

## Preclinical Study of $^{177}\text{Lu}$ -DOTA-Trastuzumab: A Potential Radiopharmaceutical for Therapy of Breast Cancer Positive HER-2

### (Uji Preklinis $^{177}\text{Lu}$ -DOTA-Trastuzumab: Radiofarmaseutika Potensial untuk Terapi Kanker Payudara HER-2 Positif)

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**Abstract:** Radiopharmaceutical  $^{177}\text{Lu}$ -(1,4,7,10-tetraazacyclododecane-N,N',N'',N''')-tetra acetic acid)-trastuzumab ( $^{177}\text{Lu}$ -DOTA-trastuzumab), based on antihuman epithelial receptor type 2 (HER-2) monoclonal antibody which is expected to be potential for diagnostic and therapeutic agent of breast cancer positive HER-2, had been successfully prepared with radiochemical purity of > 99%. Preclinical studies aimed in providing basic data for clinical trial and particularly in finding out the effectiveness of  $^{177}\text{Lu}$ -DOTA-trastuzumab in killing cancer cells which over express HER-2, have been performed. The data included urine and faeces clearance tests, imaging with gamma camera and cytotoxicity test. The results showed that the excretion of radioactivity post injection of  $^{177}\text{Lu}$ -DOTA-trastuzumab in normal rats were more rapid through urine as compared to the excretion through faeces. The gamma camera image on normal rat 144 hours post injection of  $^{177}\text{Lu}$ -DOTA-trastuzumab showed that there was remaining a trace of radioactivity in hepatic area. The residue of radioactivity (< 5%, quantified by biodistribution test) was found to be relatively lower than reported for  $^{111}\text{In}$ -NSL-trastuzumab. However, this residue of radioactivity has to be seriously considered when  $^{177}\text{Lu}$ -DOTA-trastuzumab is going to be applied for treatment of cancer. Cytotoxicity test showed that  $^{177}\text{Lu}$ -DOTA-trastuzumab was far more effective in killing cancer cells positive HER-2 (SKOV-3 cell lines) than that of trastuzumab.

**Key Words:** breast cancer,  $^{177}\text{Lu}$ -DOTA-trastuzumab, preclinical, radiolabelled Anti-HER-2 monoclonal antibody.

**Abstrak:** Radiofarmaka  $^{177}\text{Lu}$ -(1,4,7,10-tetraazacyclododecane-N,N',N'',N''')-tetra acetic acid)-trastuzumab ( $^{177}\text{Lu}$ -DOTA-trastuzumab), berbasis *antihuman epithelial receptor type 2* (HER-2) antibodi monoklonal, yang diharapkan potensial untuk diagnosis dan terapi kanker payudara positif HER-2, dengan kemurnian radiokimia besar dari 99% telah berhasil dipreparasi. Uji preklinis yang dimaksudkan untuk mendapatkan data dasar sebelum pelaksanaan uji klinis dan khususnya untuk melihat efektifitas  $^{177}\text{Lu}$ -DOTA-trastuzumab dalam membunuh sel kanker yang mengekspresikan HER-2, telah berhasil dilakukan. Data dasar tersebut diantaranya adalah data *clearance*, citra dengan gamma kamera dan data hasil uji sitoksisitas. Hasil uji *clearance* memperlihatkan bahwa ekskresi radioaktif setelah pemberian  $^{177}\text{Lu}$ -DOTA-trastuzumab pada tikus sehat lebih cepat melalui urin dibandingkan dengan ekskresi melalui feses. Hasil pencitraan dengan kamera gamma pada tikus normal 144 jam

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setelah pemberian  $^{177}\text{Lu}$ -DOTA-trastuzumab memperlihatkan adanya residu radioaktif di daerah hati (< 5%, berdasarkan hasil uji biodistribusi). Residu ini lebih rendah jika dibandingkan dengan residu radioaktif pada hati yang dilaporkan untuk  $^{111}\text{In}$ -NSL-trastuzumab. Walaupun residu radioaktif ini relatif rendah, tetapi harus tetap menjadi perhatian yang serius pada saat  $^{177}\text{Lu}$ -DOTA-trastuzumab akan digunakan pada pasien penderita kanker. Uji sitotoksitas memperlihatkan  $^{177}\text{Lu}$ -DOTA-trastuzumab jauh lebih efektif dalam membunuh sel kanker positif HER-2 (SKOV-3 *cell lines*) dibandingkan dengan trastuzumab tidak bertanda.

