

**Preparation, Stability and Biodistribution Studies of  $^{177}\text{Lu}$ -DOTA-Trastuzumab,  
A Potential Radiopharmaceutical for Radioimmunotherapy of Breast Cancer**

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## Outline

- Background
- Aim of the project
- Methods and Results
- Summary and Future Work

# Background

## World's Statistic (2000)

- 10 million new cases of cancer
- 22 million people living with cancer
- 6 million death were caused by cancer

## Estimation

- 15 million new cases of cancer
- 10 million death were caused by cancer

D.M. Parkin, *The Lancet*, 2000, 2, 533.

## Indonesia's Statistic

### Estimation (2008)

1.1 million people living with cancer

*www. Cancer. CureResearch™.com* (accessed 19 June 2008)

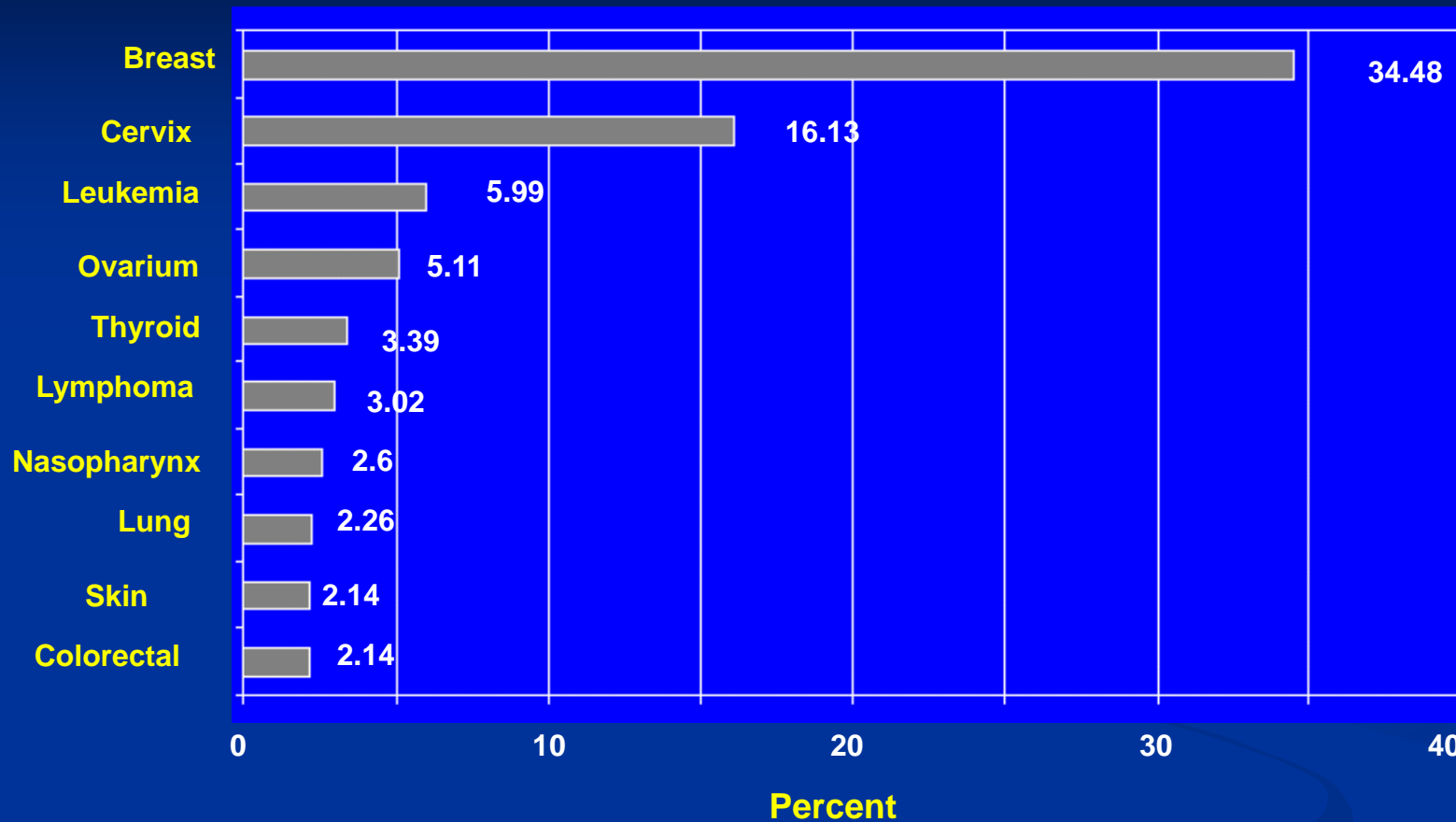
## Estimation

170 - 190/ 100 000 population/ year/ new cancer cases

The most frequent cancers (1991): cervix (19.18%), breast (12.10%), skin (7.69%), lymph node (5.62%),

D. Tjindarbumi, R. Manggunkusoma. *Jpn. J. Clin. Oncol.* 2002. 32 (Supplement 1). S17-S21.

## The Ten Most Frequent Malignancy in Female in 24 Hospitals, 2006



*Evlina Suzanna, MD, Coordinator Collecting and Data Analysis  
