203Pb labeled panitumumab for SPECT imaging of EGFR+ head and neck cancer

Nasim Sarrami[1], Bryce Nelson[2], Samantha Leier[2], John Wilson[2], Conrad Chan[3], Jalna Meens[4], Teesha Komal[5], Laurie Ailles[4,6], Raymond Reilly[3], Frank Wuest[2] and Afsaneh Lavasanifar[1*]

1 Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta
2 Department of Oncology, Cross Cancer Institute, University of Alberta
3 Faculty of Pharmacy and Pharmaceutical Sciences, University of Toronto
4 Princess Margaret Cancer Centre, Toronto
5 STTARR Innovation Centre, University Health Network, Toronto
6 Department of Medical Biophysics, University of Toronto
*afsaneh@ualberta.ca

Abstract

Introduction:Radiotheranostics are radiolabelled compounds that target cancer biomarkers such as EGFR with the application of diagnosis and treatment in the cancer field. The objective of this research was to develop a new radiotheranostic by labeling panitumumab with 203Pb and assessment of its potential as an immuno-SPECT probe. Besides 203Pb has a complementary radionuclide (212Pb) which can be substituted with 203Pb and used for therapeutic purposes.

Methods: PSC-NCS reacted with panitumumab then purified by size exclusion chromatography and its composition was confirmed by MALDI. Radiolabeling with 203Pb was done at a pH of 4.5, room temperature for 5 minutes and the incorporation efficiency was measured using radio-TLC. The uptake of 203Pb-panitumumab by FaDu cell line was studied and 203Pb-panitumumab was injected into NRG mice bearing subcutaneous head and neck squamous cell carcinoma PDX (n=5) via vein tail. SPECT/CT images were acquired and for biodistribution studies, mice were euthanized 5 days after injection with 203Pb-panitumumab; γ-photons of tissues were counted via γ-counter and the tissue uptake was calculated. Blocking experiments were performed by pretreating a group of mice (n=5) with 1 mg of unlabeled panitumumab 1 hour before receiving 203Pb-panitumumab.

Results:~5 PSC chelators were attached per antibody and radiolabeling efficiency was 99.2+0.7%. The 203Pb-panitumumab homed and accumulated in the tumor (26% ID%/g) significantly higher than other organs (<6.2 ID%/g).

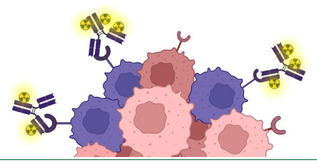
Conclusions: labeling with 203Pb was successful and it retained in EGFR+ tumor; making it a suitable immuno-SPECT probe for imaging EGFR+ tumor.

Introduction

Radiotheranostics are traceable radiolabelled compounds targeting cancer biomarkers for application in cancer diagnosis and treatment (1).

Epidermal growth factor receptor (EGFR) is one of these biomarkers which is overexpressed in many solid tumors. This receptor is overexpressed in >90% of head and neck cancer tumors (2,3).

Recognition of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including the human IgG2 monoclonal antibody panitumumab.



The objective of this research was to develop a new radiotheranostic based on 203Pb-panitumumab and assessment of its potential as an immuno-SPECT probe. Application of 203Pb (t1/2:51.9 hours) as the radiotracer is expected to provide an optimum timeframe for the imaging of solid tumors. Besides 203Pb has a complementary radionuclide (212Pb) which can be substituted with 203Pb and used for therapeutic purposes

