^{153}Sm-DTPA-Cetuximab targeted to tumors xenografted in mice

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ABBREVIATION

Sm : Samarium

DTPA : Diethylene triamine pentaacetic acid

EGFR : Epidermal growth factor receptor

RIT : Radioimmunotherapy

ITLC-sg : Instant thin layer chromatography - silica gel

% ID/g : Percentage injected dose per gram

SDS-PAGE : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SPECT : Single photon emission computed tomography

CT : Computed tomography

keV : Kiloelectron volt

MBq : Megabecquerel

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ABSTRACT

The radionuclide labeled with monoclonal antibodies can be delivered to the target cells and showed successful results in cancer, which is known as radioimmunotherapy (RIT). ^{153}Sm ($T_{1/2}$; 46.3 hr) is a medium beta energy emitter [E_{max} = 810 (20%), 710 (50%) and 640 (30%) keV] with 30% abundance of gamma emission (103 keV) that is suitable for radioimmunoscintigraphy and radioimmunotherapy. In addition to the desirable emission properties of ^{153}Sm, it also readily forms complexes with bifunctional chelating agents that can be conjugated to antibodies. Cetuximab, a chimeric monoclonal antibody against epidermal growth factor receptor (EGFR), is currently used to treat several solid tumors.

In this study, ^{153}Sm-Diethylene triamine pentaacetic acid (DTPA)-Cetuximab was assessed by immuno-single photon emission computed tomography (immuno-SPECT) imaging and RIT as a potential diagnostic and therapy application in a EGFR expression tumor (A431) model. The antibody was labeled with ^{153}Sm-chloride after conjugation with freshly prepared DTPA and their immunoreactivity exceeded 71% for A431 tumor. The *in vitro* stability test of ^{153}Sm-DTPA-Cetuximab in serum indicated that >96% of the radionuclide remained bound to the antibody for 24 hr. The biodistribution study using ^{153}Sm-DTPA-Cetuximab indicated the highest tumor uptake of 10.65 ± 0.66% ID/g at 120 hr post-injection and tumor-to-blood and tumor-to-muscle ratios of 4.83 ± 1.41 and 21.43 ±

4.54% ID/g at 168 hr post-injection, respectively. This data shows that the urinary system is a predominant clearance system for the ^{153}Sm-DTPA-Cetuximab. A quantitative analysis was also performed using SPECT/CT and autoradiography images. ^{153}Sm-DTPA-Cetuximab was selectively localized in EGFR-positive A431 tumors. The RIT efficacy of ^{153}Sm-DTPA-Cetuximab was evaluated once a week for 4 week in A431-xenografted mice. RIT with ^{153}Sm-DTPA-Cetuximab resulted in delayed tumor growth.

The biodistribution, SPECT/CT and autoradiography results suggest that ^{153}Sm-DTPA-Cetuximab can be applicable for *in vivo* diagnosis of cancers and RIT with ^{153}Sm-DTPA-Cetuximab is effective in inhibition of EGFR-expressing tumors with acceptable toxicity.

Key words : ^{153}Sm, monoclonal antibody, bifunctional chelate, immuno-SPECT, radioimmunotherapy

I. INTRODUCTION

Radioimmunotherapy (RIT) developed from the spectacular growth in cancer therapy that resulted in the production of highly purified monoclonal antibodies for clinical use. Numerous radiolabeling has been successful with specific and purified monoclonal antibodies, which are excellent targets for diagnostic and therapeutic purpose [1]. In RIT, use of monoclonal antibodies as a means of targeting radionuclides to specific cells or tissues has recently become more promising in both diagnostic and therapeutic applications [2]. The use and acceptance of radiolabeled antibody has resulted from the ability to design compounds that have suitable biological characteristics, such as rapid blood clearance, low toxicity, high uptake into tumor and low immunogenicity [2, 3].

A significant number of monoclonal antibody (mAb) products have been developed in clinical trials, and more are currently in clinical evaluation [4, 5]. Cetuximab (Erbitux; C225) is an immunoglobulin G1 chimeric (human/mouse) monoclonal antibody that acts as an inhibitor of epidermal growth factor receptor (EGFR) [6]. Cetuximab can cause cell-cycle arrest, potentiation of apoptosis, inhibition of angiogenesis and inhibition of tumor-cell invasion and metastasis [7, 8]. Cetuximab received approval from the Food and Drug Administration (FDA) USA in 2006 for use in combination with radiation therapy for treating squamous cell carcinoma of the head and neck [9].

The radiolantanides (^{153}Sm, ^{166}Ho, ^{177}Lu, etc.) are considered excellent

candidates for RIT because of their desirable physical characteristics and ready availability [10]. The potential application of ^{153}Sm in peptide-targeted RIT is indicated by successful use in both bone palliation and in radiation synovectomy [2]. ^{153}Sm has a half-life of 46.3 hr, appropriate to the kinetics of whole immunoglobulins and F(ab´)$_2$ fragments, a mean beta energy [E_{max} = 810 (20%), 710 (50%) and 640 (30%) keV] suitable for treating small lesions while limiting irradiation of adjacent normal tissues and a gamma emission with an energy of 103 keV (28%), which facilitates gamma camera detection [11]. ^{153}Sm can be produced from enriched ^{152}Sm by the (n, ɣ) reaction in a high yield. In addition to the desirable emission properties of ^{153}Sm, this lantanide also readily forms complexes with diethylene triamine pentaacetic acid anhydride (DTPA)-type chelates that can be conjugated to small peptide based molecules [2, 12].

Single-photon-emitting radionuclides have many advantages in these applications, including a range of half-life, relatively simple radiolabeling chemistry, low cost, and broad availability. Recently growth in nuclear imaging applications has motivated academic and industrial development of both single photon emission computed tomography (SPECT) and positron emission tomography (PET) systems designed specifically for small-animal imaging. SPECT has several features suited for small-animal imaging. For example, gamma rays are recorded to SPECT after radionuclide emission, thereby gaining a theoretic advantage in resolution. SPECT uses many radiopharmaceuticals widely applied in clinical nuclear medicine and can be obtained from fundamental radiopharmacies.

Small-animal SPECT studies generally cost less than other small-animal imaging methods, such as small-animal MRI or PET [13, 14, 15, 16].

In this study, Cetuximab (anti-EGFR antibody) conjugated with DTPA and radiolabeled with ^{153}Sm. The prepared ^{153}Sm-DTPA-Cetuximab was assessed the immuno-SPECT imaging and RIT potential as a diagnostic and therapeutic agent in a EGFR-positive tumor model.

II. MATERIALS AND METHODS

1. Materials

^{153}Sm was provided by Korea Atomic Energy Research Institute (KAERI, Korea). Cetuximab (Erbitux) was a pharmaceutical sample purchased from Merck Inc. (Germany). DTPA anhydride, ethylene diamine tetra acetic acid (EDTA), acetic acid, sodium acetate, sodium hydroxide and sodium bicarbonate were purchased from Sigma-Aldrich Chemical Co. (USA). Antibodies and conjugates were concentrated with Vivaspin-20 centrifugal filter devices (Sartorius, Germany), Slide-A-Lyzer® dialysis cassette (Pierce, USA) and a Allegra X-15R centrifuge (Beckman Coulter, USA). The radiochemical purity and radiolabelling yield were analyzed with instant thin layer chromatography using silica gel (ITLC-sg, Pall Co., USA) and measured by a thin-layer chromatogram scanner (Aloka, Japan). The radioactivity was counted on a 1480 WIZARD well-type gamma counter (PerkinElmer, Wallac, Finland). SPECT/CT images were obtained with a SPECT/CT system (Inveon, Siemens Preclinical Solution, USA). Digital whole body autoradiography was performed with a cryostat microtome (Nakagawa Seisakusho, Japan) and phosphor imager (BAS-5000, FUJIFILM Co., Japan).