of Indonesia National Cancer Registry, Department of Health*

## Aims

- To prepare a monoclonal antibody based radiopharmaceutical for breast cancer (which express HER-2 in particular).
- To study the potential (preclinical and clinical) the prepared monoclonal antibody based radiopharmaceutical
- The prepared a radioimmunotherapeutic agent is expected to be more effective than the than the available agent (Trastuzumab)

### Work Plan

3 year2 (2009 - 2011)

2009: First year from 3 years of the planned work

# The Choice of Radionuclide

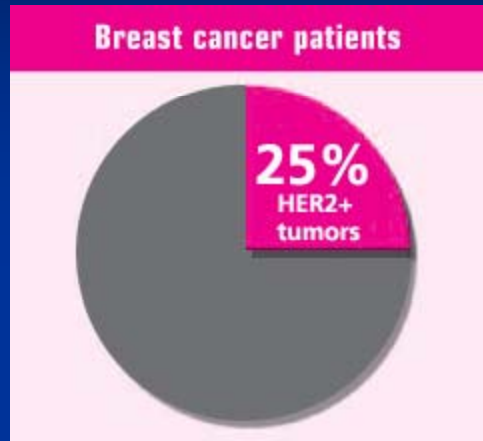
## Lutetium-177 ( $^{177}\text{Lu}$ )

- New emerging radionuclide (in the last 5 years)
- $\beta$  [ $E_{\text{max}}$  497 (78,6%) dan 176 (12,2%) keV ] particle emitter → Considered to be suitable for therapy of small size of cancer (tissue penetration ~ 1,5 mm)
- $\gamma$  [113 (6,4%) dan 208 (11%) keV], emitters (ideal for imaging)
- Procurement of  $^{177}\text{Lu}$  with high radioactivity is relatively easy, [ $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ ], cross section (2100 barn)]

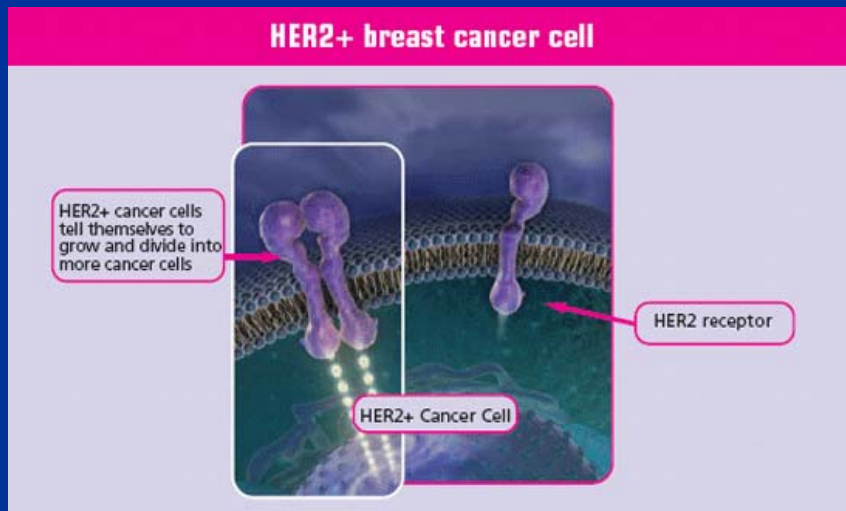
## Final Stages of Clinical Trial/ Waiting to be marketed

