Evaluation of $^{111}In/^{177}Lu$ antibody fragments for diagnostic and therapeutic purpose

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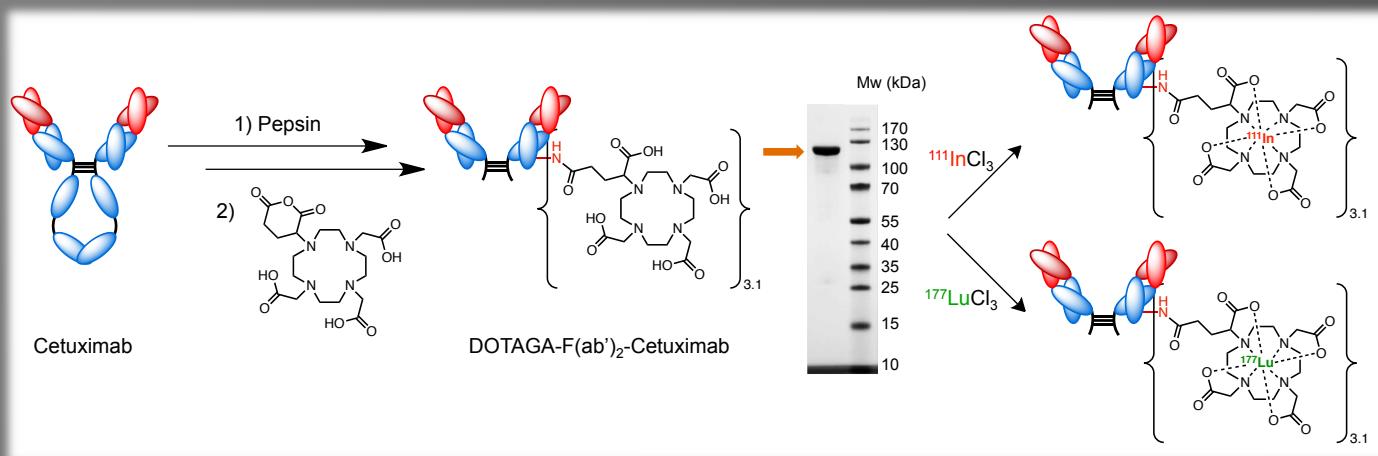
Introduction

The Epidermal Growth Factor Receptor (EGFR) has evolved over years into a main molecular target for the treatment of different cancer entities. This receptor is often overexpressed in various malignancies such as Head and Neck Squamous Cell Carcinoma (HNSCC), gastrointestinal / abdominal carcinomas, lung carcinomas, carcinomas of the reproductive tract, melanomas, glioblastomas and thyroid carcinomas [1]. In the era of personalized medicine, theranostic strategy based on monoclonal antibodies and their deriving structures could represent an exciting approach. Given their pharmacokinetic properties, $F(ab')_2$ (110 kDa) could be valuable theranostic agents. Indeed, they are bivalent (as the native monoclonal antibody), less immunogenic, have a shorter blood clearance, have higher tumor to background ratios and reduced non-specific distribution [2].

In this work, our aims were to design $F(ab')_2$-cetuximab-based theranostic agent and to assess it in preclinical EGFR-overexpressing cancer models. The first part focused on the two types of emission of ^{177}Lu (β- and γ) which enabled us to evaluate ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab dose escalation in epidermoid carcinoma tumor-bearing mice. Then, we made the proof of concept that ^{111}In-DOTAGA-F(ab')2-cetuximab was suitable for monitoring the down-regulation of EGFR as a biomarker of the efficacy of a targeted therapy with a HSP90 inhibitor in colorectal tumor-bearing mice.

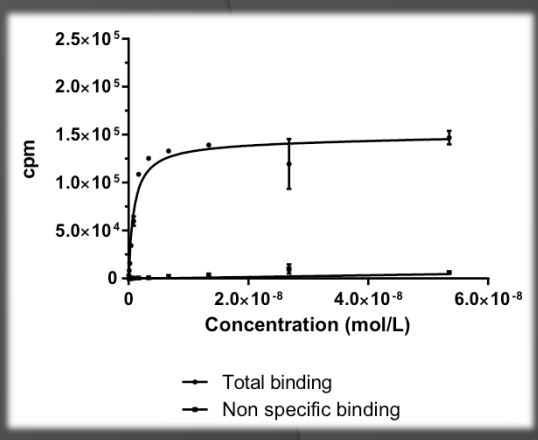
Digestion, bioconjugation and radiolabeling

The $F(ab')_2$-cetuximab was produced by pepsin digestion. Purity was assessed by SDS-PAGE gel electrophoresis. The fragment was then conjugated to DOTAGA anhydride (3.1 DOTAGA/$F(ab')_2$) following previously described method [3], prior to radiolabeling with ^{111}In or ^{177}Lu (540 MBq/mg and 850 MBq/mg respectively).