2. Cell lines and culture

Human epithelial carcinoma A431 cell line, human lung adenocarcinoma epithelial A549 cell line and human glioblastoma U87-MG cell line were purchased from American Type Culture Collection (Manassas, USA). A431 and U87-MG cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA). A549 cells were maintained in RPMI 1640 medium (Gibco, USA). All the cells was cultured in mediums supplemented with 10% Fetal Bovine Serum (FBS, JR scientific Inc., USA), 100 units/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 37℃.

3. Evaluation of EGF receptor expression in cancer cells

Western blot analysis was performed to evaluate EGFR expression in cells. A431, A549 and U87-MG cells (5×10^6 cells) were lysed in a cell lysis buffer (M-PER Mammalian Protein Extraction Reagent, Pierce, USA) with protease inhibitor cocktail for 1 hr in 4℃ cold chamber. The cellular total protein concentration was determined by the micro-protein assay (DC Protein Assay, USA), measuring at 655 nm of wavelength with microplate reader 550 (Bio-Rad, USA). The extracted proteins (10 μg) were separated by a 4 - 15% SDS-polyacrylamide gel (100 V, 70 min) and transferred (100 V, 1 hr, 4℃) to nitrocellulose membranes (Micron separations Inc., USA). The membrane were blocked with TBST containing 5% (w/v) skim milk for overnight in 4℃ cold chamber and triple-washed with TBST (Tris-buffered saline Tween 20) for 10 min. The washed membrane was incubated with a 1:200 dilution of the anti-EGFR rabbit antibody (Cell signaling Technology, USA) for 2 hr at room temperature and triple washed with TBST for 10 min. The membranes were incubated with a 1:1,000 dilution of the horseradish peroxidase conjugated to anti-mouse and rat antibodies (Santa Cruz Biotechnology, USA) for 1 hr at room temperature and triple washed with TBST for 100 min. The immunolabeled proteins were visualized ECL (Amersham, UK) and exposed onto X-ray film (Agpa, Belgium). The anti-alpha tubulin (Sigma, USA) was used as an internal control.

4. Preparation of ^{153}Sm-DTPA-Cetuximab

4.1. Synthesis of DTPA-Cetuximab

Cetuximab (10 mg) was incubated for 30 min at room temperature with 50 μL of 0.1 M sodium acetate buffer containing 50 mM EDTA (pH 6) to remove any metal ions and concentrated with vivaspin-20 (50K MWCO). The Cetuximab buffer was changed to 0.1 M sodium bicarbonate buffer (pH 8.5) and concentrated to 5 mg/mL. The Cetuximab in the solution (5 mg) was conjugated with DTPA anhydride at a molar ratio of 1:1,000, and pH was adjusted to 8.5 by adding 1 M sodium hydroxide. The conjugation mixtures were incubated with gentle mixing at room temperature for 1 hr and followed by incubation at 4℃ overnight with end-over-end rotation. DTPA-Cetuximab conjugates were purified and concentrated by Slide-A-Lyzer dialysis cassette (2K MWCO), and the buffer was then changed with 0.1 M sodium acetate buffer (pH 6). The concentration of conjugation mixture was measured on UV absorbance at 280 nm.

4.2. Production of ^{153}Sm

A detailed procedure for production of ^{153}Sm has been reported [17]. A ten milligram of $^{152}Sm_2O_3$ was irradiated in the HANARO reactor at the Korea Atomic Energy Research Institute, Daejeon, Korea (reactor

power = 20MW) under a neutron flux of 1.7×10^{13} $n/cm_2 \cdot sec$ for 1 hr. Radionuclidic purity of the $^{152}Sm_2O_3$ was determined by a multichannel analyzer, GEM25185 (EG&G Ortec, USA) and the radioactivity of the $^{152}Sm_2O_3$ was determined by a radioisotope calibrator, Atomlab 200 (Biodex, New York, USA). $^{152}Sm_2O_3$ was transferred to a glass vial and dissolved in 2 mL of 4 N HCl and then evaporated at 100℃ under a stream of argon gas. The residue was dissolved in an appropriate volume of d-HCl (pH 2.8) to yield 0.5% ^{153}Sm-chloride ($^{153}SmCl_3$) solution.

4.3. Radiolabeling of DTPA-Cetuximab with ^{153}Sm

Radiolabeling of DTPA-Cetuximab with ^{153}Sm was performed by addition of 13 MBq of $^{153}SmCl_3$ in 0.1 M sodium acetate buffer (pH 6) to 200 μg of the conjugate followed by 1 hr incubation at room temperature with gentle mixing. The radiochemical purity was confirmed with instant thin-layer chromatography (ITLC) using 0.15 M sodium acetate buffer (pH 4.2) as the mobile phase. The developed radiochromatographs were analyzed by a TLC scanner.

4.4. *In vitro* serum stability test of ^{153}Sm-DTPA-Cetuximab

In vitro serum stability test of ^{153}Sm-DTPA-Cetuximab was analysed in the presence of an equivalent of human serum. A 1:1 mixture of

freshly prepared ^{153}Sm-DTPA-Cetuximab to human serum was incubated at 37℃ for 24 hr. At each time point (5 min, 1, 2, 4, 8, 12, 18 and 24 hr), the stability of ^{153}Sm-DTPA-Cetuximab was confirmed by ITLC.

5. Immunoreactivity of ^{153}Sm-DTPA-Cetuximab

The immunoreactive fraction of radiolabeled antibody using the Lindmo method [18, 19] and *in vitro* cell binding assay (comparison of EGFR positive A431 cell with negative U87-MG cell) were determined.

In the Lindmo method, the ^{153}Sm-DTPA-Cetuximab antibody in a volume of 100 μL (100 ng/mL) was mixed with 100 μL of 6 different dilutions of A431 tumor cells ranging from 2 × 10^6 cells/mL to 1 × 10^8 cells/mL. A431 cells were prepared in a PBS solution containing 1% BSA. The reaction mixtures were incubated with continuous shaking at room temperature for 90 min. The cell suspensions were washed three times with 2 mL of cold PBS and centrifuged at 2,000 rpm for 5 min. The radioactivity associated with the cell pellet (cell binding activity) was determined by counting the tubes in a gamma counter with corresponding standards (for nonspecific binding).

In *in vitro* cell binding assay of A431 and U87-MG cancer cells, A431 and U87-MG cells (1 × 10^6 cells/tube for specific binding and non-specific binding) suspensions prepared in an 1% BSA-PBS. For determination of non-specific binding of ^{153}Sm-DTPA-Cetuximab, one set of tubes was incubated with unlabeled Cetuximab (10 μg) at room

temperature for 15 min to block receptors. The ^{153}Sm-DTPA-Cetuximab (100 ng) was added to the cells in the tubes which were then incubated at room temperature for 90 min. The tube was washed three times with 2 mL of cold PBS. The radioactivity associated with the cell pellet was determined by counting the tubes in a gamma counter with corresponding standards.

6. An experimental animal model

All animal studies were conducted in accordance with the highest standards of care as outlined in the Korea Institute of Radiological and Medical Science Animal Care and Use Committee. Experimental animal models were established in 6 week-old athymic female nude mice (BALB/c-nu/nu, Japan SLC inc., Japan). Mice were maintained in autoclaved microisolator cages housed in a positive pressure containment rack. A431 tumor cells (1×10^7 cells/mouse; single cell suspensions of over 98% viability as determined by trypan blue exclusion) in 100 μL PBS were inoculated subcutaneously into the right flank of mice. Tumor volumes in mm^3 were measured using a formula: $(L \times W^2) \times 1/2$ [20]. Biodistribution, SPECT/CT and autoradiography were conducted when tumor volumes reached 100 mm^3 at 3-4 weeks after tumor implantation.