- $^{177}\text{Lu}$ -TOC/TATE (Erasmus Medical Centre, Netherlands,) for therapy of lung cancer
- $^{177}\text{Lu}$ -BB2 Bombesin GRP (Bracco) for therapy of lung cancer
- $^{177}\text{Lu}$ -PSMA Antibody (Cytogen) for therapy of prostate

## The choice Monoclonal Antibody [Human Epidermal Growth Factor Receptor-2 (HER-2)]

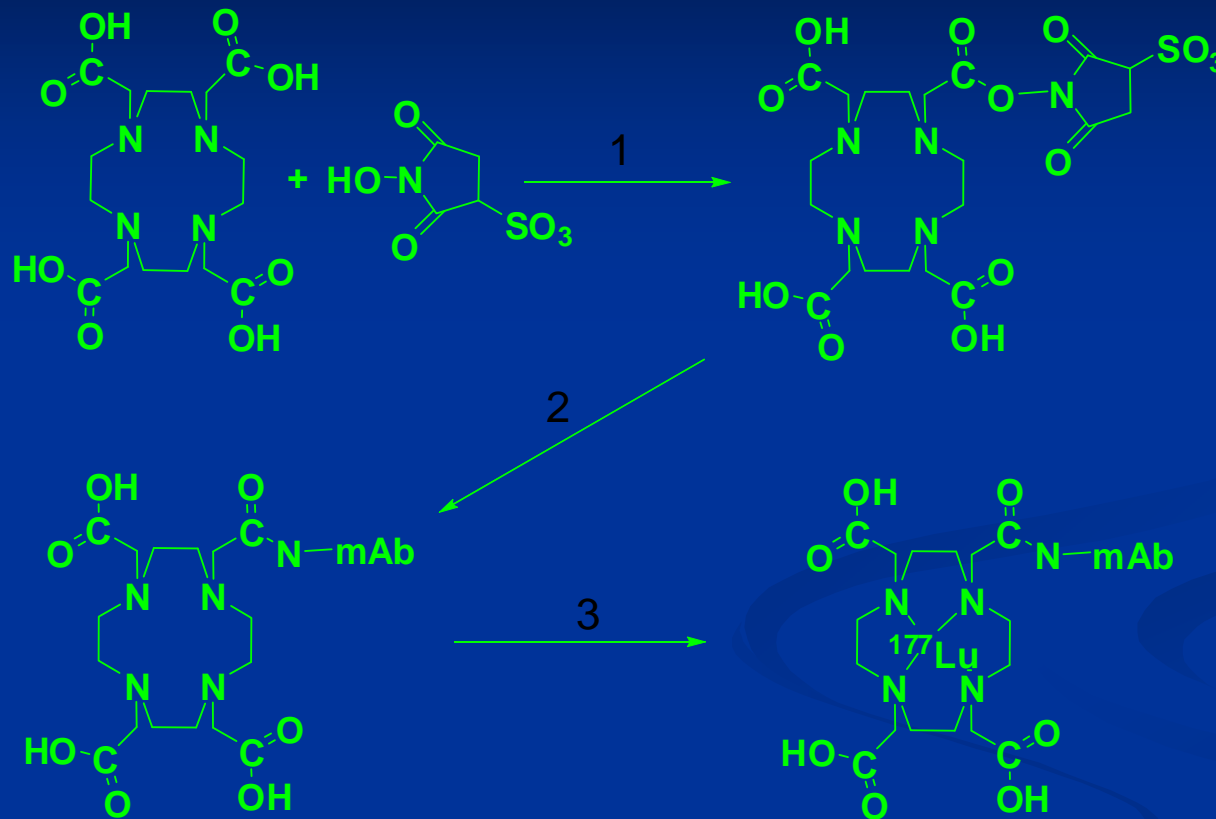


- 25% breast cancer cases have been found to have over expression of HER-2
- Over expression ~ seven times higher than on normal tissue
- Over expression of HER-2 in breast cancer is associated with increased disease recurrence and worse prognosis
