Theranostic ^{155}Tb- and ^{225}Ac-Labelled Trastuzumab F(ab')$_2$ for HER2-Positive Cancers

Alessia M.C. Pileggi[1], Conrad Chan[1], Joshua Mojica[2], Hua Yang[3,4,2], Raymond M. Reilly[1,5,6]

[1] Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada, [2] Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada, [3] Life Sciences Division, TRIUMF, Vancouver, BC, Canada, [4] The University of British Columbia, Vancouver, BC, Canada, [5] University Health Network, Toronto, ON, Canada, [6]Princess Margaret Cancer Centre, Toronto, ON, Canada.

UNIVERSITY OF TORONTO
LESLIE DAN FACULTY OF PHARMACY

BACKGROUND & AIM

Breast cancer remains a major global health concern, as the second leading cause of cancer-related deaths among women worldwide. Approximately 15–20% of these breast cancer cases are classified as human epidermal growth factor receptor 2 (HER2)-positive. While commonly associated with breast cancer, HER2 is also overexpressed in about 10–15% of gastric cancers and in several other malignancies, contributing to aggressive tumour behaviour and poorer prognoses. Trastuzumab is a humanized monoclonal antibody that binds to the extracellular domain of HER2, inhibiting the growth of HER2-dependent tumours and representing a major advancement in targeted cancer therapy. An example of a well-known commercial formulation of trastuzumab is Herceptin. However, despite its initial effectiveness, many patients have become resistant to Herceptin and other biosimilars, driving the need for alternative therapeutic strategies. Radioimmunotherapy (RIT) with trastuzumab complexed to the α-particle emitter Actinium-225 (^{225}Ac) may overcome resistance. Trastuzumab F(ab')$_2$ conjugated with DOTA to complex ^{225}Ac were effective for RIT of HER2-positive breast cancer (BC) xenografts in mice but F(ab')$_2$ were less toxic than intact IgG **(1).** Labelling DOTA-trastuzumab F(ab')$_2$ with ^{225}Ac required heating at 37°C for 2 hours (hrs) achieving 70% labelling efficiency requiring purification. Crown (3A-crown-NCS), a novel bifunctional chelator, more efficiently complexes ^{225}Ac in 1 hour at room temperature (RT) **(2)**. Our aim was to study the labelling of trastuzumab F(ab')$_2$ conjugated to crown with ^{225}Ac.

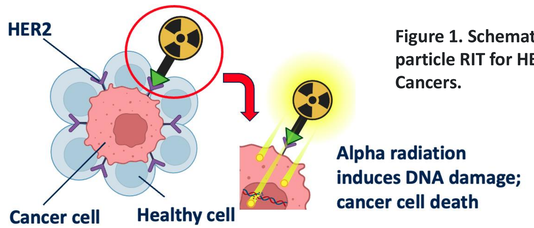
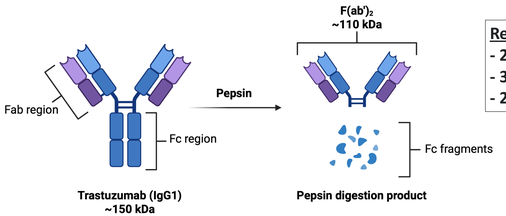


Figure 1. Schematic of α-particle RIT for HER2-Positive Cancers.

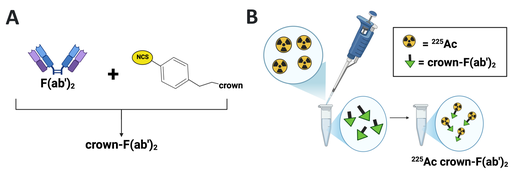
METHODS



Reaction Conditions
- 20 mM NaOAc, pH 4.5
- 37°C, 15 hrs
- 200 rpm mixing

Figure 2. Production of Trastuzumab F(ab')$_2$. F(ab')$_2$ was produced by proteolysis of trastuzumab with immobilized pepsin followed by purification by ultrafiltration on an Amicon device [molecular weight cut-off (MWCO)= 30 kDa]. F(ab')$_2$ purity was assessed by sodium-dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing and reducing conditions.