7. Biodistribution of ^{153}Sm-DTPA-Cetuximab

The biodistribution of ^{153}Sm-DTPA-Cetuximab conjugates were evaluated in A431 tumor-bearing nude mice. Each tumor-bearing mouse was injected with 100 μL of ^{153}Sm-DTPA-Cetuximab conjugates (7.4 MBq/100 μg) via tail vein. Groups of mice (n = 5) were sacrificed by ether asphyxiation at selected times post-injection (2, 24, 48, 72, 120 and 168 hr). Blood was collected by cardiac puncture. Tumors and organs (heart, liver, lung, spleen, kidney, stomach, small intestine, large intestine, thyroid, muscle and femur) were excised, blotted and weighed. All samples were counted in a well-type gamma counter. Results of accumulated activity were expressed as the percentage of injected dose per gram of tissue (% ID/g), as tumor-to-blood (T/B) ratios and tumor-to-muscle (T/M) ratios.

8. SPECT/CT imaging of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mouse

The A431 tumor-bearing nude mouse was injected with ^{153}Sm-DTPA-Cetuximab (22.2 MBq/100 μg) via tail vein. After 120 hr post-injection, the small animal SPECT/CT images were obtained for 70 min using the SPECT/CT systems. The injection was performed after the mouse had been anesthetized with 2% isoflurane.

9. Autoradiography imaging of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mouse

Autoradiography study was performed in A431 tumor-bearing nude mouse injected with ^{153}Sm-DTPA-Cetuximab (22.2 MBq/ 100 μg) via tail vein. After 120 hr post-injection, the mice were euthanized, the tumors were frozen with 3% carboxy methyl cellulose (CMC) and dry-ice (-70℃) and prepared for sectioning with a cryostat (-20℃) microtome. The sections were obtained with the dorsal plane at 20 μm thickness. Tape-mounted cryosections were freeze-dried for 24 hr at -20℃, and placed on imaging plates and put in a BAS cassettes for 2 hr exposure at 4℃. All digital autoradiographic systems were scanned on a BAS-5000.

10. Radioimmunotherapy of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mice

RIT studies were performed using 0.9 % sodium chloride, Cetuximab, ^{153}Sm-DTPA-Rituximab and ^{153}Sm-DTPA-Cetuximab (radioactivity of 9.5 MBq and 100 μg/100 μL antibody concentration). The highest radioactive dose that caused less than 20% decrease in body weight [21, 22]. 4 groups of mice (n = 8) tumors (A431) with a mean volume of 103.2 ± 61.8 mm^3 were treated three times once a week with saline (100 μL), Cetuximab (100 μg/100 μL), ^{153}Sm-DTPA-Rituximab (9.5 MBq/100 μg/100 μL) or ^{153}Sm-DTPA-Cetuximab (9.5 MBq/100 μg/100 μL) by intravenous injection.

During treatment the tumors were measured three times a week by external caliper, and tumor volumes relative to the volume at the start of treatment were calculated: $(L \times W^2) \times 1/2$ [20]. Their toxicities were monitored by measurement of body weight three times a week.

III. RESULTS

1. Evaluation of EGF receptor expression in cancer cells

According to the Western blotting analysis of EGFR protein expression, the EGFR was highly expressed in the A431 cells more than the A549 cells. The EGFR was little expressed in the U87-MG cells (Figure 1). According to this data, the EGFR over-expression A431 cells were utilized as an *in vitro* and *in vivo* tumor model in this study. The U87-MG cells were used as a negative control of EGFR expression cells.

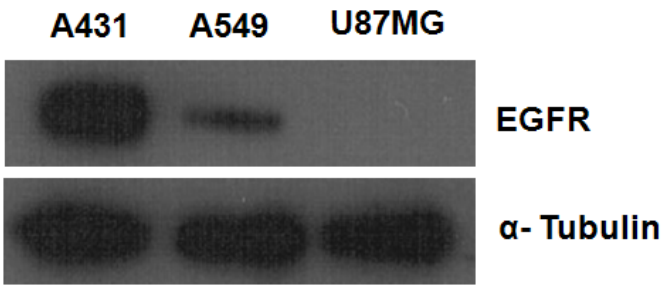

Figure 1. Analysis of EGFR expression in A431, A549 and U87-MG by Western blot. The EGFR was highly expressed in the A431 cells more than the A549 cells. The EGFR expression was little in U87-MG cells.

2. Radiolabeling of DTPA-Cetuximab with ^{153}Sm

The ^{153}Sm-DTPA-Cetuximab was prepared to a specific activity of 13 MBq/200 μg protein. The radiolabeling yield of ^{153}Sm-DTPA-Cetuximab was confirmed with ITLC and to be >99%. On ITLC-sg with a mobile phase of 0.15 M sodium acetate buffer (pH 4.2), radiolabeled antibodies remains at the origin (R_f = 0) and the free $^{153}SmCl_3$ moves with the solvent front as ^{153}Sm-acetate (R_f = 1) (Figure 2).

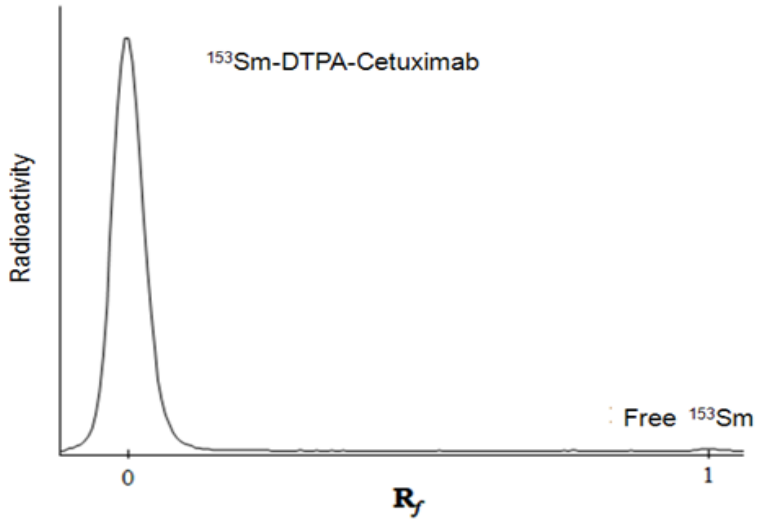

Figure 2. Analysis of radiolabeling yield of ^{153}Sm-DTPA-Cetuxiamb by instant thin layer chromatograpy (ITLC). ITLC-sg as a stationary phase and 0.15 M sodium acetate buffer (pH 4.2) as a mobile phase were used for ITLC. The ^{153}Sm-DTPA-Cetuxiamb remained at the origin (R_f = 0) and free $^{153}SmCl_3$ moves with the solvent front as ^{153}Sm-acetate (R_f = 1).

3. *In vitro* serum stability of ^{153}Sm-DTPA-Cetuximab

In vitro serum stability of ^{153}Sm-DTPA-Cetuximab was evaluated in the presence of human serum at 37℃ for 24 hr. During the incubation, intact ^{153}Sm-DTPA-Cetuximab was analyzed by ITLC. The radiochemical purity was 99.21 ± 0.28, 99.18 ± 0.21, 98.96 ± 0.26, 98.48 ± 0.33, 98.22 ± 0.20, 97.87 ± 0.22, 97.42 ± 0.38 and 96.98 ± 0.50 at 5 min, 1, 2, 4, 8, 12, 18 and 24 hr of incubation, respectively (Figure 3).

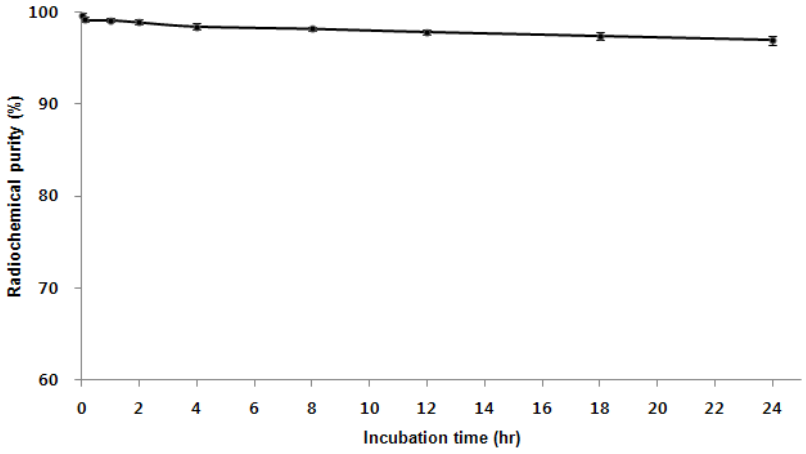

Figure 3. *In vitro* serum stability of ^{153}Sm-DTPA-Cetuximab.