**Kata Kunci:** kanker payudara,  $^{177}\text{Lu}$ -DOTA-trastuzumab, preklinis, Anti-HER-2 antibodi monoklonal bertanda.

## INTRODUCTION

CANCER has now become one of major Indonesia's health problems. Globocan reported that in 2008 there were 292,600 new cancer cases with mortality number of 214,600<sup>(1)</sup>. Globocan also reported that breast cancer is the most common type of cancer suffered by Indonesian women. In 2008 there were 39,831 Indonesian women who suffered breast cancer with mortality number of 20,052. This statistic shows that how cancer has become a heavy burden to our society welfare physically and emotionally.

It has been reported that between 25-30% of breast cancer have high expression of antihuman epithelial receptor type 2 (HER-2)<sup>(2,3,4)</sup>. The existence of HER-2 on cancer cells, which was reported to be seven time higher as compared to those on normal cells, indicated that such breast cancer is an aggressive, tends to metastases, short recurrences and difficult to handle kind of cancer. Over expression of HER-2 is also found on other cancers such ovary, gastric and uterine endometrial carcinoma. Clinically HER-2 has been a target for trastuzumab, a humanised monoclonal antibody that is marketed as Herceptin for treatment of breast and gastric cancers positive HER-2<sup>(5,6,7)</sup>. The inhibition on cancer cells proliferation by trastuzumab is believed to be caused by the binding of trastuzumab onto HER-2 which in turn stop HER-2 signalling for proliferation process of cells.

Radiopharmaceutical is a pharmaceutical compound which is one or more of its atoms replaced by radioisotope(s) of  $\gamma$  (gamma)-,  $\beta$  particle (beta)- and or  $\alpha$  (alfa)-particle emitters<sup>(8)</sup>.

Radiopharmaceuticals containing gamma emitting radionuclide are commonly used as imaging agents for diagnostic, identification and/ or localisation of cancer. It is also used in monitoring and evaluation the progress of a therapy. Radiopharmaceutical containing with  $\alpha$ - or  $\beta$ -particle emitter is mainly used for internal radiation therapy of cancer. Therapeutic radiopharmaceutical with radionuclide which emits  $\alpha$ - or  $\beta$ -particle as well as an imageable emission of  $\gamma$ -ray is advantageous as it allows accurate

assessment of dosimetry and direct observation on radiopharmaceutical route to the target tissue.

In recent developments, the deposition of radioisotopes at the targeted site has been aided by the emergence of sophisticated molecular carriers, which are able to selectively transport the radioisotopes to the target site (such as cancer). Such molecular carriers include low molecular weight ligands (MW 300-500) to large molecules such as peptides, monoclonal antibodies and liposomes.

Lutetium-177 ( $^{177}\text{Lu}$ ) is a radionuclide that has useful physical properties that make it suitable for treatment and imaging of target tissue. Its  $\gamma$ -emissions [Emax 497 keV (78.6%) and 176 keV (12.2%)] are useful for internal radiotherapy of small cancer and its  $\gamma$  emissions [113 keV (6.4%) and 208 keV (11%) keV] are very close to the  $^{99\text{m}}\text{Tc}$   $\gamma$ -emission, the most versatile and popular radionuclide in nuclear medicine, therefore it is ideal for imaging<sup>(9)</sup>. In order for this radionuclide can be effective for the above-mentioned procedure, the radionuclide should be targeted to the targeted organ or disease. The availability of  $^{177}\text{Lu}$  is relatively easy. It can be produced by irradiation of  $^{176}\text{Lu}$  with nuclear reaction of  $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$  (cross section of 2050 barn)<sup>(10)</sup>.

