# P209. COMPARISON OF TWO FLUORINE-18 LABELED TETRAZINE TRACERS FOR PRETARGETING OF INTERNALIZING ANTIBODIES, 5B1 AND CETUXIMAB

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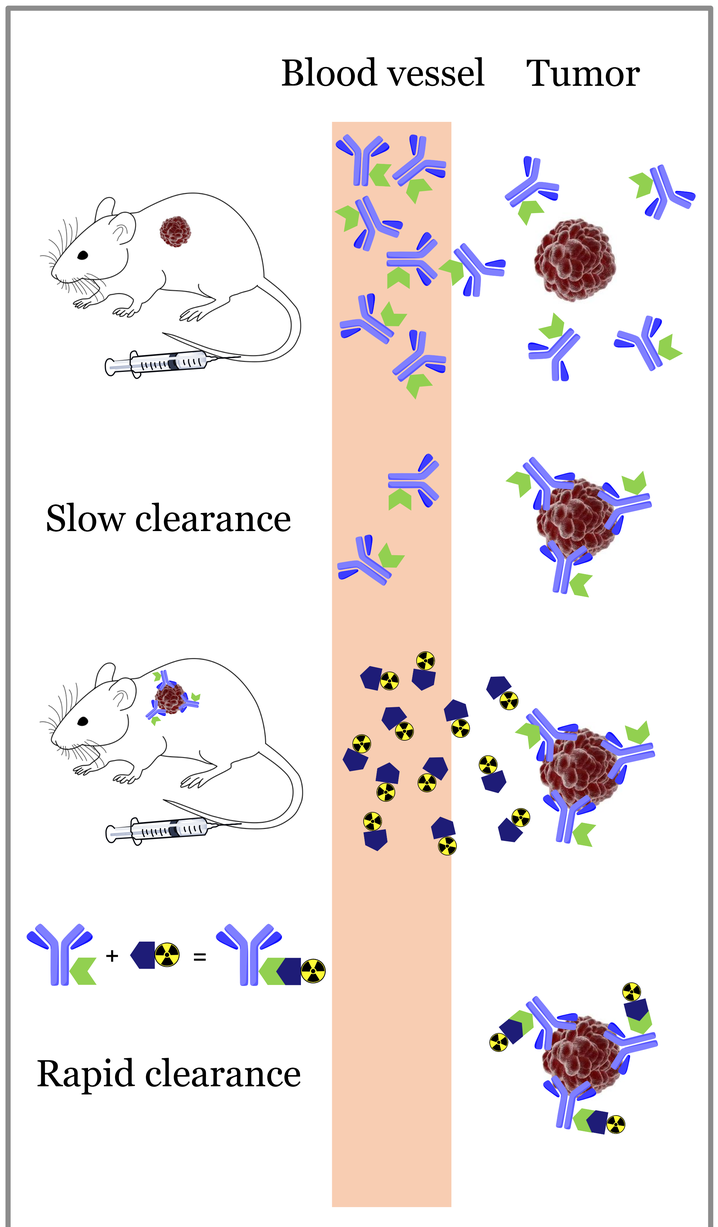
## BACKGROUND

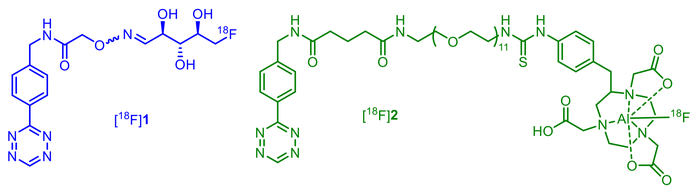
We have used the inverse electron-demand Diels-Alder (IEDDA) reaction for pretargeting two monoclonal antibodies (mAbs), 5B1 and cetuximab, with two structurally different tetrazine radiotracers. While 5B1 and cetuximab share similarities like internalization over time and long blood half-life, the concentration, degree of internalization and recycling rate of the antigens on the cell surface are different. We wanted to explore if these differences affect the pretargeted approach.




## TCO-mAb

200 µg of TCO-5B1 (5.0±0.1 TCO/mAb) or 75 µg of TCO-cetuximab (6.1±0.1 TCO/mAb) was injected into BxPC3 or A431 tumor bearing nude mice 72 h prior the injection of radiotracer.