The radiochemical purity of ^{153}Sm-DTPA-Cetuximab was 99.21 ± 0.28, 99.18 ± 0.21, 98.96 ± 0.26, 98.48 ± 0.33, 98.22 ± 0.20, 97.87 ± 0.22, 97.42 ± 0.38 and 96.98 ± 0.50 at 5 min, 1, 2, 4, 8, 12, 18 and 24 hr of incubation, respectively.

4. Immunoreactivity of ^{153}Sm-DTPA-Cetuximab

The results of antibody immunoreactivity measured by the Lindmo method are shown in Figure 4. Data were plotted as a double inverse plot: total applied radioactivity over specific binding as a function of the inverse cell concentrations. By means of linear extrapolation a to the ordinate, the immunoreactivity was determined to be 71.4%. The influence of radionuclide and specific activity on cell bound radioactive fraction is shown in Figure 4A [23].

The result of *in vitro* cell binding assay is shown in Figure 4B. Immunoreactivity of ^{153}Sm-DTPA-Cetuximab to A431 cells was 77.00 ± 2.05%. Immunoreactivity of ^{153}Sm-DTPA-Cetuximab to A431 cells was significantly different from that to U87-MG cells.

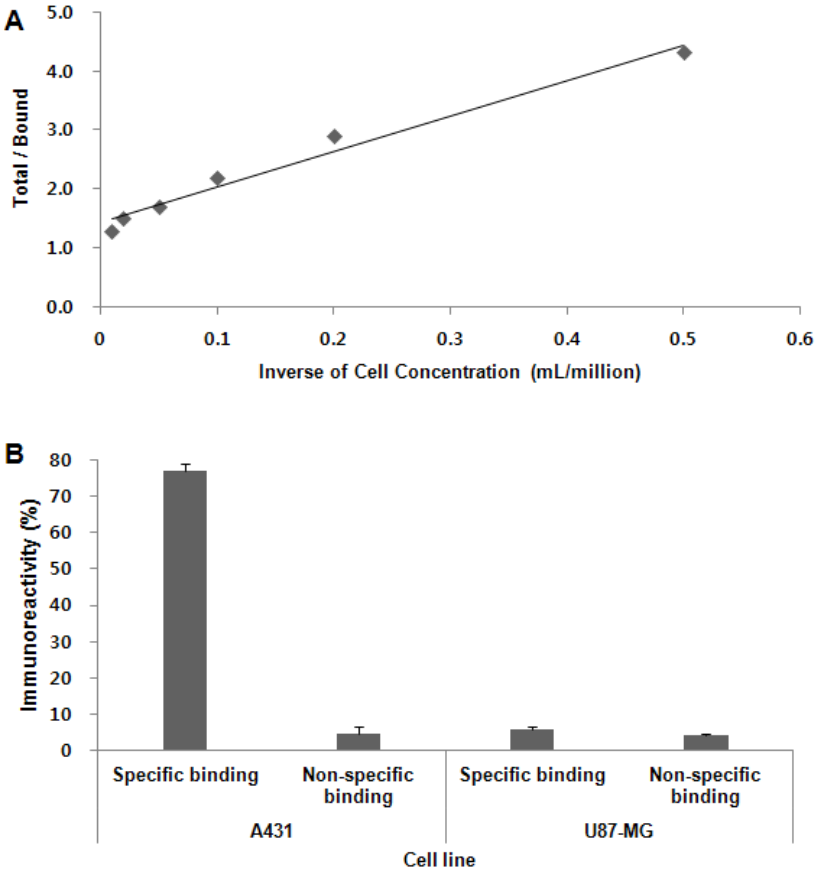

Figure 4. Immunoreactivity of ^{153}Sm-DTPA-Cetuximab. (A) The results of antibody immunoreactivity by the Lindmo method. (B) A431 and U87-MG cells was measured by cell binding assay.

5. Biodistribution of ^{153}Sm-DTPA-Cetuximab

^{153}Sm-DTPA-Cetuximab conjugates were intravenously injected to A431 tumor-bearing nude mice. At various time points post injection, radioactivities of bloods, tumors and organs were counted by a well-type gamma counter. The tumor (A431) uptake of ^{153}Sm-DTPA-Cetuximab was 1.26 ± 0.25, 5.12 ± 0.46, 6.90 ± 1.80, 8.77 ± 0.95, 10.65 ± 0.66 and 6.77 ± 0.64% ID/g at 2, 24, 48, 72, 120 and 168 hr post-injection, respectively (Table 1, Figure 5). Specific A431 tumor uptake peaked at 120 hr. ^{153}Sm-DTPA-Cetuximab was highly accumulated in blood, liver and spleen in early time. Accumulation of ^{153}Sm-DTPA-Cetuximab was increased over time in bone. The tumor-to-blood ratio of ^{153}Sm-DTPA-Cetuximab was 0.11 ± 0.03% ID/g at 2 hr post-injection and increased to 4.83 ± 1.41% ID/g at 168 hr. The tumor-to-muscle ratio of ^{153}Sm-DTPA-Cetuximab also increased to 21.43 ± 4.54% ID/g at 168 hr post-injection (Table 2).

Table 1. Biodistribution of ^{153}Sm-DTPA-Cetuximab in A431-xenografted mice

Unit : % ID/g

Organ	2 hr	24 hr	48 hr	72 hr	120 hr	168 hr
Blood	12.07±1.06 *	10.15±1.16	7.11±1.96	5.59±1.64	2.06±0.71	1.50±0.45
Heart	5.58±0.41	2.97±0.30	2.47±0.49	1.66±0.32	1.23±0.26	0.86±0.15
Liver	12.51±0.82	11.40±0.69	11.72±0.65	12.48±1.17	13.44±1.33	10.76±0.26
Lung	7.82±0.88	4.10±0.57	3.38±0.93	2.46±0.35	1.99±0.33	1.49±0.28
Spleen	10.87±1.85	7.14±1.97	7.00±1.11	6.95±2.11	6.68±1.42	6.14±0.39
Kidney	9.12±0.59	4.78±0.47	3.38±0.60	2.84±0.31	1.97±0.26	1.50±0.24
Stomach	2.21±0.69	1.48±0.71	1.29±0.27	0.80±0.10	0.85±0.33	0.46±0.12
S. Intestine	3.98±0.40	1.52±0.25	1.19±0.25	0.85±0.16	0.68±0.17	0.42±0.08
L. Intestine	1.32±0.41	2.07±0.27	1.63±0.23	1.15±0.13	0.80±0.08	0.66±0.29
Thyroid	0.13±0.02	0.08±0.03	0.05±0.03	0.06±0.04	0.05±0.03	0.07±0.02
Muscle	1.07±0.09	1.79±0.16	1.42±0.04	1.11±0.34	0.55±0.12	0.33±0.06
Femur	2.47±0.27	3.38±0.74	4.60±0.98	5.08±0.27	6.15±0.52	6.40±0.31
A431	1.26±0.25	5.12±0.46	6.90±1.80	8.77±0.95	10.65±0.66	6.77±0.64

* Values at each time point represent the mean ± S.D of percentage of injected dose per gram of tissue weight (n = 5).