In the last couple of years  $^{177}\text{Lu}$  has been investigated for research and development of new radiopharmaceuticals<sup>(10,11,12)</sup>. Several peptides and monoclonal antibodies (mAbs) have been conjugated with bifunctional chelating agents and concomitantly radiolabelled with  $^{177}\text{Lu}$ . These radiopharmaceuticals were  $^{177}\text{Lu}$ -Octreotate-and  $^{177}\text{Lu}$ -BB2 bombesin GRP for therapy of neuroendocrine and lung cancers respectively, and  $^{177}\text{Lu}$ -PSMA antibody for therapy of prostate cancer. These radiopharmaceuticals are on their final stages of their clinical trial and are about to be launched to the market in a very near future.

Based on the above-mentioned phenomena it can be hypothesized that monoclonal antibody anti-HER-2, labelled with  $\gamma$ - and  $\alpha$ - and/ or  $\beta$ -particle radionuclide emitters while maintaining its bio-character, will interact specifically with HER-2. Monoclonal antibody anti-HER is meant to stop the

cancer cell proliferation and the emitted  $\gamma$ -particles will transfer its energy to surrounding cancer cells which in turn will destroy them. The  $\gamma$ -radiation helps in imaging of lesion and size of cancer. The synergy of these two components is expected to increase the effectiveness of the radiolabelled monoclonal antibody anti-HER-2 as compared to the use of the monoclonal antibody anti-HER-2 or radiation alone in treatment of cancer.

The study is meant, in collaboration with the Hasan Sadikin General Hospital, Bandung and Dharmais Cancer Hospital, Jakarta, to provide basic data for future clinical trial and in particular in finding out the effectiveness of  $^{177}\text{Lu}$ -DOTA-trastuzumab in killing cancerous cells which over express HER-2. The results will then be used as a reference and a basic consideration in performing the following clinical trial of  $^{177}\text{Lu}$ -DOTA-trastuzumab.

## MATERIALS AND METHODS

**MATERIALS.** Chemicals and materials used in this work included  $^{177}\text{Lu}$ , prepared in situ by irradiation of isotopic enriched  $^{176}\text{Lu}_2\text{O}_3$  (60.60%) supplied by Isoflex USA. Hydroxysuccinimide-1,4,7,10-tetraazacyclotridecane-1,4,7,10-tetraacetic acid (NHS-DOTA) (Macrocyclic), bovine serum albumin (BSA), ethylene diamine tetraacetic acid (EDTA), sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), sodium hydroxide and sodium chloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS) were obtained from Sigma. Commercially available Trastuzumab (Herceptin<sup>TM</sup>) was obtained from Roche. HER-2 positive SKOV-3 cell lines were given by the Centre for Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology (LAPTIAB-BPPT). Normal mice were supplied by National Veterinary Drug Assay Laboratory. Dialysis cassettes (20 KD MWCO) (Pierce) and silica gel impregnated glass fibre sheets (ITLC-SG) (Pall). Protein assay kit (Bio-Rad). Sephadex-G-25 resin (medium) (Pharmacia). High purity water was obtained from Sartorius Stedimä system ( $\Omega 18$  MegaOhm).

**Instruments.** The equipments used for preparation and analysis of  $^{177}\text{Lu}$ -DOTA-trastuzumab included a thin layer chromatographic scanner (Bio Scan), a plate reader (Bio Tek), CRC-15R dose calibrator (Capintec), thermomixer (Bio Rad), refrigerated centrifuge (Beckman), and a plate reader (BioTek).

**METHODS.**  $^{177}\text{Lu}$ -DOTA-trastuzumab was prepared in two-step reactions (Figure 1). Preparation and stability test of  $^{177}\text{Lu}$ -DOTA-trastuzumab has been

reported elsewhere<sup>(13)</sup>.