In vitro validation

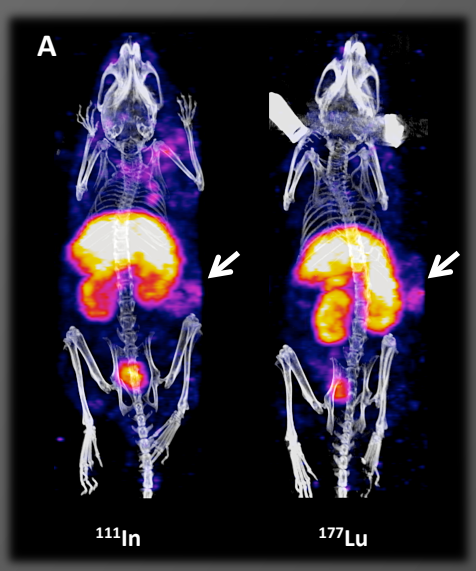
Biological activity of ^{111}In-DOTAGA-$F(ab')_2$-cetuximab was maintained: the affinity (Kd) determined on EGFR-overexpressing A-431 cells, was close to 1 nM, and immunoreactivity was ~50%.



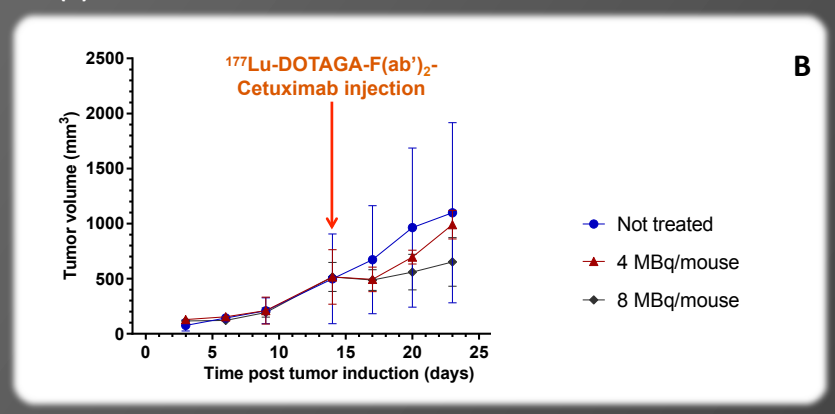
^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab dose escalation

In this first experiment, we studied the tolerance of SWISS Nude mice xenografted with A-431 cells (epidermoid carcinoma) to ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab RIT.
2 groups of 4 mice received a single injection IV of 4 and 8 MBq of ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab (20-25 µg) 14 days after tumor induction (vol. ≈ 500 mm^3).

4 mice injected either with ^{111}In- or ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab (8-10 MBq, 20-25 µg) were imaged 24h p.i. using a NanoSPECT/CT® small animal imaging tomographic γ-camera (Bioscan Inc). Despite the high uptake in liver and kidneys, both tumor was clearly observed regardless of the radionuclide used. (A)



Subcutaneous A-431 human epidermoid carcinoma imaging by SPECT/CT: representative MIP 24h post-injection of ^{111}In- (left) or ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab. The tumor is indicated by an arrow.



- No weight loss was observed during the study → the ^{177}Lu-radioimmunoconjugate is well tolerated by the mice.
- Tumor growth was slowed down in groups receiving the ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab. (B)
- But high kidneys and liver uptake → limitation for a treatment ?