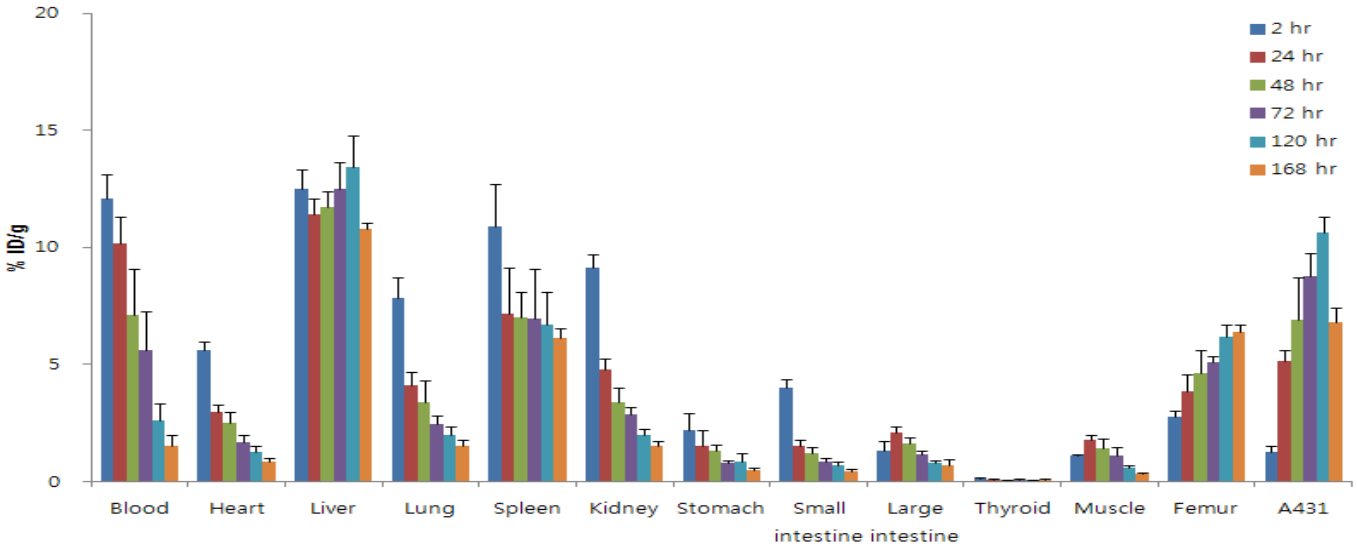

Figure 5. Biodistribution of ^{153}Sm-DTPA-Cetuximab in A431-xenografted mice. Animals were injected with ^{153}Sm-DTPA-Cetuximab (7.4 MBq/100 μg) and sacrificed at 2, 24, 48, 72, 120 and 168 hr post-injection.

Table 2. Tumor per blood and muscle ratios of ^{153}Sm-DTPA-Cetuximab in A431-xenografted mice

	2 hr	24 hr	48 hr	72 hr	120 hr	168 hr
Tumor/Blood ratio	0.11±0.03 *	0.51±0.05	1.01±0.28	1.67±0.46	4.32±1.09	4.83±1.41
Tumor/Muscle ratio	1.17±0.19	2.87±0.17	4.99±1.14	8.23±1.48	19.99±4.62	21.43±4.54

* Mean ± S.D (n = 5).

6. SPECT/CT imaging of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mouse

The SPECT/CT image of the A431 tumor-bearing nude mouse was acquired to evaluated *in vivo* tumor targetability of ^{153}Sm-DTPA-Cetuximab. The ^{153}Sm-DTPA-Cetuximab (22.2 MBq/100 μg) were injected via the tail vein. According to biodistribution data, A431 tumor uptake of ^{153}Sm-DTPA-Cetuximab was peaked at 120 hr post-injection. Therefore, the SPECT/CT image was acquired at 120 hr post-injection (Figure 6). ^{153}Sm-DTPA-Cetuximab was selectively localized in the right flank with EGFR-positive A431 tumor. The quantitative analysis of conventional SPECT/CT imaging gave values comparable to those of the biodistribution study in tissues accumulating radioactivity moderately, such as tumour, liver and spleen (Table 1). The analysis with the SPECT/CT imaging gave comparable results to those of the biodistribution for all tissue.

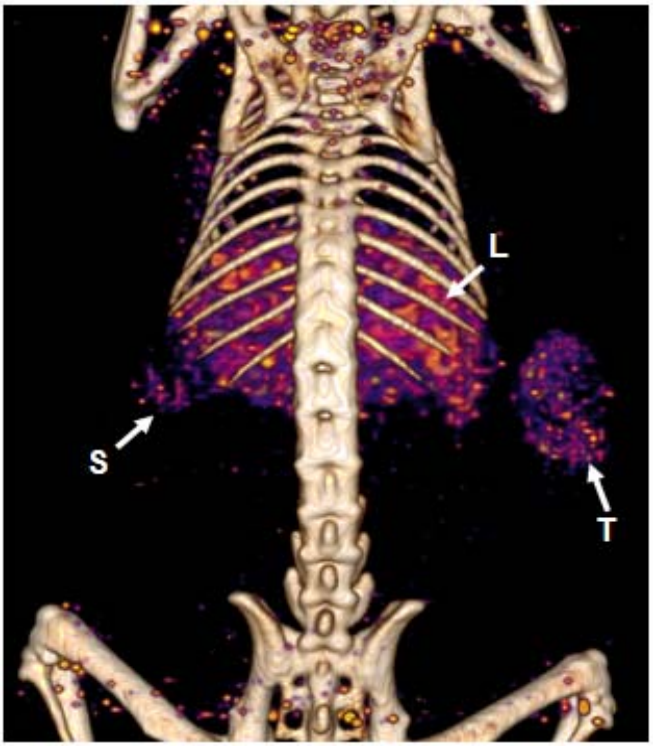

Figure 6. SPECT/CT imaging of ^{153}Sm-DTPA-Cetuximab in A431-xenografted mouse. SPECT/CT images were acquired at 120 hr post-injection of ^{153}Sm-DTPA-Cetuximab. This image is a dorsal view. (T: A431 tumor, L: Liver, S: Spleen)

7. Autoradiography imaging of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mouse

The autoradiography study was performed in A431 tumor-bearing nude mouse injected with ^{153}Sm-DTPA-Cetuximab (22.2 MBq/ 100 μg) via the tail vein. After 120 hr post-injection. The autoradiography images of the A431 tumor-bearing nude mouse with ^{153}Sm-DTPA-Cetuximab showed ^{153}Sm-DTPA-Cetuximab uptake restricted to viable tumor areas (Figure 7). ^{153}Sm-DTPA-Cetuximab was selectively localized in the right flank with EGFR-positive A431 tumor. The quantitative analysis of conventional autoradiographs gave values comparable to those of the biodistribution study in tissues accumulating radioactivity moderately, such as tumour, liver and spleen (Table 1). The analysis with the imaging gave comparable result to those of the biodistribution for all tissues.