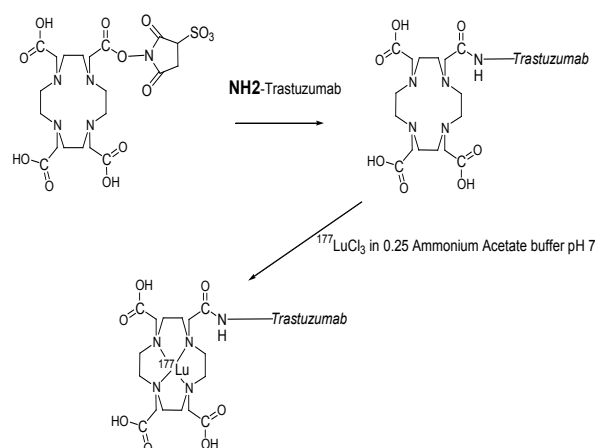


Figure 1. Reaction scheme of  $^{177}\text{Lu}$ -DOTA-trastuzumab<sup>(13)</sup>.

Preparation of  $^{177}\text{Lu}$ -DOTA-trastuzumab was initiated by reacting ester active of NHS-DOTA to form an amide bond with  $\text{NH}_2$ -functional group of trastuzumab. The DOTA-trastuzumab formed was then purified from un-reacted NHS-DOTA by dialysis process. The purified DOTA-trastuzumab was then radiolabeled with  $^{177}\text{Lu}$  to form  $^{177}\text{Lu}$ -DOTA-trastuzumab.

**Preparation of immunoconjugate DOTA-trastuzumab<sup>(13)</sup>.** The formation of immunoconjugate, DOTA-trastuzumab, was carried out by addition of NHS-DOTA (33  $\mu\text{mol}$ ) to 2 mL of trastuzumab (5 mg/mL) in 0.1 M phosphate buffer pH 7.5. The mixture was left to react for 24 hours at 4  $^\circ\text{C}$  which was then followed by purification using dialysis cassette (Molecular Cut off 20 KD) with 0.2 M ammonium acetate pH 7.5 buffer for 24 hours (four time buffer changes).

**Preparation of  $^{177}\text{LuCl}_3$ .** A solution of  $^{177}\text{LuCl}_3$  ( $\sim 7.5$  Ci/mg Lu) was prepared by irradiating of 0.3–0.4 mg of isotopically enriched of  $^{176}\text{Lu}_2\text{O}_3$  (60.60%) in Multi Purposes GA Siwabessy Reactor for four days. To the irradiated target was then added 2 mL of 6 M HCl and left to stand for 30 minutes after which 2 mL of  $\text{H}_2\text{O}_2$  was added. The reaction mixture was then heated to dryness. The residue was re-dissolved with 3 mL of HCl 0.05 M.

**Radiolabelling of immunoconjugate DOTA-trastuzumab with  $^{177}\text{Lu}$ .** To an aliquot of DOTA-trastuzumab was added an aliquot of  $^{177}\text{LuCl}_3$  (diluted in 0.25 M ammonium acetate pH 7.0,  $\frac{1}{2}$  v/v). The pH of reaction mixture was adjusted to 5.5 by addition of 0.1 M HCl. The reaction mixture was then incubated at 37  $^\circ\text{C}$  for one hour which was then followed by addition of an excess amount of 0.005 M EDTA solution (mol EDTA : mol Lu = 50:1). The formation of  $^{177}\text{Lu}$ -DOTA-trastuzumab was monitored using

a TLC system with ITLC-SG as a stationary phase and saline solution as the mobile phase. The  $R_f$  values in this system were about 0.3 for  $^{177}\text{Lu}$ -DOTA-trastuzumab and about 0.7 for free  $^{177}\text{Lu}$  (in form of  $^{177}\text{Lu}$ -EDTA or  $^{177}\text{Lu}$ -DOTA).