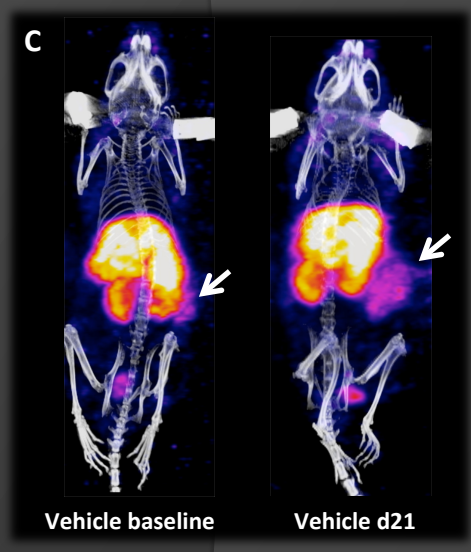
Monitoring of HSP90 inhibitor treatment by SPECT-CT imaging

In this second experiment, we made the proof of concept that ^{111}In-DOTAGA-$F(ab')_2$-cetuximab was suitable for monitoring the down-regulation of EGFR as a biomarker of the efficacy of a targeted therapy with a HSP90 inhibitor in colorectal tumor-bearing mice.

Balb/c Nude mice were xenografted with CR-LRB-014P tumor (primary model of human colon cancer overexpressing EGFR). 30 days after tumor induction (vol. ≈ 180 mm^3), mice were seperated in 2 groups as follow:

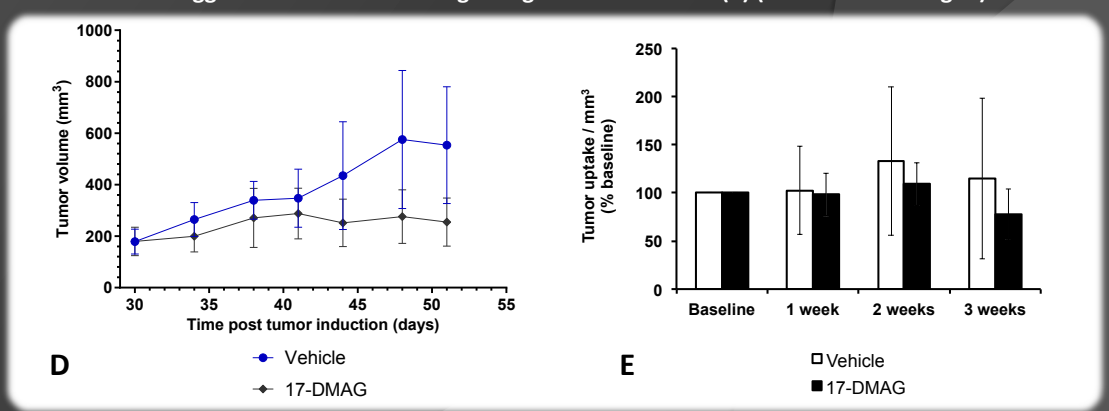
- Vehicle: 10 mice received an IP injection of a vehicle three times a week during 3 weeks.
- 17-DMAG: 10 mice received an IP injection of 17-DMAG (geldanamicin derivative, HSP-90 inhibitor) three times a week during 3 weeks.

4 mice per group were selected and injected with ^{111}In-DOTAGA-$F(ab')_2$-cetuximab (3-12 MBq, 20-25 µg) before the first day of treatment (baseline), and 7, 14, and 21 days after the beginning of the treatment. Those mice were imaged 24h p.i.



Subcutaneous CR-LRB-014P human colon tumor imaging by SPECT/CT: representative MIP 24h post-injection of ^{111}In-DOTAGA-$F(ab')_2$-cetuximab before (left) and 21 days (right) after the beginning of the treatment with vehicle (same mouse).

17-DMAG treatment induced a weight loss (~2 g), but tumor growth was significantly slowed down (x3 for mice injected with vehicle vs. x~1.5 for 17-DMAG treated mice at 3 weeks) (D). In the same manner, tumor uptake determined by image analysis increased for mice injected with vehicle, whereas tumor uptake of mice treated with 17-DMAG decreased by ~25 % (E). SPECT-CT images reflects this evolution for vehicle group as tumor looks much bigger 3 weeks after the beginning of the treatment (C) (same mouse imaged).



Conclusion and perspectives

- ^{177}Lu-DOTAGA-$F(ab')_2$-cetuximab showed interesting results for RIT as it is well tolerated by mice and inhibits tumor growth. However, similar studies have to be done with higher dose to increase the inhibition of tumor growth.
- ^{111}In-DOTAGA-$F(ab')_2$-cetuximab is a promising tool for the evaluation of EGFR downregulation by HSP90 inhibitors by nuclear imaging.
- Other cetuximab derivatives should be investigated in order to reduce kidneys and liver uptake as it may be an issue for treatment by radioimmunotherapy.

References

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[3] M. Moreau *et al.*, *Bioconjugate Chemistry* 2012, *23*, 1181-1188.

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