Title of the report: PET of EGFR with $^{64}\text{Cu}$-cetuximab-$\text{F(ab')}_{2}$ in UT-SCC xenografted mice

HOST INSTITUTE:
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Patient selection is of great clinical relevance. Imaging expression of the epidermal growth factor (EGFR) in head and neck squamous cell carcinomas (HNSCC) could aid in stratification of patients for treatment with radiotherapy and/or EGFR-inhibitors like cetuximab. $^{111}$In-cetuximab-F(ab')$_2$, a SPECT tracer, visualizes in vivo accessibility of EGFR. It has shown a differential uptake in multiple head and neck xenografts, each with varying amounts of EGFR expression and differential response to treatment with radiotherapy +/- cetuximab. After development of the SPECT tracer, it was of interest to develop a PET tracer, thereby creating a potentially clinically relevant agent to predict and/or monitor treatment.

The Turku PET Center has a large database of patient derived squamous cell carcinoma biopsies (UT-SCC) of which their expertise is extremely useful. The University of Turku also has a stable and continuous production of Copper-64 (64Cu), enabling the labelling of our protein with a PET radionuclide. For cetuximab-F(ab')$_2$ to be labelled to 64Cu, the protein fragment was conjugated to NODAGA. At the Radiochemistry department, I got to see the accelerator and familiarized myself with the method of collection after the production of 64Cu, thereby for me personally completing the process from radionuclide generation to tracer application. After collection, the tracer was labelled to 64Cu and radiochemical purity was investigated by instant thin layer chromatography (ITLC). It superseded 98% in all experiments. Specific activity of the tracer was high at 5 TBq/mmol. The tracer proved stable in mouse and human plasma (86% and 66% after 26 h, respectively).

The tracer was subjected to in vitro and in vivo experiments, using two head and neck xenografts lines, UT-SCC 45 and UT-SCC 8. From previous experiments, it is known that UT-SCC 8 is sensitive to cetuximab treatment, while UT-SCC 45 is cetuximab resistant.

Cells were cultured and subcutaneously injected in the right hind leg of nude mice. After tumor formation, mice were injected with 22 ± 1.4 MBq of 64Cu-cetuximab-F(ab')$_2$ (15µg/250µl/mouse) and scanned 24 h post injection. Images showed a higher uptake in UT-SCC 8 tumours compared to UT-SCC 45 tumours, which was effectively inhibited in mice receiving a blocking dose of cetuximab 3 days prior to tracer injection. SUVmax was 1.5 ± 0.5 for UT-SCC 8 and 0.7 ± 0.1 for UT-SCC 8, respectively.

Directly after PET scans, tumours were harvested and the bio-distribution was evaluated by gamma counter. Uptake in kidneys and liver was elevated (7% of injected dose per gram tissue) compared to other organs due to clearance kinetics. Tumours were frozen and cut for autoradiography analysis to evaluate intra-tumoral distribution of the tracer. This proved heterogeneous for both UT-SCC xenografts.
The acquired knowledge related to the labelling of cetuximab-F(\(ab\')_2\) with positron-emitting radionuclide Copper-64, builds on the development of (PET) tracers being applicable as markers for prediction/early response monitoring in treatment of patients with radiotherapy combined with EGFR-inhibitors. The results of these experiments will add to existing knowledge, combining the two disciplines of radiation oncology and nuclear medicine.

I thoroughly enjoyed my stay at the departments of the Turku University and would like to thank all the people I met for their friendship and helpfulness. Special thanks go out to Dr. Cheng-Bin Yim and Dr. Tove Grönroos for guiding me and making this such a successful visit.