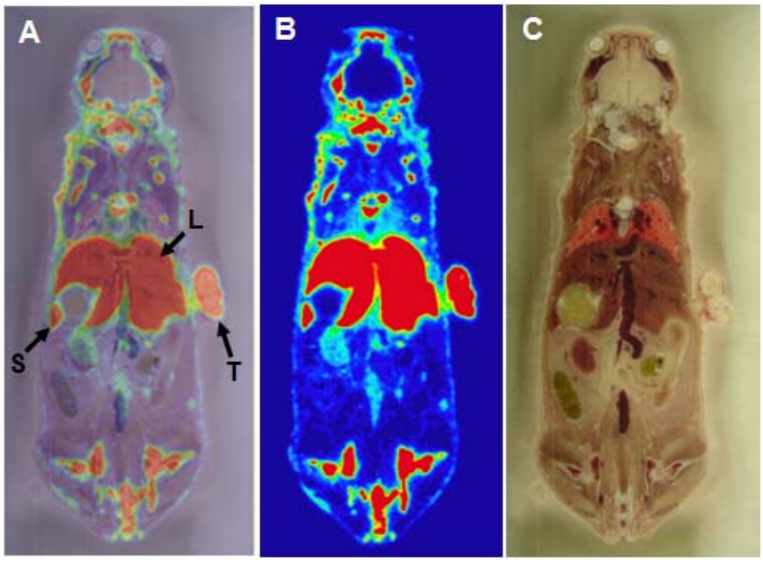

Figure 7. Digital Whole Body Autoradiography (DWBA) imaging of ^{153}Sm-DTPA-Cetuximab in A431-xenografted mouse. DWBA images were acquired at 120 hr post-injection of ^{153}Sm-DTPA-Cetuximab. Fused images (A) were merged with DWBA images (B) and frozen section photo images (C). All images are dorsal view. (T: A431 tumor, L: Liver, S: Spleen)

8. Radioimmunotherapy of ^{153}Sm-DTPA-Cetuximab in A431 tumor-bearing nude mice

Tumor growth, calculated as the tumor volume at each time point post-treatment is shown in Table 4 and Figure 9. The saline group and the unlabeled Cetuximab-treated group showed considerable tumor growth. In contrast, the mice treated with the ^{153}Sm-DTPA-Cetuximab and ^{153}Sm-DTPA-Rituximab groups showed a significantly delayed tumor growth. The ^{153}Sm-DTPA-Cetuximab-treated group showed regressive tumor growth at days 8, 10, 13 and 15 post first-treatment. A continuous body weight loss was observed in both groups treated with the ^{153}Sm-DTPA-Cetuximab or ^{153}Sm-DTPA-Rituximab from day 8 after therapy was initiated (Table 3, Figure 8), presumable due to treatment related toxicity meadiated by ^{153}Sm-DTPA-Cetuximab or ^{153}Sm-DTPA-Rituximab. Consequenty, the mice had to be sacrificed at day 16.

Table 3. Non-hematology toxicity was assessed by measuring body weight

unit : g

Group	Day after RIT (d)						
	0	3	6	8	10	13	15
Saline	19.7±2.2 *	20.0±2.4	20.8±2.9	21.7±2.8	21.9±3.0	21.2±3.7	21.6±3.3
Cetuximab	19.2±1.9	19.2±1.5	19.9±1.4	20.7±1.5	20.9±1.7	19.8±1.8	20.8±1.8
^{153}Sm-DTPA-Rituximab	18.9±1.6	19.3±1.9	19.0±2.2	18.8±2.0	18.8±2.0	18.8±2.3	18.8±2.2
^{153}Sm-DTPA-Cetuximab	19.1±1.3	19.5±1.4	19.6±1.5	19.2±1.8	18.6±1.7	18.4±1.8	18.4±2.1

* Mean ± S.D (n = 8).

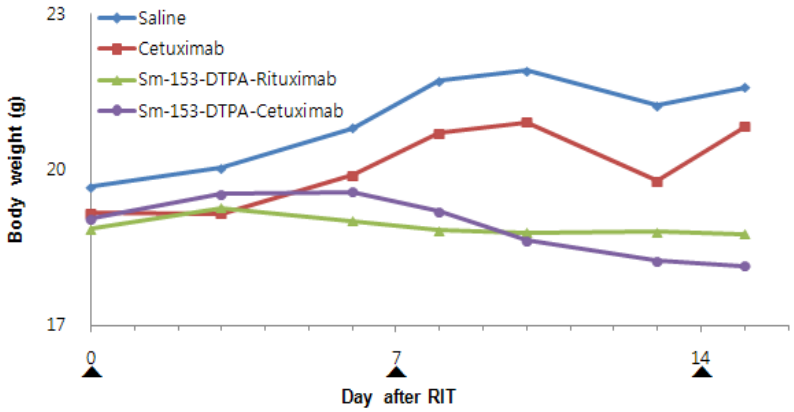

Figure 8. Non-hematology toxicity was assessed by measuring body weight. Toxicity was monitored by measurement of body weight three times a week. Data are shown as average body weight (n = 8). ▲ : treatment

Table 4. Temporal assessment of tumor volume in A431-xenografted mice

Unit : mm^3

Group	Day after RIT (d)						
	0	3	6	8	10	13	15
Saline	119.2±86.2*	145.6±93.9	246.4±148.7	337.1±244.1	360.9±240.8	385.2±242.4	409.4±277.7
Cetuximab	100.2±62.1	128.7±66.7	179.9±93.5	249.3±158.1	258.3±163.4	253.1±194.3	280.1±197.7
^{153}Sm-DTPA-Rituximab	101.9±58.4	128.3±67.0	157.9±81.7	149.6±73.8	141.8±57.3	146.1±59.1	146.3±91.6
^{153}Sm-DTPA-Cetuximab	91.4±45.1	118.8±51.6	123.3±55.7	116.7±57.0	96.4±58.7	87.7±34.1	73.6±23.4

* Mean ± S.D (n = 8).

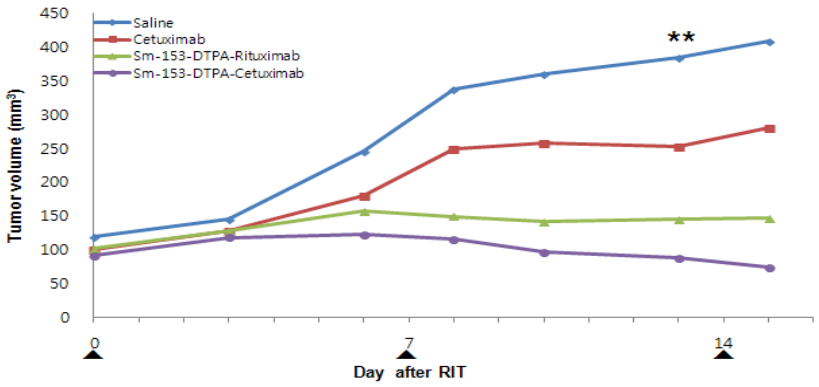

Figure 9. Temporal assessment of tumor volume in A431-xenografted mice. RIT studies were performed using 0.9% sodium chloride, Cetuximab, ^{153}Sm-DTPA-Rituximab or ^{153}Sm-DTPA-Cetuximab (radioactivity of 9.5 MBq and antibody concentration of 100 μg). Data are shown as average body weight (n = 8). **, $P < 0.01$; P-value of saline group and ^{153}Sm-DTPA-Cetuximab group. ▲ : treatment.

IV. DISCUSSION

Radioimmunotherapy (RIT) is a highly promising oncological therapeutic modality, with good reported potency for incurable or relapsed cancers. In the future, prospective trials involving RIT will determine and certainly expand the scope of useful therapeutic modality in the clinical management of cancers [1].

Ideal agents for diagnostic and RIT relies on radioactivity to destroy cells distant from immuno-targeted cells. Therefore, tumors (for antigen recognition) can be treated, because not all cells have to be targeted [4]. Cetuximab binds to EGFR, which is expressed in a wide variety of tumors, with high affinity [24]. Many radiolabeled Cetuximab has been developed to visualize and monitor EGFR tumors in animals and humans using techmetium-99m, yttrium-86 and copper-64 or for therapeutic purposes including lutetium-177 and yttrium-90 in tumor bearing nude mice as well as iodine-131 [9]. ^{153}Sm has many desirable physical characteristics to recommend its use for internal RIT, such as a short half-life, medium beta energy (soft tissue penetration 2.5 mm) and low gamma energy, which is easily detectable by SPECT/CT systems [17, 25].

Radiolabeling of Cetuximab with ^{153}Sm for RIT requires the use of a suitable bifunctional chelating agent. The cation of Sm^{3+} is known to form stable complexes with Diethylene triamine pentaacetic acid anhydride (DTPA) [26].

In the present study, the potential of a diagnostic and RIT agent, the ^{153}Sm radiolabeled to DTPA-Cetuximab, was assessed for treatment of

EGFR-expressing tumors.