Methods

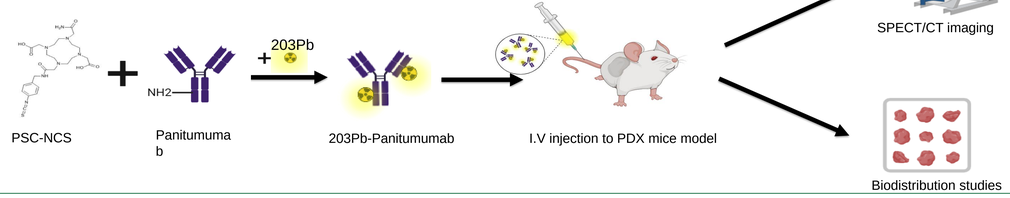
1. Chelator attachment to panitumumab
2'-(4-(2-amino-2-oxoethyl)-10-(2-((4-isothiocyanate benzyl)amino)-2-oxoethyl)-1,4,7,10 tetraazacyclododecane-1,7-diyl) diacetic acid (PSC-NCS) reacted with panitumumab at pH of 8 at room temperature for 2.5 hours. Then, PSC-panitumumab was purified by size exclusion chromatography and its composition was confirmed by matrix-assisted laser desorption/ionization (MALDI).

2. Labeling panitumumab with 203Pb
This step was done at a pH of 4.5 at room temperature for 5 minutes, and the incorporation efficiency of 203Pb into PSC-panitumumab was measured using radio-TLC.

3. *In vitro* studies on 203Pb labeled panitumumab
FaDu cells were treated with 203PB labeled panitumumab and the cell uptake at different time points was determined by counting γ-photons via γ-counter. Also, cell uptake when co-treating with different amounts of cold panitumumab was studied.

4. *Micro SPECT/CT images*
~10MBq in 140µl NaOAc was injected to NRG mice with subcutaneous patient-derived xenograft (PDX) head and neck squamous cell carcinoma (HNSCC) (n=5) via vein tail. SPECT/CT images were acquired 48- and 120 hours post-injection. For blocking studies, mice were injected with 1mg of cold panitumumab one hour prior to receiving 203Pb-panitumumab.

5. Biodistribution studies
For biodistribution studies, mice were euthanized 5 days after they were injected with 203Pb labeled panitumumab, tissues were collected, weighed, and γ-photons were counted via γ-counter. The uptake was calculated as injected dose percentage per gram of each tissue (ID%/g). Blocking experiments were performed by pretreating a group of mice (n=5) with 1 mg of unlabeled panitumumab 1 hour before receiving 203Pb labeled panitumumab. [BioRender object]



Results

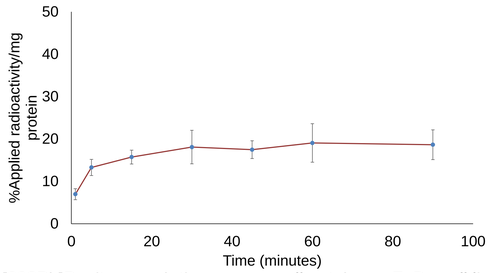


Fig1. [203Pb]Panitumumab time course cell uptake on FaDu cell line at 1,5,15,30,45,60 and 90 minutes time points.

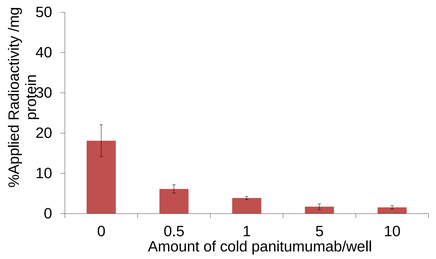


Fig2. [203Pb]Panitumumab blocking cell uptake onFaDu cell line by co-treating cells with different amounts of cold and labeled panitumumab

In Vivo Studies: SPECT/CT images

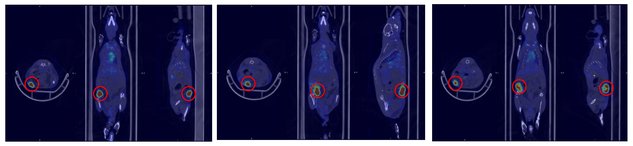


Fig 3. SPECT/CT images of mice injected with [203Pb]Panitumumab after 48h. Tumors are marked with a red circle.

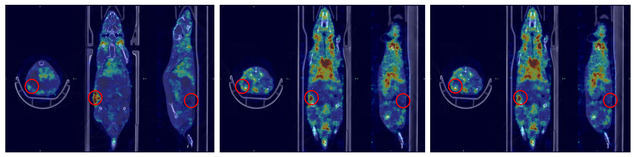


Fig 4. SPECT/CT images of mice injected with cold panitumumab one hour prior to [203Pb]Panitumumab injection after 48h. Tumors are marked with a red circle.

