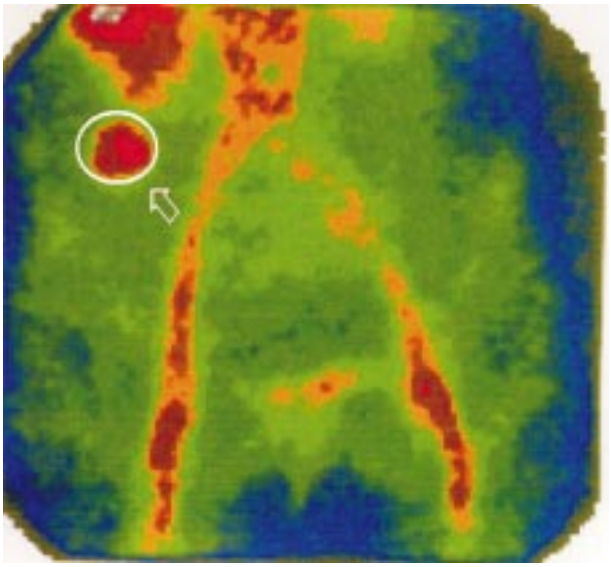

Fig. 4. Tumor localization through planar images (circle and arrow) taken at 24 h after injection of ^{99m}Tc-ior C5 at a dose of 3 mg i.v. in a patient with colon adenocarcinoma.

and 82.8% for ^{99m}Tc-ior C5 in Group B. Overall, the sensitivity of ^{99m}Tc-ior C5 was 89.3% and for ^{99m}Tc-ior cea1 91.8%.

The specific tumor localization of i.v. ^{99m}Tc-ior C5 was also studied in colorectal cancer patients. Tumor localization was obtained from multiple planar γ camera and SPECT images. The geometric mean of the serial anterior and posterior planar images performed at 24 h is shown in Figure 4, showing tumor uptake with an area of increased ^{99m}Tc-ior C5 activity (circle and arrow) in the colon adenocarcinoma of patient No. 18, a 68-year-old man with a primary tumor in the ascending colon. In most of the patients with positive immunoscintigraphy, there was rapid uptake in the tumor by the first 1-3 h postadministration of ^{99m}Tc-ior C5. Tumor uptake was maintained for up to 24 h, whereas blood vessels were barely seen at this time point. Other studies have also demonstrated the ability of the compound to localize metastases from colon adenocarcinoma tumors (Fig. 5). These data also demonstrate the selective binding of MAb ior C5 to colon carcinoma lesions *in vivo*.

Phase II clinical diagnostic studies were performed in 22 adult female patients with ovarian tumors. Before inclusion in the trial, all patients underwent clinical, radiological and ultrasound examinations (25). For the trial, patients were divided into 2 groups. Group 1 included 9 patients with palpable ovarian tumors and without any previous treatment or any past history of ovarian cancer. All patients from this group were subject to laparoscopic biopsy or exploratory laparotomy to confirm the entry diagnosis. Group 2 included 13 patients with a past history of ovarian cancer who were entered into the study for follow-up to detect possible recurrences or metastases. All 13 patients also underwent second-look laparotomy after immunoscintigraphy to confirm the diagnosis (25). From the histological studies, the 9 patients in Group 1 were negative for ovarian tumors, while 10 (76.9%) of 13 patients in Group 2 had histologically active malignant disease, with 6 of 13 being in stage III at the time of the study. Immunoscintigraphy revealed absence of disease in 7 (77.7%) of 9 patients in Group 1. There were 2 (22.2%) false-positives in this group: an ovarian fibroma with a broad inflammatory area around it and a mature cystic teratoma. All 9 locoregional and 3 distant lesions were correctly identified in the 10 histologically positive patients from Group 2. The 3 histologically negative cases were also negative in the immunoscintigraphy