To select an appropriate *in vitro* model of tumors, EGFR expression was analysed with varied cancer cell lines (A431, A549 and U87-MG) by western blot analysis. This analysis showed that A431 cells expressed a higher level of EGFR than U87-MG cells. Previous studies have also reported that A431 cells a over-expressed EGFR and U87-MG cells is expressed a low level of EGFR [27, 28].

The *in vitro* stability test for ^{153}Sm-DTPA-Cetuximab showed that the radiolabeled antibody was very stable in serum proteins. The radiochemical purity of ^{153}Sm-DTPA-Cetuximab had decreased only less than 3% for 24 hr. It has been reported that the serum stability of radiolabeled antibodies depend on the type of radioisotope and chelate [29]. The prepared ^{153}Sm-DTPA-Cetuximab in this study had excellent stability in serum.

Immunoreactivity was performed by the Lindmo method, in which the immunoreactive fraction of immunoreactive radiolabeled antibody is accurately determined by linear extrapolation to binding at infinite antigen excess. The value determined for the immunoreactive fraction was high (71.4%), showing good possibilities for *in vivo* diagnostic applications.

To assess the potential for ^{153}Sm-DTPA-Cetuximab as a SPECT/CT agent, a preliminary study was performed in which ^{153}Sm-DTPA-Cetuximab was injected into nude mice bearing tumors derived form A431 epithelial carcinoma cells. The biodistribution analysis demonstrated a high uptake of ^{153}Sm-DTPA-Cetuximab in the implanted A431 tumors at 120 hr after injection (10.65 ± 0.66% ID/g). This result is comparable to the highest

level of uptake of other radiolabeled antibodies previously reported [2, 11]. The tumor-to-blood ratio of ^{153}Sm-DTPA-Cetuximab was 0.11 ± 0.03% ID/g at 2 hr post-injection and increased to 4.83 ± 1.41% ID/g at 168 hr. The tumor-to-muscle ratio of ^{153}Sm-DTPA-Cetuximab was also increased to 21.43% ID/g at 168 hr post-injection. This data shows that the urinary system is a predominant clearance system for the ^{153}Sm-DTPA-Cetuximab.

The SPECT/CT and autoradiography images of the A431 tumor-bearing nude mouse were acquired to evaluated *in vivo* tumor targetability of ^{153}Sm-DTPA-Cetuximab. According to the biodistribution data, A431 tumor uptake of ^{153}Sm-DTPA-Cetuximab peaked at 120 hr post-injection. Therefore, the SPECT/CT and autoradiography images were acquired at 120 hr post-injection. According to the SPECT/CT and autoradiography, ^{153}Sm-DTPA-Cetuximab was selectively localized in the right flank with EGFR-positive A431 tumor. This result shows that ^{153}Sm-DTPA-Cetuximab has good possibilities for *in vivo* diagnostic applications.

The radiotherapeutic efficacy of ^{153}Sm-DTPA-Cetuximab was demonstrated here by finding that even a single 9.5 MBq/100 μg dose per animal was sufficient to attenuate tumor growth as compared to untreated controls. The saline-treated group and the unlabeled Cetuximab-treated group of mice showed considerable tumor growth. The ^{153}Sm-DTPA-Rituximab-treated mice showed delayed tumor growth unexpectedly as a RIT control. However, as expected, RIT with ^{153}Sm-DTPA-Cetuximab induced a significant tumor growth delay and showed regression of tumor growth at days 8, 10, 15 post-first treatment, presumably due to selective death of EGFR positive tumor cells. In this study, radiation response of

EGFR-expressing tumor cells was improved by anti-EGFR treatment. However, ^{153}Sm-DTPA-Cetuximab showed certain radiotoxicity to the treated mice. The parameter used to evaluate the toxicity of treatment was body weight loss. After the third treatment, the consumption of water and diet decreased significantly in the ^{153}Sm-DTPA-Cetuximab-treated group of mice (data not shown), probably due to radiotoxicity. The clinical application of RIT with ^{153}Sm-DTPA-Cetuximab might be promising only if the radiotoxicity is controled. This pilot study needs to confirm the maximal tolerated activity dose of ^{153}Sm before clinical application of RIT [30].

In conclusion, the SPECT/CT and autoradiography results in this study suggest that ^{153}Sm-DTPA-Cetuximab has a good candidate for *in vivo* diagnosis of cancer, and RIT with ^{153}Sm-DTPA-Cetuximab may be clinically applicable to treat EGFR-expressing tumors within acceptable toxicity.

VI. REFERENCE

1. Ng D. Radioimmunotherapy: a brief overview. *Biomed Imaging Interv J*. 2006;2:1-6.

2. Bugaj JE, Erion JL, Johnson MA, Schmidt MA, Srinivasan A. Radiotherapeuticefficacy of ^{153}Sm-CMDTPA-Tyr^{3}-octreotate in tumor-bearing rats. *Nucl Med Biol*. 2001;28:327-334.

3. Anderson CJ, Jones LA, Bass LA, Sherman EL, McCarthy DW, Cutler PD, Lanahan MV, Cristel ME, Lewis JS, Schwarz SW. Radiotherapy, toxicity and dosimetry of copper-64-TETA-octreotide in tumor-bearing rats. *J Nucl Med*. 1998;39:1944-1951.

4. Kairemo KJ. Radioimmunotherapy of solid cancer. *Acta Oncol*. 1996;35:343-355.

5. Sharkey RM, Goldenberg DM. Targeted therapy of cancer: New prospects for antibodies and immunoconjugates. *CA Cancer J Clin*. 2006;56:226-243.

6. Cai W, Chen K, He L, Cao Q, Koong A, Chen X. Quantitative PET of EGFR expression in xenograft-bearing mice using ^{64}Cu-labeled Cetuximab, a chimeric anti-EGFR monoclonal antibody. *Eur J Nucl Med Mol Imaging*. 2007;34:850-858.

7. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and tratment of cancer. *J Clin Oncol*. 2003;21:2787-2799.

8. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, Peyrade F, Benasso M, Vynnychenko I, De Raucourt D, Bokemeyer C, Schueler A, Amellal N, Hitt R. Platinum-Based Chemotherapy plus Cetuximab in Head and Neck Cancer. *N Engl J Med*. 2008;359:1116-1127.

9. Simindokht SA, Ali BS, Amir RJ, Amirsaleh SA, Mohammad GM. Development of ^{170}Tm-DOTA-Cetuximab for radioimmunotherapy. *J Label Compd Raiopharm*. 2012;55:103-107.

10. Li WP, Ma DS, Higginbotham C, Hoffman T, Ketring AR, Cutler CS, Jurisson SS. Development of an in vitromodel for assessing the in vivostability of lanthanide chelates. *Nucl Med Biol*. 2001;28:145-154.

11. Kraeber-Bodéré F, Mishra A, Thédrez P, Faivre-Chauvet A, Bardiès M, Imai S, Le Boterff J, Chatal JF. Pharmacokinetics and biodistribution of samarium-153-labelled OC125 antibody coupled to CITCDTPA in a xenograft model of ovarian cancer. *Eur J Nucl Med*. 1996;23:560-567.

12. Stimmel JB, Kull FC Jr. Samarium-153 and Lutetium-177 Chelation Properties of Selected Macrocyclic and Acyclic Ligands. *Nucl Med Biol*.

1998;25:117-125.

13. Flux G, Bardies M, Monsieurs M, Savolainen S, Strands SE, Lassmann M. The impact of PET and SPECT on dosimetry for targeted radionuclide therapy. *Z Med Phys*. 2006;16:47-59.

14. Franc BL, Acton PD, Mari C, Hasegawa BH. Small-animal SPECT and SPECT/CT: important tools for preclinical investigation. *J Nucl Med*. 2008;49:1651-1663.

15. Baum RP, Adams S, Kiefer J, Niesen A, Knecht R, Howaldt HP, Hertel A, Adamietz IA, Sykes T, Boniface GR, et al. A novel technetium-99m labeled monoclonal antibody (174H.64) for staging head and neck cancer by immuno-SPECT. *Acta Oncol*. 1993;32:747-751.

