Preclinical Evaluation of Indium 111 Radiololabeled Trastuzumab and Pertuzumab for HER2-positive Breast Cancer Molecular Imaging

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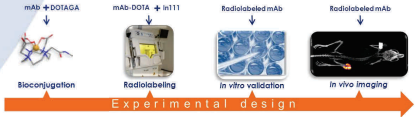
Introduction

- The accurate and reliable **assessment of HER2 status** is an essential step in the diagnostic workup and in the selection of targeted treatment strategy in breast cancer patients. In this context, non-invasive imaging of HER2 expression could be a relevant alternative to primary tumor biopsy with additional benefits such as a better selection of patients for HER2-targeted therapies and the possible optimization of treatment strategies.
- **The aim of our study was to evaluate the use of radiolabeled ^{111}In-DOTAGA** (2,2',2'-(10-(2,6-dioxotetrahydro-2H-pyran-3-yl)-1,4,7,10-tetraazacyclodo decane-1,4,7-triyl)-triacetic acid) **- conjugated trastuzumab and pertuzumab for SPECT/CT (Single Photon Emission Computed Tomography / Computed Tomography) HER2 imaging purposes.**

Methods

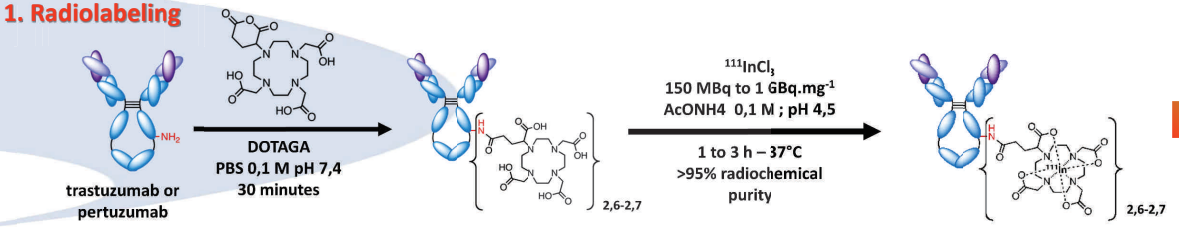
- **Bioconjugation / radiolabeling :** Trastuzumab and pertuzumab were conjugated to a new bifunctional chelating agent: DOTAGA anhydride (DOTA analog) and subsequently radiolabeled with the gamma-emitter Indium 111.

- **In vitro validation:** The functionality of ^{111}In-DOTAGA-antibodies analogs was evaluated by in vitro saturation assays using HER2-overexpressing human breast cancer cell line (HCC1954).
- **In vivo biodistribution:** biodistribution was studied by SPECT/CT imaging at 24h, 48h and 72h after intravenous injection of ^{111}In-DOTAGA-antibodies in BT-474 tumor-bearing mice using a NanoSPECT/CT® small animal imaging tomographic γ-camera (Bioscan Inc). After the last image acquisition, animals were sacrificed. Blood, tumor and organs were collected and radioactivity in these samples was measured with a γ-counter.



Results

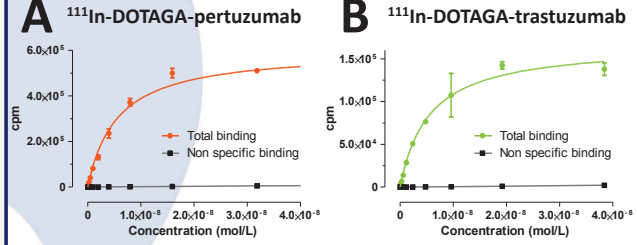
1. Radiolabeling



^{111}In-DOTAGA-trastuzumab and ^{111}In-DOTAGA-pertuzumab showed respectively 2.6 and 2.7 DOTAGA /antibody. Both ^{111}In-DOTAGA-trastuzumab and ^{111}In-DOTAGA-pertuzumab were radiolabeled with a radiochemical purity > 95%.

2. *In vitro* saturation assays

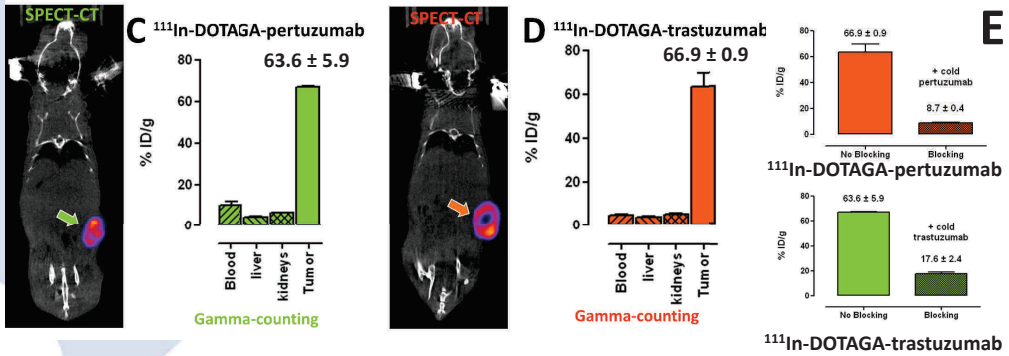
^{111}In-DOTAGA-pertuzumab (**A**) and ^{111}In-DOTAGA-trastuzumab (**B**) dissociation constants (Kd) were determined by incubating increasing concentrations of the radiolabeled probes with a known fixed concentration of HER2-expressing human tumoral cells (HCC1954). Dissociation constants were estimated at 5.6 10^{-9}M for radiolabeled pertuzumab (**A**) and 5.5.10^{-9} M for radiolabeled trastuzumab (**B**) respectively.



Biological activity of ^{111}In-DOTAGA-trastuzumab and ^{111}In-DOTAGA-pertuzumab was maintained: the affinity determined on HCC1954 cells, was close to 5 nM.

3. *In vivo* SPECT/CT imaging and *ex vivo* organs gamma-counting.

Biodistribution of ^{111}In-DOTAGA-pertuzumab (**C**) and ^{111}In-DOTAGA-trastuzumab (**D**) obtained after intravenous injection of 15-20 MBq (25 µg) of the radiolabeled probes in BT-474 tumor-bearing mice. Tumor uptake was dramatically decreased (**E**) after injection of either excess (x 1000) of cold pertuzumab or trastuzumab, underlying the specific targeting of the HER2+ tumor.



*** *In vivo* experiments showed a high accumulation of both antibodies in HER2+ breast tumors with a similar intensity**
*** An excess of non-radiolabeled corresponding antibody significantly shifted down tumor-targeting**

Conclusion

- **Our results demonstrated a similar HER2 binding of both radiolabeled ^{111}In-DOTAGA-pertuzumab and ^{111}In-DOTAGA-trastuzumab suggesting that lower efficacy of pertuzumab in HER2+ breast cancer is probably not due to a reduced HER2 binding.**
- **These radiolabeled probes should therefore be considered as promising tools for molecular imaging diagnosis of HER2 overexpression in breast cancer.**

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