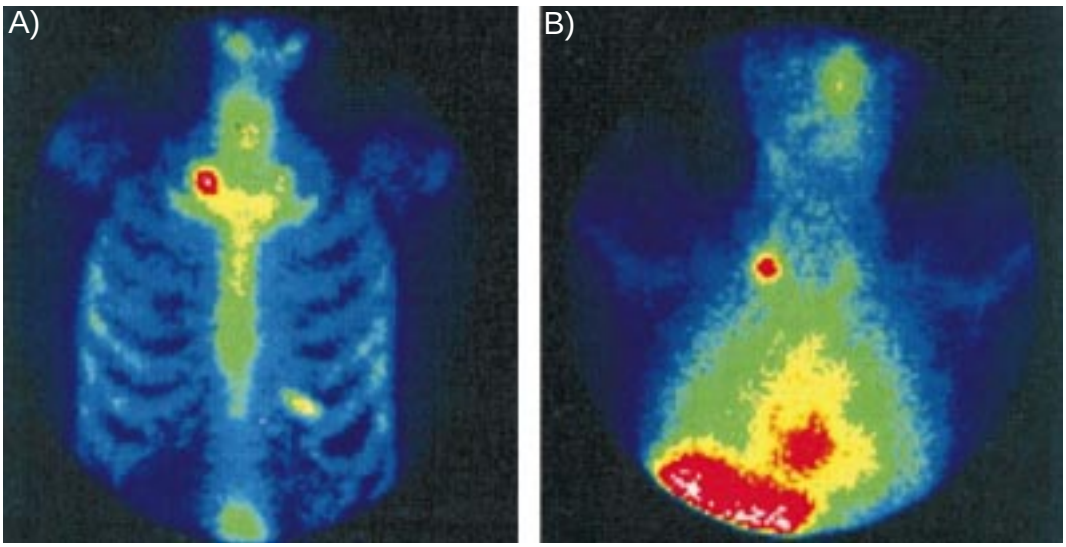

Fig. 5. Imaging from bone metastases of a primary colon adenocarcinoma measured for ^{99m}Tc-MDP at 3 h after injection (A) and for ^{99m}Tc-ior C5 at 24 h after injection (B).

study. The overall sensitivity, specificity and accuracy of immunoscintigraphy with ^{99m}Tc-ior C5 were 83.3%, 100% and 90.9%, respectively. The kappa coefficient of agreement (k) was 0.82 ± 0.12, showing good agreement between immunoscintigraphy and histopathology (25).

Conclusions

Data presented in this monograph demonstrate the selective binding of ^{99m}Tc-ior C5 to well-established xenografts in experimental animal models and to colorectal and ovarian tumors in patients (1, 18). The MAb ior C5 shows great promise for both radioimmunodetection and radioimmunotherapy. It binds to the TAA ior C2, which is expressed on 83% of human colon adenocarcinomas (1, 24) but not on normal tissues. This antibody could also be used in the management of recurrent colon and ovarian cancer. The impact of such monitoring on the overall mortality of patients with recurrent colon or ovarian cancer is limited by the relatively small proportion of patients in whom localized, potentially curable metastases are found. To date, there have been no large-scale randomized trials documenting the efficacy of a standard postoperative monitoring program (26-28). Postoperative monitoring should be reserved primarily for the detection of asymptomatic recurrences that can be curatively resected, and for the early detection of metachronous tumors (28).

Although studies in humans demonstrated that patients did not develop a human anti-mouse antibody (HAMA) response following administration of the murine antibody, a humanized form has been prepared and is being tested in preclinical studies. Reduced immunogenicity would permit multiple injections, which in turn would expand the diagnostic and therapeutic usefulness of this MAb. It would also permit fractionation of the doses when the antibody is used for radioimmunotherapy, which may be required for limiting toxicity to normal tissues.

Finally, the cytotoxic effect of this antibody may be enhanced if it is coupled to β- or α-emitting radioisotopes.

Source

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