16. Wojcik R, Bradley EL, Brewer P, Majewski S, Qian J, Ranck A, Saha M.S, Smith K, Smith MF, Welsh RE. SPECT-CT system for small animal imaging. *IEEE Transactions on Nuclear Science*. 2002;50:1840-1844.

17. Shin BC, Park KB, Jang BS, Lim SM, Shim CK. Preparation of ^{153}Sm-chitosan complex for radiation synovectomy. *Nucl Med Biol*. 2001;28:719-725.

18. Lindmo T, Boven E, Cuttitta F, Fedorko J, Bunn PA Jr.

Determination of the immunoreactive function of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J immunol Meth*. 1984;72:77-89.

19. Konishi S, Hamacher K, Vallabhajosula S, Kothari P, Bastidas D, Bander N, Goldsmith S. Determination of immunoreactive fraction of radiolabeled monoclonal antibodies: what is an appropriate method?. *Cancer Biother Radiopharm*. 2004;19:706-715.

20. Euhus DM, Hudd C, LaRegina MC, Johnson FE. Tumor measurement in the nude mouse. *J Surg Oncol*. 1986;31:229-234.

21. Niu G, Sun X, Cao Q, Courter D, Koong A, Le QT, Gambhir SS, Chen X. Cetuximab-based immunotherapy and radioimmunotherapy of head and neck squamous cell carcinoma. *Clin Cancer Res*. 2010;16:2095-2105.

22. Tijink BM, Neri D, Leemans CR, Budde M, Dinkelborg LM, Stigter-van Walsum M, Zardi L, van Dongen GA. Radioimmunotherapy of head and neck cancer xenografts using ^{131}I-labeled antibody L19-SIP for selective targeting of tumor vasculature. *J Nucl Med*. 2006;47:1127-1135.

23. Morales AA, Ducongé J, Alvarez-Ruiz D, Becquer-Viart ML, Núñez-Gandolff G, Fernández E, Caballero-Torres I, Iznaga-Escobar N.

Humanized versus murine anti-human epidermal growth factor receptor monoclonal antibodies for immunoscintigraphic studies. *Nucl Med Biol*. 2000;27:199-206.

24. Milenic DE, Wong KJ, Baidoo KE, Ray GL, Garmestani K, Williams M, Brechbiel MW. Cetuximab: preclinical evaluation of a monoclonal antibody targeting EGFR for radioimmunodiagnostic and radioimmunotherapeutic applications. *Cancer Biother Radiopharm*. 2008;23:619-631.

25. Deutsch E, Brodack JW, Deutsch KF. Radiation synovectomy revised. *Eur J Nucl Med*. 1993;20:1113-1127.

26. Fani M, Vranjes S, Archimandritis SC, Potamianos S, Xanthopoulos S, Bouziotis P, Varvarigou AD. Labeling of monoclonal antibodies with ^{153}Sm for potential use in radioimmunotherapy. 2002;57:665-674.

27. Masui H, Castro L, Mendelsohn J. Consumption of EGF by A431 cells: evidence for receptor recycling. *J Cell Biol*. 1993;120:85-93.

28. Nagane M, Levitzki A, Gazit A, Cavenee WK, Huang HJ. Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-X_L and caspase-3-like proteases. *Proc Natl Acad Sci U S A.* 1998;95:5724-5729.

29. Cole WC, DeNardo SJ, Meares CF, McCall MJ, DeNardo GL, Epstein AL, O'Brien HA, Moi MK. Serum stability of ^{67}Cu chelates: comparison with ^{111}In and ^{57}Co. *Nucl Med Biol*. 1986;13:363-368.

30. Salaun PY, Bodet-Milin C, Frampas E, Oudoux A, Saï-Maurel C, Faivre-Chauvet A, Barbet J, Paris F, Kraeber-Bodéré F. Toxicity and efficacy of combined radioimmunotherapy and bevacizumab in a mouse model of medullary thyroid carcinoma. *Cancer*. 2010;116:1053-1058.

국문 요약

마우스 종양 모델에서 ^{153}Sm-DTPA-Cetuximab의 암세포 표적 및 항암 활성 분석

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치료용 방사성동위원소에 표지된 항체는 표적치료가 가능하며, 이러한 치료를 방사면역치료라 하며 암 치료에 성공적인 결과를 보여주고 있다. Sm-153(반감기; 46.3시간)은 중간 베타 에너지 방사체 [E_{max} = 810(20%), 710(50%) and 640(30%) keV]이며 30%의 감마선(103 keV)을 방출한다. ^{153}Sm의 이러한 특징은 방사면역진단과 방사면역치료에 적합하다. 이러한 ^{153}Sm의 물리적 특성 외에도, bifunctional chelate가 결합된 항체와의 방사성표지도 용이한 장점이 있다. Cetuximab은 상피세포 성장 인자 수용체를 억제하는 키메릭 단일클론 항체로서 최근 여러 가지의 고형암 치료에 사용되고 있다. 본 연구에서는, ^{153}Sm-DTPA-Cetuximab을 제조하여 immuno-SPECT 영상과 방사면역치료 가능성이 있는지 알아보기 위하여 상피세포 성장 인자 수용체를 발현하는 암을 이식한 실험동물을 이용하여 암세포 친화성을 평가하였다.

^{153}Sm-DTPA-Cetuximab는 Cetuximab과 두 자리 킬레이트인 DTPA를 접합시킨 후 ^{153}Sm을 표지하였다. ^{153}Sm-DTPA-Cetuximab을 상피세포

성장 인자 수용체를 발현하는 A431 세포에 대한 면역반응성 실험을 실시한 결과 71.4%의 면역반응성을 나타내었다. ^{153}Sm-DTPA-Cetuximab과 사람 혈청을 이용하여 24시간 동안의 체외 안정성 실험을 한 결과 96% 이상의 방사화학적순도를 유지하였다. 앞의 실험을 바탕으로 생체 분포 실험을 실시하였다. ^{153}Sm-DTPA-Cetuximab을 A431 암세포를 이식한 마우스에 정맥을 통하여 투여 후 120시간이 지났을 때 ^{153}Sm-DTPA-Cetuximab의 암 축적 율이 10.65 ± 0.66% ID/g 가장 높았으며, 암/혈액 비율과 암/근육의 비율은 투여 후 점차 증가하였으며, 168시간 후에 4.83 ± 1.41 과 21.43 ± 4.54% ID/g으로 가장 높았다. 이 결과에서 비뇨기관이 ^{153}Sm-DTPA-Cetuximab의 주요한 clearance 시스템이라는 것을 알 수 있었다. ^{153}Sm-DTPA-Cetuximab의 영상분석을 위하여 단일광자 단층촬영(single photon emission computed tomography)과 단층촬영(computed tomography)이 결합된 SPECT/CT와 자기방사법(autoradiography)을 이용하였다. 두 영상 결과 ^{153}Sm-DTPA-Cetuximab이 암 세포에 축적되어 있는 영상을 얻었다. ^{153}Sm-DTPA-Cetuximab의 방사면역치료제로서의 효능을 평가하기 위하여 A431 암 세포를 이식한 마우스들에게 ^{153}Sm-DTPA-Cetuximab을 1주일에 한 번 3주 동안 실시하였다. 그 결과 치료를 하지 않는 그룹과 눈에 띄게 암 세포 성장이 느렸으며, 암 세포의 크기가 줄어드는 마우스들도 있었다. 이 결과들을 통하여 ^{153}Sm-DTPA-Cetuximab이 상피세포 수용 인자 수용체를 발현하는 암에서의 진단과 방사면역치료제로서의 이용가능성을 확인하였다.

핵심어 : Sm-153(사마륨-153), 단일클론 항체, 두 자리 킬레이트, 면역단일광자 단층촬영, 방사면역치료