METHODS



Reaction Conditions
- 0.1 M $NaHCO_3$, pH 8.2
- RT, 2 hrs
- 150 rpm mixing

Reaction Conditions
- 1 M NH_4OAc, pH 5.5
- RT, 1 hr
- No mixing

Figure 3. A) Conjugation and B) Radiolabelling of the Radiopharmaceutical. F(ab')$_2$ was conjugated to crown with either a 20, 30, 50, 80, 110 or 120-fold molar excess (ME) of crown and purified by ultrafiltration. The purity of crown-F(ab')$_2$was determined by SDS-PAGE and the number of crown per F(ab')$_2$ was measured by electrospray ionization mass spectrometry (ESI-MS). Crown-F(ab')$_2$ was then radiolabelled with ^{225}Ac (1-10 kBq/µg) and followed by instant thin layer-silica gel chromatography (ITLC-SG) in 0.1 M Na citrate buffer, pH 5.5, to determine the radiochemical purity (RCP) of the radiopharmaceutical.

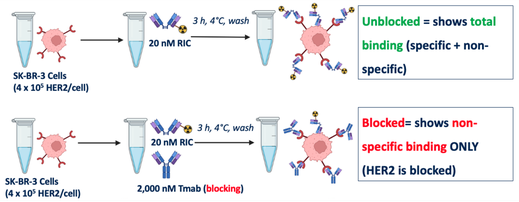


Figure 4. Single-Concentration Binding Assay. HER2 binding of ^{225}Ac-crown-F(ab')$_2$ was evaluated by incubation with HER2-positive human SK-BR-3 cells with or without excess trastuzumab to measure total and specific binding (SB), respectively.

RESULTS

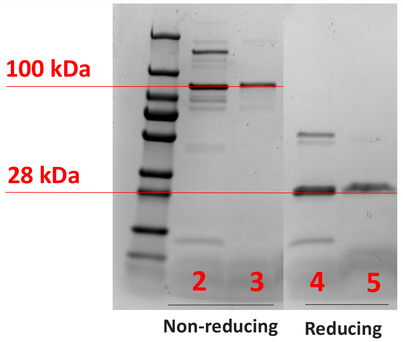


Figure 5. SDS-PAGE of Trastuzumab F(ab')$_2$ and 110-fold ME Crown-Trastuzumab F(ab')$_2$ under Non-Reducing and Reducing Conditions. SDS-PAGE revealed pure, homogenous crown-F(ab')$_2$. Under non-reducing conditions, F(ab')$_2$ (lane 2) displayed a prominent band at ~100 kDa, corresponding to the expected molecular weight (MW) of F(ab')$_2$, confirming successful production of F(ab')$_2$. The higher MW band in lane 2 represented any undigested trastuzumab. Conjugation with a 110-fold ME of crown (lane 3) caused a slight upward band shift with a MW= 101 kDa. Under reducing conditions [dithiothreitol (DTT)], F(ab')$_2$ (lane 4) showed a MW = 28 kDa, corresponding to the light chains of the antibody, and 110-fold ME crown-F(ab')$_2$ showed a slight upward band shift (lane 5). SDS-PAGE of all other ME F(ab')$_2$ conjugates tested showed similar results.

F(ab')$_2$: Crown Ratio	Number of Crown per F(ab')$_2$
1:20	0-1
1:30	1-2
1:50	1-2
1:80	1-3
1:110	1-3
1:120	1-3

Table 1. Number of Crown Conjugated per F(ab')$_2$. ESI-MS was used to determine the number of crown chelators conjugated to each F(ab')$_2$. Reaction of F(ab')$_2$ with a 20-fold ME introduced 0-1 crown/F(ab')$_2$ Reactions with 30 and 50-fold MEs introduced 1-2 crown/F(ab')$_2$. Although the 80-, 110-, and 120-fold MEs showed overlapping distributions (1–3 crown per F(ab')$_2$), the 110-fold ME spectrum demonstrated a higher abundance of F(ab')$_2$ conjugated to 3 chelators, while also showing the lowest proportion of unmodified F(ab')$_2$. This 110-fold ME condition was therefore selected as the most suitable for radiolabelling.