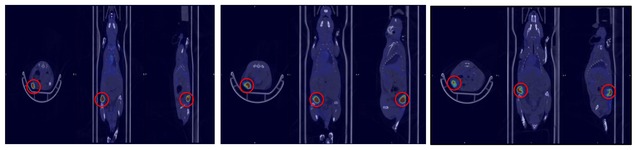


Fig 5. SPECT/CT images of mice injected with [203Pb]Panitumumab after 120h. Tumors are marked with a red circle.

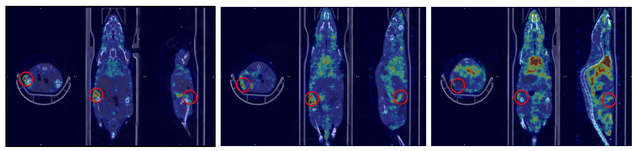


Fig 6. SPECT/CT images of mice injected with cold panitumumab one hour prior to [203Pb]Panitumumab injection after 120h. Tumors are marked with a red circle.

Results

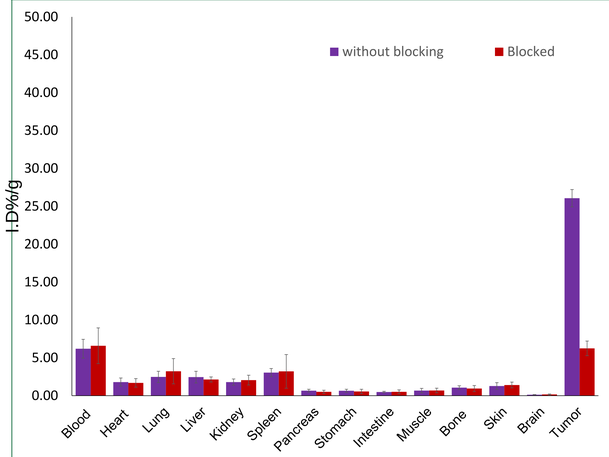


Fig 7. Biodistribution of [203Pb]Panitumumab in mice bearing head and neck PDX model 5 days post-injection.

Conclusions

- Panitumumab was successfully and reproducibly labeled with 203Pb in high radiochemical yields.
- 203Pb labeled panitumumab was specifically accumulated and retained in EGFR+ tumors in NRG mice with s.c HNSCC PDX tumors.
- 203Pb labeled panitumumab is a a suitable immuno-SPECT probe for imaging EGFR+ tumor.

Acknowledgments

Nasim Sarrami acknowledges funding from the Canadian Institution of Health Research (CIHR) and Nano Medicine Innovation Network (NMIN). Alberta Innovates, NSERC-CREATE grant to Polymer Nanoparticles for Drug Delivery (PoND) program and Graduate scholarships from the Faculty of Pharmacy and Pharmaceutical Sciences of the University of Alberta. The authors would like to acknowledge the Spatio-Temporal Targeting and Amplification of Radiation Response (STTARR) program and its affiliated funding agencies; also, New Frontiers in Research Fund (NFRF).

References

1. Gomes Marin JF, Nunes RF, Coutinho AM, Zaniboni EC, Costa LB, Barbosa FG, et al. Theranostics
in Nuclear Medicine: Emerging and Re-emerging Integrated Imaging and Therapies in the Era of Precision
Oncology. RadioGraphics. 2020 Oct;40(6):1715–40.
2. Thomas R, Weihua Z. Rethink of EGFR in Cancer With Its Kinase Independent Function on Board.
Front Oncol. 2019 Aug 23;9:800.
3. Rogers SJ, Harrington KJ, Rhys-Evans P, O-Charoenrat P, Eccles SA. Biological significance of c-erbB
family oncogenes in head and neck cancer. Cancer Metastasis Rev. 2005 Jan;24(1):47–69