- Over expression also occurs in other cancer such as ovarian cancer, stomach cancer, and biologically aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma
- Clinically, HER2 is important as the target of the monoclonal antibody trastuzumab (marketed as Herceptin)



## Methods

### Preparation of $^{177}\text{Lu}$ -DOTA-Trastuzumab



*Reactions and conditions: (1) 1-ethyl-3-(3-dimethylpropyl) carbodiimide (EDC), (2) dialysed trastuzumab, (3)  $^{177}\text{LuCl}_3$ , 0.1 M Ammonium acetate buffer pH 5.5*

**Note:**

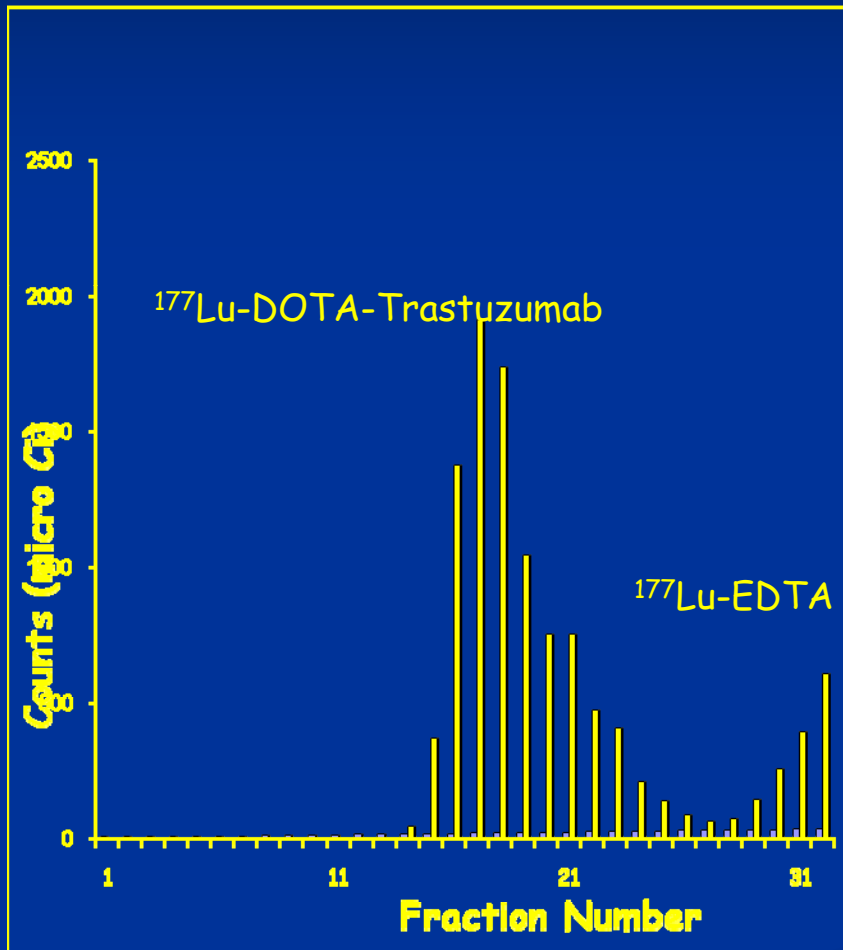
1,4,7,10-tetraazacyclotridecane-1,4,7,10-tetraacetic acid (DOTA)  
Sulfo-hydroxysuccinimide (sulfo-NHS)