## TETRAZINES

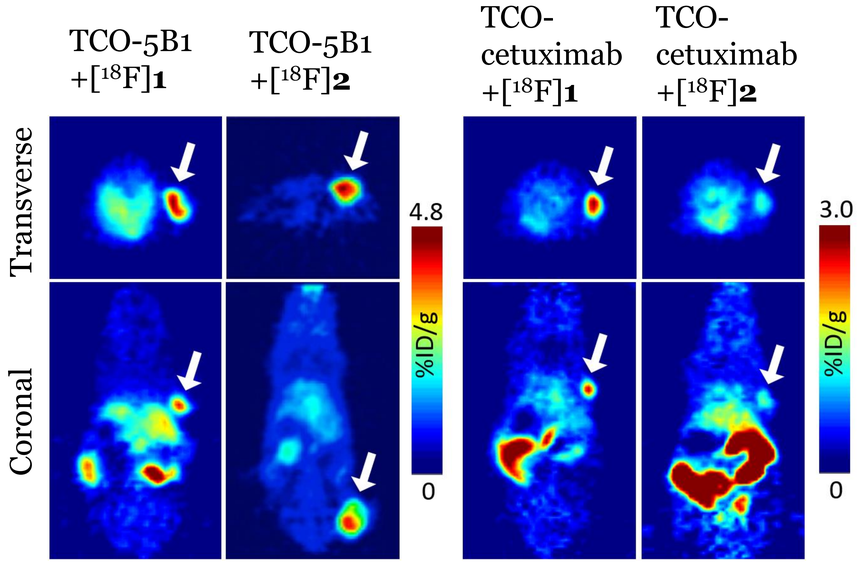



Fluorine-18 labeled tetrazine tracers, [$^{18}$F]**1** and [$^{18}$F]**2** were synthesized as previously described.[1,2] The amount of [$^{18}$F]**1** was adjusted approximately equimolar either to the amount of mAb or TCO. [$^{18}$F]**2** was adjusted approximately equimolar to mAb amount in 5B1 experiments and to TCO amount in cetuximab experiments.

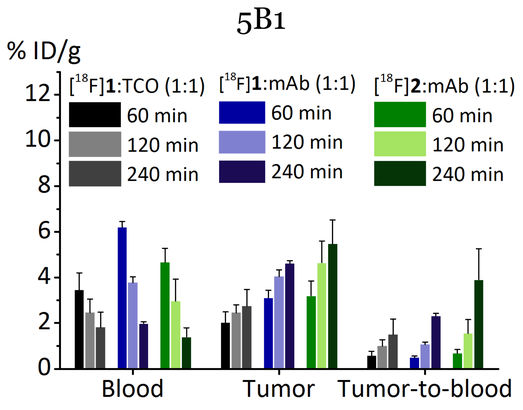
## CONCLUSIONS

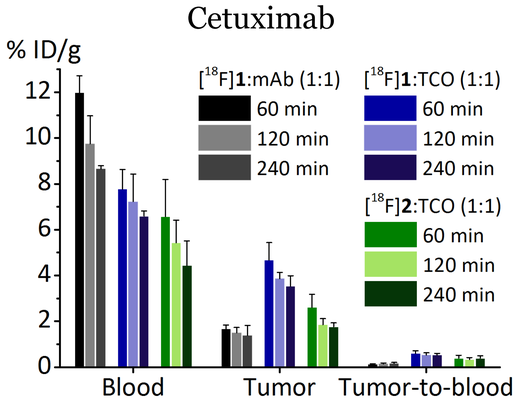
Our results indicate that the optimal molar ratio between the radiotracer and TCO-modified mAb varies depending on the biopharmaceutical properties of the antibody and the success of pretargeted PET imaging is not governed by the pharmacokinetic properties of the radiotracer alone.

## RESULTS




PET images 240 min. after i.v. injection of radiotracer ([$^{18}$F]**1** or [$^{18}$F]**2**).




With 5B1, the radioactivity levels in the blood decreased and the tumor uptake increased during time. This indicates that the tetrazine tracers reacted with the unbound antibody in the blood and then accumulated at the tumor. With cetuximab this was not observed. This is most likely due to different recycling and internalization rates of the antigens at the tumor cell surface. With cetuximab higher tumor uptake was obtained by using ratio [$^{18}$F]**1**:TCO (1:1) than [$^{18}$F]**1**:mAb (1:1). With 5B1 slightly higher tumor uptake was observed using ratio [$^{18}$F]**1**:mAb (1:1) than ratio [$^{18}$F]**1**:TCO (1:1).

## ACKNOWLEDGEMENTS

Supported by the Academy of Finland (278056 and 298481), CHEMS Doctoral Program (University of Helsinki), and Inkeri and Mauri Vänskä Foundation. The MSKCC Small Animal Imaging Core Facility as well as the Radiochemistry and Molecular Imaging Probe core, which were supported in part by NIH grant P30 CA08748. Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research and The Center for Experimental Therapeutics at Memorial Sloan Kettering Cancer Center is also gratefully acknowledged. NIH Shared Instrumentation Grant No 1 S10 RR020892-01, which provided funding support for the purchase of the Focus 120 microPET, is gratefully acknowledged.

## REFERENCES

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