Purification of  $^{177}\text{Lu}$ -DOTA-trastuzumab was carried out using a Sephadex G-25M column (15 X 1.2 cm, pre-blocked with one mL of 10 % BSA, and pre-equilibrated with 0.01 M PBS pH 7.2). An aliquot of raw product of  $^{177}\text{Lu}$ -DOTA-trastuzumab was loaded onto Sephadex G-25 column. The column was then eluted with 0.01 M PBS pH 7.2 at ~ 1 mL/min flow rate. Eluent was retrieved in 0.5 mL fraction and its radioactivity and radiochemical purity were then measured with dose calibrator and TLC system respectively. Each fraction which was associated with  $^{177}\text{Lu}$ -DOTA-trastuzumab (radiochemical purity > 95%) was pooled and then used for further studies.

**Imaging test.** Imaging test was carried out by intravenous injection of 200  $\mu\text{Ci}$  of  $^{177}\text{Lu}$ -DOTA-trastuzumab into a rat for each time point. At a pre-determined time (3, 24, 48, 72 or 144 hours) post injection two rats were anesthetized and then imaged with gamma camera.

**Clearance test.** Clearance test was carried out by intravenous injection of 400  $\mu\text{Ci}$  of  $^{177}\text{Lu}$ -DOTA-trastuzumab into two rats. Each rat was then kept in a metabolic cage. Urine and faeces of each rat were collected in separate container. At a determined-time (3, 24, 48, 72 or 144 hours) post injection, then urine and faeces were transferred into counting tubes which then counted with gamma counter.

**Cell culture.** SKOV-3 cell lines (ovary cancer positive HER-2 cell lines) were cultured in a growth medium which consisted of RPMI with 10% of FBS, and 1% of penicillin-streptomycin in 5%  $\text{CO}_2$ -incubator at 37 °C. Passage was carried out every two or three days until the number of cells met with cytotoxicity and binding affinity test's requirement.

**Cytotoxicity test.** Cytotoxicity test was carried out in order to determine the ability of  $^{177}\text{Lu}$ -DOTA-trastuzumab in killing cells which express HER-2 as compared to that of trastuzumab. In vitro test was performed on HER-2 positive SKOV-3 cell lines.

SKOV-3 cells (~ 5,000 cells) in RPMI /10 % FBS/ 1% penicillin-streptomycin were transferred into each well of a sterile 96-microplate. The plate was then incubated for 24 hours at 37 °C with atmosphere of 5%  $\text{CO}_2$  which was followed by removal of the growth medium. The  $^{177}\text{Lu}$ -DOTA-trastuzumab and unlabeled trastuzumab (50, 100, 200 dan 250 ppm) in growth medium were then added into a separate wells on the plate. To all wells were then added a growth medium up to a final volume of 150  $\mu\text{L}$ . The plate was then

left to incubate for 24 hours at 37 °C with atmosphere of 5%  $\text{CO}_2$ . The growth medium was then removed from each well by pipette which was then followed by washing process with 150  $\mu\text{L}$  of PBS. To each well was then added 10  $\mu\text{L}$  of MTT (5 mg/ml) and 90  $\mu\text{L}$  of growth medium which was then followed by incubation for four hours with atmosphere of 5%  $\text{CO}_2$ . Finally, to each well was added 100  $\mu\text{L}$  of SDS 10% which was followed by incubation in dark room for 24 hours. The absorbance of each well was then read at 570 nm by using a plate reader.

## RESULTS AND DISCUSSION

Trastuzumab is a protein which can not be directly labeled with  $^{177}\text{Lu}$ , therefore trastuzumab has to be modified to enable a stable binding with  $^{177}\text{Lu}$ . In this project 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), a bifunctional chelating agent (BFCA) which has been known to be able in chelating with high stability of several metals including lanthanides, had been used as BFCA for  $^{177}\text{Lu}$ . Conjugation of DOTA to trastuzumab was performed by reacting NHS-DOTA with trastuzumab at pH 7.3 (Figure 1). The resulting immunoconjugate DOTA-trastuzumab was purified by dialysis process and then radiolabeled with  $^{177}\text{Lu}$  to produce  $^{177}\text{Lu}$ -DOTA-Trastuzumab. The radiochemical purity of the resulting radio-immunoconjugate  $^{177}\text{Lu}$ -DOTA-trastuzumab was determined by a thin layer chromatography (TLC) using a stationary phase and a mobile phase of ITLC-SG and saline solution respectively. The  $R_f$  for this system were about 0.3 for  $^{177}\text{Lu}$ -DOTA-trastuzumab and about 0.6 for free  $^{177}\text{Lu}$  (in form of  $^{177}\text{Lu}$ -EDTA or  $^{177}\text{Lu}$ -DOTA).

