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DOTA-conjugated antibodies for scintigraphic evaluation of new anti-cancer treatments

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Introduction / Abstract

The treatment of cancer is being revolutionized by the development of targeted therapeutic biologics. Translational pharmaco-imaging represents a key step in the evaluation of these new targeted anti-cancer therapies. In this respect, we have implemented and validated an antibody radiolabeling process for scintigraphic SPECT imaging.
A new macrocyclic reagent (DOTAGA, bearing an anhydride function able to react with primary amines) was used for DOTA-conjugation of trastuzumab. DOTA-trastuzumab was synthesized by directly reacting DOTAGA with trastuzumab. The number of DOTA per antibody was determined using mass spectrometry.
The DOTA-conjugated antibodies were metallated with Indium-111. The functionality of DOTA-trastuzumab analogs was evaluated by in vitro saturation assays using two human breast cancer cell lines (SK-BR-3 and HCC1954). *In vivo* evaluation was performed in subcutaneous BT474 tumor-bearing rodents.
Two DOTA-trastuzumab analogs were synthesized. The first analog (MM205) was bearing one DOTA per antibody while the second (MM343.1) was bearing 3 DOTA per antibody. The labeling yield reached 75% for both conjugates and high specific activities were obtained (>700 MBq.mg^{-1}). The affinity of the DOTA-trastuzumab analogs, determined on Her2 expressing SK-BR-3 and HCC1954 cell lines, was around 1 nM. SPECT/CT imaging experiments in tumor-bearing rodents were performed for *in vivo* validation of the MM343.1 analog only.
Together, these results demonstrate the efficiency of our new DOTA-synthesis and conjugation processes for biologics radiolabeling to assess biodistribution, anti-cancer efficacy and related toxicity of anti-cancer drugs by *in vivo* non invasive imaging.

Materials and Methods

Synthesis of the DOTA-trastuzumab conjugates MM205 and MM343.1

In brief, 47 µl of a 4.5 mg.mL^{-1} suspension of DOTAGA-anhydride in dry chloroform was pipetted under ultrasonication and transferred to a polypropylene microtube. The chloroform was evaporated under a gentle stream of nitrogen. Purified trastuzumab (3 mg = 750 µL) in PBS 1X was subsequently added and gently mixed on a vortex at room temperature for 30 min. Unbound DOTAGA was then removed by ultrafiltration (twice 12 min at 7670*g* in Nanosep® 30 KDa). The immunoconjugate was finally diluted in ammonium acetate buffer (0.1 M, pH 5.8) to a final concentration of 5 mg.mL^{-1}.

In vitro biological characterization of the DOTA-trastuzumab conjugates MM205 and MM343.1

Breast cancer cells

Two Her2 expressing cell lines were investigated in this study: the human breast cancer cell lines HCC1954 and SK-BR-3. Cells were cultured as adherent monolayer in RMPI 1640 medium supplemented with 10% fetal bovine serum (Lonza).

Ability of DOTA-trastuzumab to bind Her2 antigen

The ability of DOTA-trastuzumab to bind Her2 antigen was assessed by FACS analysis. Approximately 2-3x10^5 HCC1954 and SK-BR-3 tumor cells were incubated with trastuzumab, DOTA-trastuzumab or control isotype (10 µg.mL^{-1}). Cells were then washed twice with binding medium and then incubated with a FITC-conjugated secondary anti-human IgG antibody for FACS analysis.

Saturation receptor-binding experiments

The Her2 antigen density and the dissociation constant (Kd) of the ^{111}-In-DOTA-trastuzumab were determined in radioligand binding saturation assays. Approximately 3.10^5 HCC1954 and SK-BR-3 tumor cells were incubated with increasing concentrations of ^{111}In-DOTA-trastuzumab (5.8x10^{-11} to 6.0x10^{-8} M, 10^{18} cpm.mol^{-1}) in a total volume of 0.2mL for 1 hour at +4°C. Cells were then centrifuged, rinsed twice with ice cold binding medium. Cells were then dissolved in 0.1 N NaOH and radioactivity associated to cells was counted with a γ-counter. Assays were performed in the absence (total binding) or presence (non specific binding) of a >100-fold excess unlabelled trastuzumab.

Immunoreactivity

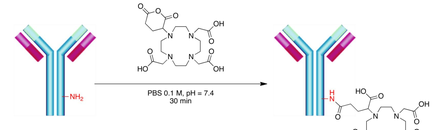
The fraction of ^{111}In-DOTA-trastuzumab able to bind to Her2 antigen was determined by incubating trace amounts of ^{111}In-DOTA-trastuzumab (7.0x10^{-9} M) with increasing concentrations of HCC1954 tumor cells (0.4 to 24 x 10^6 cells.mL^{-1}) in a total volume of 0.2mL for 1 hour at +4°C. At the end of the incubation period, cells were processed as described for the saturation binding experiments.

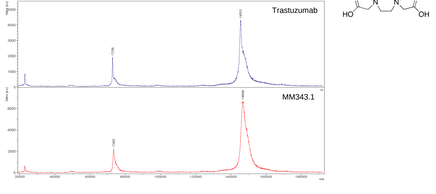
In vivo tumor targeting (MM343.1 analog)

Imaging was performed 24, 48 and 72 hours after the IV injection of 25 µg of ^{111}-In-DOTA-trastuzumab (MM343.1, 13-15 MBq) 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 terminated. Blood, tumor and organs were collected and radioactivity in these samples was measured with a γ-counter.