## Methods

### Preparation of $^{177}\text{Lu}$ -DOTA-Trastuzumab

- 0.3 – 0.4 mg of  $^{176}\text{Lu}_2\text{O}_3$  (60.60%) was irradiated at the Multi Purposes GA Siwasbessy Reactor for four day
- Irradiated of  $^{176}\text{Lu}_2\text{O}_3$  was dissolved in 6M HCl/  $\text{H}_2\text{O}_2$  solution, heated to dryness
- Re-dissolved in 0.5 M HCl
- $^{177}\text{LuCl}_3$  (0.3 mg  $^{176}\text{Lu} \sim 2$  Ci: Specific Activity  $^{177}\text{Lu} \sim 7$  Ci/ mg.)

## Elution Profile of Purification of $^{177}\text{Lu}$ -DOTA-Trastuzumab (1.2 x 20 cm Sephadex-G25 Column, saturated with 1 mL of 10 % BSA, eluted with 0.1 PBS pH 7.2)



- Fraction was checked for its radiochemical purity with ITLC-SG (mobile phase: saline solution)

$R_f$   $^{177}\text{Lu}$ -DOTA-Trastuzumab < 0.2

$R_f$   $^{177}\text{Lu}$ -EDTA > 0.7

- Fractions (15 - 23) which contained  $^{177}\text{Lu}$ -DOTA-Trastuzumab > 95 % was pooled and used for further studies

Radiochemical purity of  $^{177}\text{Lu}$ -DOTA-Trastuzumab in this work ~ 99.9%

## Stability Test of $^{177}\text{Lu}$ -DOTA-Trastuzumab (Stored at 4°C and Room Temperature)

### Stored at Room Temperature

Duration	% Radiochemical Purity
0 Hour	99.9 ± 0.05
24 Hours	99.63 ± 0.20
48 Hours	99.26 ± 0.25
72 Hours	99.36 ± 0.03
96 Hours	99.06 ± 0.10

### Stored at 4°C

Duration	% Radiochemical Purity
0 Hour	99.96 ± 0.05
24 Hours	99.79 ± 0.01
48 Hours	98.91 ± 0.41
72 Hours	99.41 ± 0.01
96 Hours	99.62 ± 0.10

5 mCi ( 1mL) of  $^{177}\text{Lu}$ -DOTA-Trastuzumab  
Radiochemical Purity was monitored using ITLC-SG (mobile phase: saline solution)

