Introduction

Recently, new strategies emerged in the field of monoclonal antibodies radiolabeling for PET imaging with the use of positron emitters such as zirconium-89 or gallium-68. Despite their important role in the therapeutic world, antibodies have many disadvantages related to their structure. Moreover, conjugation of chelating agent often occurs on lysines, which is non-regioselective and leads to a heterogeneous mixture of products. In addition, the slow clearance of antibodies can be a problem to obtain a good contrast when they are used in imaging.

To address these different limitations, we developed a chemistry-free chelating system consisting of a phosphorylatable peptide tag. A specific phosphorylation step can generate a nanocluster of phosphate moieties that can interact strongly with metal ions like zirconium\(^{[1]}\). We used a peptide sequence which has been selected for its capacity to chelate lanthanide ions such as terbium(III) to optimize this peptide tag and fuse it genetically to a Nanofitin, a protein scaffold developed as an alternative to antibodies, to ensure an efficient targeting of the radionuclide.

Objectives:
1) Adapt the labeling tag to the stereoselective chelation of gallium-68 for PET imaging.
2) Validate the use of Nanofitin as a potent alternative tool for in vivo imaging.

Chelation with gallium

Method:

\[
\text{LBT} \times \text{NF} + \text{Ga} \xrightarrow{\text{phosphorylation}} \text{NF} \text{tagged} + \text{Ga}^{3+} \text{complex}
\]

To increase the affinity for radionuclide, we worked on a sequence derived from calcium-binding proteins to chelate specifically lanthanides\(^{[2]}\). We optimized this sequence by incorporating a phosphate nanocluster to improve the chelation with radionuclides\(^{[3]}\):
- Affinity for terbium(III) is in the sub-micromolar range for the lanthanide-binding tag fused to the Nanofitin and in the micromolar range for the mono-phosphorylated.
- Chelation of zirconium and gallium by the peptide tag was observed by a competition study.

Labelling with fluorine-18

Method:

1) Automatic synthesis of \(^{18}\text{F}\)-FBEM and radiolabeling of Nanofitin NF2 (Dammicco S. et al.)

2) Injection of the Nanofitin radiolabeled in both mice and PET/MRI imaging

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Conclusions and perspectives

We succeeded to generate a phosphorylatable tag able to chelate terbium(III). Through competition studies, we have shown evidence for a capacity of chelation of zirconium(V) and gallium(III). Radiolabeling studies with gallium-68 are on going to evaluate the powerfullness of such a strategy for the chelation of radionuclides. We have also obtained an hypothetic ADME profile of the Nanofitin NF2 and we are currently making use of its specific binding to a cell-surface receptor to target a very precise cell population by using a new animal model. Once the phosphorylatable tag optimized for regeselective radiolabelling and the Nanofitin targeting validated in an animal model, the next steps will be to combine these two approaches: we will fuse genetically the tag to the specific Nanofitin, radiolabel it with gallium-68 and perform the biokinetic study of this new radiopharmaceutical product.