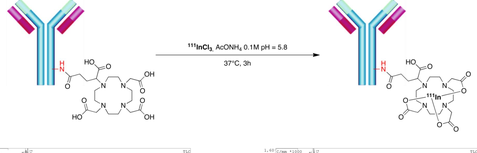
Synthesis of the DOTA-conjugated trastuzumab

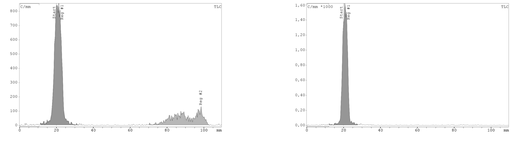
Conjugation reaction was assessed by MALDI-TOF mass spectrometry, and typically resulted to a median of 1 (MM205) or 3 DOTAs (MM343.1) per antibody.





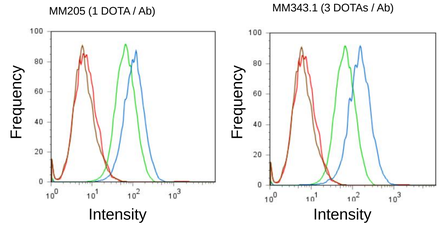
The immunoconjugates were radiolabeled with $^{111}InCl_3$ (1000 MBq.mg^{-1}) for 3h at +37°C. EDTA was used to bind free ^{111}In and radiopurity was determined by ITLC (in these conditions, ^{111}In-EDTA moved to the front while the ^{111}In-labelled monoclonal antibody remained at the starting position). The labeling yield was 75%, and radiopurity was >99% after ultrafiltration on Nanosep® 30 KDa.



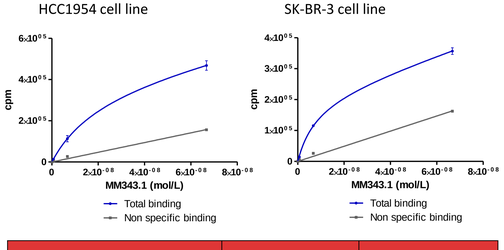


In vitro biological characterization

Determination of the ability of DOTA-trastuzumab to bind Her2 antigen. The labeling intensities observed for the two DOTA-trastuzumab analogs were similar to that of trastuzumab.



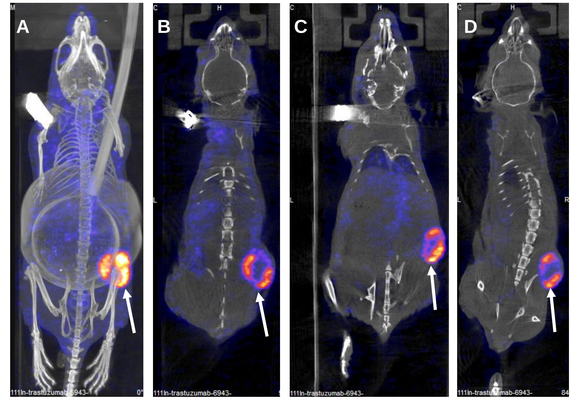
The binding dissociation constant of both ^{111}In-DOTA-trastuzumab analogs (MM205 and MM343.1) was close to 10^{-9}M as assessed in saturation binding experiments. The immunoreactivity was 65%. The average number of Her2 antigen per cell was 3x10^5. Only data obtained for MM343.1 are shown.

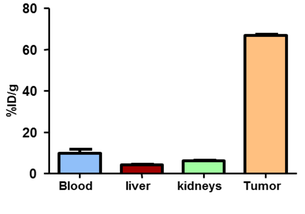


MM343.1 (3 DOTAs)	HCC1954	SK-BR-3
Binding affinity constant (Kd)	1.3 x 10^{-9}	0.5 x 10^{-9}

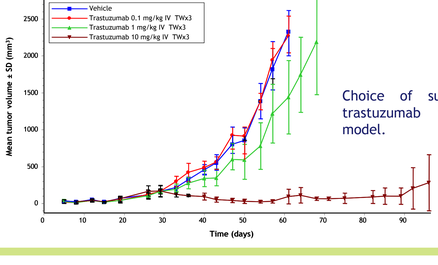
In vivo tumor targeting (MM343.1)

Subcutaneous BT-474 human breast tumor imaging by SPECT/CT: representative maximal intensity projections (A) and coronal slices at times 24h (B), 48h (C) and 72h (D) post-injection of ^{111}In-radiolabeled MM343.1. The tumor is indicated by an arrow.





Ex vivo biodistribution of ^{111}In-DOTA-trastuzumab MM343.1). Tumor and major excreting organs were collected after the last image acquisition (72h p.i.). Radioactivity uptake in the BT-474 tumors was 6.1 ± 4.7%ID/g. Tumor-to-organs uptake ratios were > 2.



Choice of suboptimal dose of trastuzumab in BT-474 tumor model.

Conclusions

- DOTA-trastuzumab analogs had high affinity properties allowing their use in *in vitro* and *in vivo* experiments.
- As expected, ^{111}In-DOTA-trastuzumab demonstrated a high specificity for human Her2 expressing tumors xenografted in mice.
- The ^{111}In-DOTA-trastuzumab conjugates together with SPECT imaging are powerful tools to evaluate the efficacy of anticancer therapies.
- Pharmimage® partners have developed qualified processes for the radiolabeling of biologics and in vivo SPECT imaging with high potential for biomarker development.