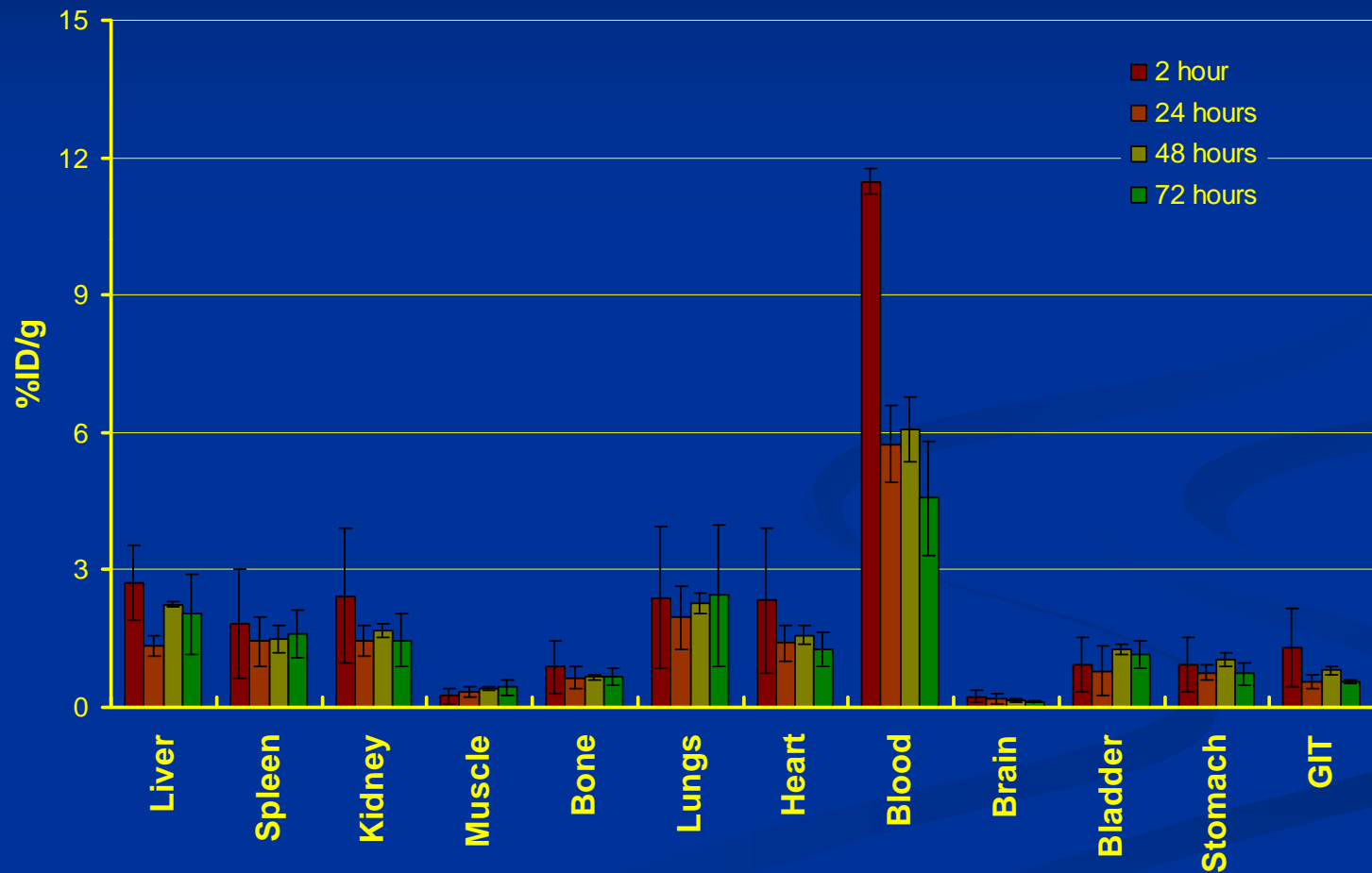
## Serum Stability of $^{177}\text{Lu}$ -DOTA-Trastuzumab

Radiochemical Purity of  $^{177}\text{Lu}$ -DOTA-Trastuzumab in human serum

Incubation Time	% Radiochemical Purity
0 Hours	$99.96 \pm 0.05$
24 Hours	$99.72 \pm 0.03$
48 Hours	$99.67 \pm 0.11$
120 Hours	$99.37 \pm 0.12$

0.5 mCi (0.050 mL) was incubated in 0.450 mL of human serum at 37 °C

## Biodistribution of $^{177}\text{Lu}$ -DOTA-Trastuzumab (Normal Mice)



100  $\mu\text{Ci}$  injected to each mouse (weighted ~ 20 gr), 3 mice/time point

## Summary and Future Works

### Summary

- $^{177}\text{Lu}$ -DOTA-Trastuzumab with radiochemical purity > 95% has been able to be prepared (99.9%)
- $^{177}\text{Lu}$ -DOTA-Trastuzumab was found to be very stable when stored at room temperature and at 4°C for up to 96 hours, and in serum for to 120 hours
- Biodistribution of  $^{177}\text{Lu}$ -DOTA-Trastuzumab in normal mice indicated that some of  $^{177}\text{Lu}$ -DOTA-Trastuzumab was cleared trough kidney

### Future Works

- Preclinical studies: binding affinity  $^{177}\text{Lu}$ -DOTA-Trastuzumab of to HER-2 (using SKBR-3 cell lines which express HER-2), Dosage, Biodistribution and imaging using tumour model (xenograph)
- Clinical trial:

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**Thank You**