Figure 2 and 3 show radiochromatograms of  $^{177}\text{Lu}$ -DOTA-trastuzumab prior and after purification respectively. Radiolabeling of DOTA-trastuzumab with  $^{177}\text{Lu}$  resulted in  $^{177}\text{Lu}$ -DOTA-trastuzumab with radiochemical purity of 94% (Figure 2).

Purification by using a Sephadex G-25M (15 x 1.2 cm) column which was previously blocked with BSA and pretreated with PBS resulted in a  $^{177}\text{Lu}$ -DOTA-trastuzumab with radiochemical purity of > 99% (Figure 3).

Clearance test was aimed in finding the tendency, the rate and route (urine or faeces) of radioactivity excretion. The test was performed by intravenous injection a certain amount of  $^{177}\text{Lu}$ -DOTA-trastuzumab into two rats which were then kept in metabolic cages. The urine and faeces were collected and counted at a certain time. The clearance of radioactivity through urine and faeces for up to 240 hours post injection can be seen in Table 1.



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	-0.6	17.5	6.7	0.033	410354.0	820708.0	94.32	96.27
Rgn 2	48.6	69.4	59.5	0.298	8320.0	12640.0	1.45	1.48
Rgn 3	69.4	91.6	79.4	0.397	9597.0	19194.0	2.21	2.25
3 Peaks					426271.0	852542.0	97.98	100.00

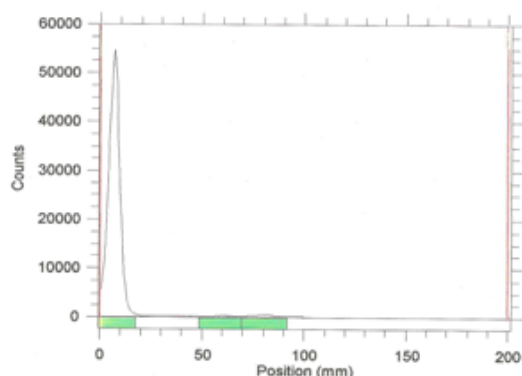


Figure 2. Radiochromatogram of <sup>177</sup>Lu-DOTA-trastuzumab prior purification. (Rf <sup>177</sup>Lu-DOTA-trastuzumab about 0.3 dan Rf free <sup>177</sup>Lu (in form of <sup>177</sup>Lu-EDTA or <sup>177</sup>Lu-EDTA) about 0.6).

Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	-0.6	19.3	8.5	0.042	446243.0	892486.0	99.20	100.00
1 Peaks					446243.0	892486.0	99.20	100.00

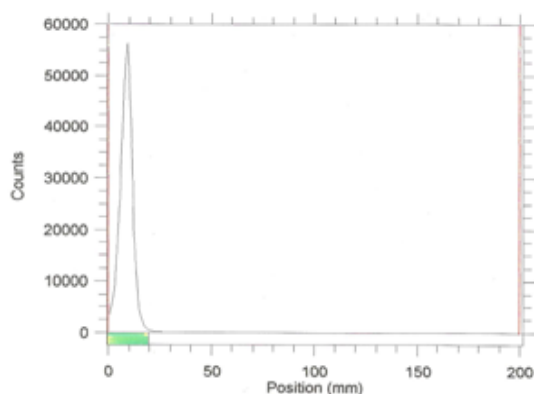


Figure 3. Radiochromatogram of <sup>177</sup>Lu-DOTA-trastuzumab after purification. (Rf <sup>177</sup>Lu-DOTA-trastuzumab ± 0.3 dan Rf free <sup>177</sup>Lu (in form of <sup>177</sup>Lu-EDTA or <sup>177</sup>Lu-EDTA) ± 0.6).