RESULTS

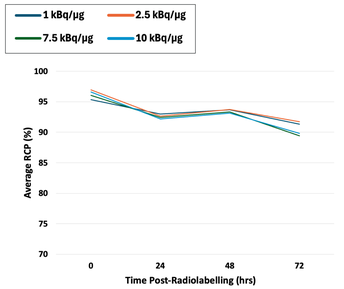
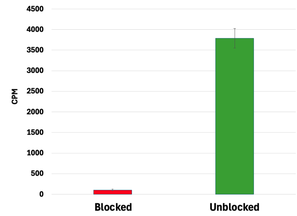


Figure 6. RCP of ^{225}Ac-crown-F(ab')$_2$ 0, 24, 48 and 72 Hours Post-Radiolabelling. The RCP of ^{225}Ac-crown-F(ab')$_2$ was evaluated via ITLC-SG at specific activities of 1, 2.5, 7.5 and 10 kBq/µg. At time 0, the RCP with ^{225}Ac was 95.3%, 97.0%, 96.0% and 96.6%, respectively. At time 24 hrs, the RCP was 93.0%, 92.6%, 92.5% and 92.2%,respectively. At time 48 hrs, the RCP was 93.7%, 93.7%, 93.3% and 93.1%, respectively. Finally, at time 72 hrs, the RCP was 91.4%, 91.7%, 89.4% and 89.8%, respectively. The radiopharmaceutical demonstrated high RCP and remained stable up to 72 hrs post-labelling while maintaining high specific activity.

Figure 7.HER2 binding of ^{225}Ac-crown-F(ab')$_2$. The % of SB was determined by subtracting nonspecific binding (blocked condition) from total binding (unblocked condition) and dividing by total binding. The unblocked samples show much higher counts per minute (CPM) indicating a substantial level of specific binding. Based on this calculation, approximately 97.0% of the cell-associated radiopharmaceutical is specifically bound to HER2, demonstrating successful targeting of the receptor.



CONCLUSION

Pure crown-trastuzumab F(ab')$_2$ were produced and labelled to a high RCP with ^{225}Ac over a specific activity range of 1-10 kBq/µg and exhibited high SB to HER2-positive BC cells.

FUTURE WORK

In-vivo translational bridge studies and in-vitro human serum stability studies will be conducted. Since crown also complexes ^{155}Tb **(3)**, we plan to investigate the labelling of crown-trastuzumab F(ab')$_2$ with ^{155}Tb for theranostic SPECT imaging and α-particle RIT of HER2-positive cancers.

REFERENCES

1. Kondo M et al. Preclinical Comparison of [^{111}In]In- and [^{225}Ac]Ac-DOTA-Trastuzumab IgG, F(ab')$_2$ and Fab for Theranostic SPECT/CT Imaging and α-Particle Radioimmunotherapy of HER2-Positive Human Breast Cancer. *Mol. Pharm*. **2025**;22:474–487.

2. Yang H et al. Synthesis and Evaluation of a Macrocyclic Actinium-225 Chelator, Quality Control and In Vivo Evaluation of ^{225}Ac-crown- αMSH Peptide. *Chem. Eur. J* **2020**;26:11435-11440.

3. Wharton L et al. *Molecules*. Preclinical Evaluation of [$^{155/161}$Tb]Tb-Crown-TATE— A Novel SPECT Imaging Theranostic Agent Targeting Neuroendocrine Tumours. **2023**;28:3155.

ACKNOWLEDGEMENTS

TRIUMF
SFU
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
New Frontiers in Research Fund / Fonds Nouvelles frontières en recherche
UHN Princess Margaret Cancer Centre