Table 1. The Percentage of clearance of radioactivity post injection of <sup>177</sup>Lu-DOTA-trastuzumab on normal rats.

No.	Duration (hours)	Average. Radioactivity in urine (%)	Average. Radioactivity in faeces (%)
1.	0 - 3	0.1	0.0
2.	4 - 24	0.6	0.5
3.	25 - 48	3.8	0.8
4.	49 - 72	5.0	0.4
5.	73- 144	11.6	1.5
6.	145- 168	2.9	0.2
7.	169 - 192	4.0	1.8
8.	193 - 216	3.8	0.8
9.	217 - 240	2.5	0.5
Sub Total		34.5	7.2

Table 1 shows that 240 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab, almost 35% radioactivity was excreted through urine as compared to that of 7% through faeces. The clearance pattern was possibly due to a negative charged radioimmunoconjugate such as <sup>177</sup>Lu-DOTA-trastuzumab.

Imaging was carried out by injection 200 µCi of <sup>177</sup>Lu-DOTA-trastuzumab to each of five rats. At a determined time 3, 24, 48, 72 and 144 hours post injection one rat was anesthetized and then imaged using a γ-camera. The γ-camera image of each rat at 3, 24, 48, 72 and 144 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab can be seen in Figure 4.

It can be seen from the above figures that there were still some radioactivity retained in hepatic area up to 144 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab. The result was similar to that of the previous biodistribution studies. The studies showed that there was ~5%ID/g residue of radio-activity in liver 72 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab<sup>(13)</sup>.

However, the radioactivity residue in liver of normal rats 72 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab was much lower as compared to that of radioactivity residues in liver of normal rats 72 hours post injection of <sup>111</sup>In-trastuzumab, <sup>111</sup>In-NLS3-trastuzumab, <sup>111</sup>In-NLS6-trastuzumab and <sup>111</sup>In-NSL3-h-IgG (11.5 ± 0.5 % ID/g, 11.0 ± 0.4 % ID/g, 12.3 ± 0.4%, 12.6 ± 0.9% ID/g respectively) as reported by Constantini *et al*<sup>(14)</sup>. The trace of radioactivity in liver of normal rats up to 144 hours post injection of <sup>177</sup>Lu-DOTA-trastuzumab indicated that some of radioimmunoconjugate was metabolized or catabolised in liver. This is not unusual phenomenon as liver has been known as an organ where high weighted molecules are metabolized or catabolised. A good radioimmunoconjugate is expected not to leave a high radioactivity residue in non-target organs such as liver, lung and other sensitive organs, so that these organs are not exposed by unnecessary radiations.

Cytotoxicity test was aimed in determining the effectiveness of <sup>177</sup>Lu-DOTA-trastuzumab in killing cancerous cells which expressed HER-2 compared to that of trastuzumab. The test was carried out in vitro by using the positive HER-2 cell lines, SKOV-3 (a positive HER-2 ovarian cancer cell lines). The use of these cells was due to the difficulty in obtaining of SK-BR-3 cell lines (a positive HER-2 breast cancer cell lines).

Cytotoxicity test is based on colorimetric method of the transformation of the yellow water-soluble MTT to form blue-formazan crystal<sup>(15)</sup>. The transformation of the yellow water-soluble tetrazolium salt (MTT) is due to the existence of succinate dehydrogenase

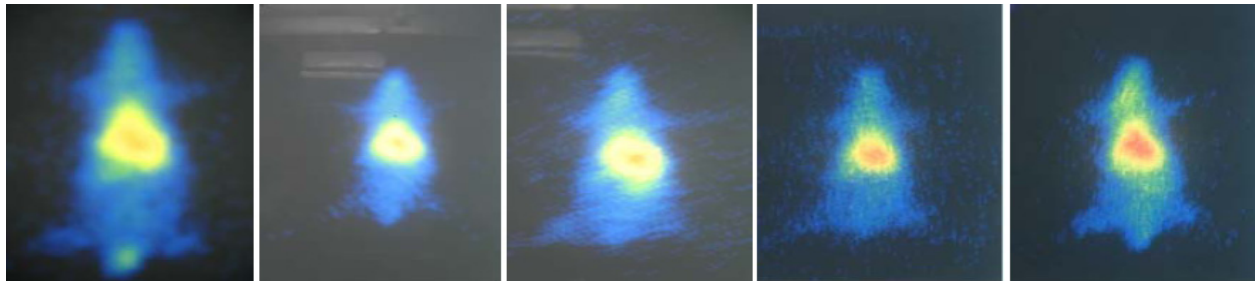


Figure 4. Image of rat at 3, 24, 48, 72 and 144 hours post injection of  $^{177}\text{Lu}$ -DOTA-trastuzumab.

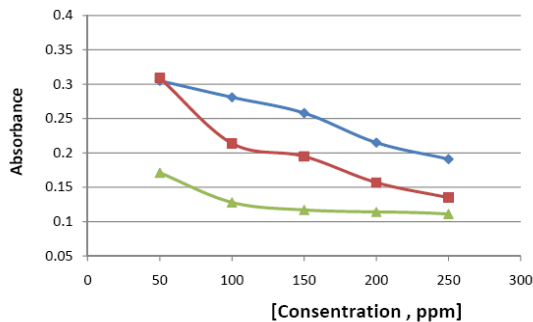


Figure 5. Absorbance of SKOV-3 cell line which was treated with naked trastuzumab and  $^{177}\text{Lu}$ -DOTA-trastuzumab.   
 ◆ = Control; ■ = Trastuzumab; ▲ = [Lu-177]-DOTA-Trastuzumab.

enzyme which is produced by metabolic process of mitochondria of living cells. The blue formazan crystal is then dissolved in a proper solvent and then quantified by spectrophotometric method. The absorbance of the blue formazan is directly proportional to the number of metabolically active cells. This method has been reported as a reliable method in the determination of apoptosis or proliferation of cells.

Figure 5 shows the absorbance of positive HER-2 SKOV-3 cell lines which were untreated and treated with unlabeled trastuzumab and treated with  $^{177}\text{Lu}$ -DOTA-trastuzumab. It can be seen that positive HER-2 SKOV-3 cell lines which were treated with  $^{177}\text{Lu}$ -DOTA-trastuzumab gave much lower absorbance as compared to that of cell lines treated with unlabeled trastuzumab.

As the absorbance is directly proportional to the concentration of blue formazan, and consequently proportional to the number of living cells, therefore it can be suggested that  $^{177}\text{Lu}$ -DOTA-trastuzumab is more effective in killing cells as compared to the trastuzumab. Figure 5 also shows that 50 ppm of  $^{177}\text{Lu}$ -DOTA-trastuzumab was able to kill almost the same number of cells which were treated with 200 ppm unlabeled trastuzumab. These results showed that  $^{177}\text{Lu}$ -DOTA-trastuzumab was more effective in killing HER-2 positive cancerous cells (SKOV-3) as compared to that of unlabeled trastuzumab.

## CONCLUSIONS

An anti HER-2 monoclonal antibody based radio-immunoconjugate,  $^{177}\text{Lu}$ -DOTA-trastuzumab, with a radiochemical purity of > 99% after purification process using Sephadex G-25 M column had been successfully prepared. In vitro and in vivo tests on  $^{177}\text{Lu}$ -DOTA-trastuzumab, including clearance test, imaging test, and cytotoxicity test had been performed. The clearance test of  $^{177}\text{Lu}$ -DOTA-trastuzumab on normal rats showed that  $^{177}\text{Lu}$ -DOTA-Trastuzumab was predominantly cleared through urine. The  $\gamma$ -camera imaging test on normal rats showed that there was still some trace of radioactivity in hepatic area up to 144 hours post injection of  $^{177}\text{Lu}$ -DOTA-trastuzumab. Cytotoxicity test showed that  $^{177}\text{Lu}$ -DOTA-trastuzumab was more effective in killing HER-2 positive cancer cell lines as compared to that of unlabeled trastuzumab.

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