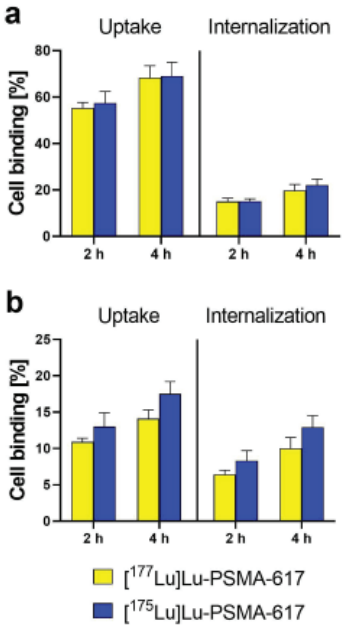
O-66 - Preparation of 211At-labeled aromatic compounds from thioarylprecursors

Clémence Maingueneau, Romain Eychenne, Jean-François Gestin, François Guérard

For proof-of-concept we employed [^{175}Lu]Lu-PSMA-617 and [^{177}Lu]Lu-PSMA-617, respectively.

Methods: For the *in vitro* cellular experiments, PSMA-617 was quantitatively complexed with the stable isotope lutetium-175. In addition, radiolabeling of PSMA-617 with lutetium-177 at a molar activity of 10 MBq/nmol was performed. Cellular uptake and internalization as well as the cellular K_D of [^{175}Lu]Lu-PSMA-617 and [^{177}Lu]Lu-PSMA-617 were investigated using PSMA-positive LNCaP and PC-3 PIP cells. All the experiments were carried out in parallel to allow a direct comparison of the two methods. The ICP-MS instrument used in this work was a triple quadrupole ICP-MS (iCAP TQ, ThermoFisher). For analysis of cell samples by ICP-MS, microwave assisted digestion of PC-3 and LNCaP cells in the presence of 69% nitric acid was used.

Results: Comparison of the uptake and internalization of the two ligands, [^{175}Lu]Lu-PSMA-617 and [^{177}Lu]Lu-PSMA-617, in PC-3 and LNCaP cells revealed, that the two methods give comparable results as shown in Figure 1. The results are shown as uptake of the ligands in percent of the total added dose. Uptake of [^{177}Lu]Lu-PSMA-617 in PC-3 PIP (Figure 1a) cells was 55.3 ± 2.4% after 2 h, and 68.3 ± 5.2% after 4 h incubation, whereas it was 57.4 ± 5.0% and 69.0 ± 6.0% for the [^{175}Lu]Lu-PSMA-617. In LNCaP cells (Figure 1b), uptake of [^{177}Lu]Lu-PSMA-617 was 10.9 ± 0.5% after 2 h and 14.1 ± 1.2% after 4 h incubation. In comparison to the radioligand, uptake of ^{175}Lu-labeled PSMA-617 was slightly higher with 13.0 ± 1.9% and 17.5 ± 1.7% uptake after 2 h and 4 h incubation, respectively. The cellular KD in PC-3 PIP cells was determined for [^{177}Lu]Lu-PSMA-617 to be 11.4-18.4 nM (95% CI), whereas it was 13.4-25.6 nM (95% CI) for [^{175}Lu]Lu-PSMA-617.



Conclusions: A robust and accurate method for quantification of lutetium-175 in *in vitro* cellular binding assays was established. Our study has proven that high sensitivity ICP-MS is a valid alternative for the characterization of metal-conjugates in conventional research laboratories with no access to radioactivity. This complements and supports the development of potential radiometal-based theranostic candidates. It enables a large-throughput pre-screen of metal-conjugates in *in vitro* experiments to select the best ones for future radiolabeling and therapy experiments.

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O-66

Preparation of ^{211}At-labeled aromatic compounds from thioarylprecursors

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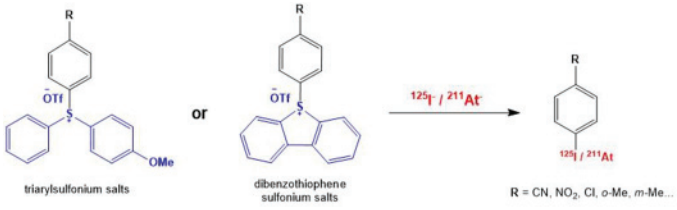
Introduction: Astatine-211 is one of the most promising alpha emitter for targeted alpha therapy of small tumors, disseminated metastases or isolated cancer cells.[1] Its development has long been hampered by a low availability and the absence of stable isotope, limiting the possibilities to investigate its chemical properties. However, recent years have seen a growing interest for this radionuclide and modern chemical investigations have led to a better understanding of this element.[2]

Early ^{211}At-labeling investigations have focused on electrophilic approaches (using At^+) such as halodeprotonation or halodemetallation. In particular, astatodestannylation has been by far the most used method in the past decades for the ^{211}At-labeling of small molecules or proteins.[3] More recently, we focused our attention on the development of nucleophilic strategies based on the At^- species, presented as much more stable than At^+, and we discovered that aryliodonium salts were highly efficient precursors.[4] Despite an improved reproducibility and efficiency as well as a simplified purification compared to electrophilic methods, these precursors are not optimal for electron rich compounds. In addition, being based on trivalent aryliodide species, the release as side product of the reaction of inseparable non-radioactive iodinated analogue of the astatinated product limits the molar activity achievable. We, and others also investigated the halodeboronation of boronic acids and esters that demonstrated a high efficiency in mild conditions, but a copper catalyst is required.[5,6]

Objectives: This work aiming at exploring the reactivity of arylsulfonium salts is part of the idea to extend the range of available precursors for astatination. These derivatives were recently reported for [^{18}F]Fluorination at an (hetero)arylposition.[7] In addition to a high thermal and chemical stability, the absence of iodine atom in the starting precursor appears as an advantage over aryliodonium salts regarding molar activity of ^{211}At-labeled compounds.

Results and Discussion: A set of triarylsulfonium and dibenzothiophene sulfonium salts were prepared with various substituents from electron withdrawing (activating) to electron donating groups. Temperature, solvent and addition of bases and/or kryptans were investigated showing that RCY up to 99% were achievable in the most favorable cases. As expected, dibenzothiophene lead to better regioselectivity of substitution compared to trialysulfonium. Interestingly, ^{211}At-labeling was far more efficient than radioiodination, confirming the superiority of astatide in this type of reaction, as already observed with aryliodonium salts.



Conclusion: Overall, these preliminary results warrant the study of functionalized precursors for the preparation of ^{211}At-labeled compounds of interest for targeted alpha therapy.

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Scientific Session 17: Other Fields

O-67
Evaluation of Affibody molecule Z09591 for PET imaging in fibrosis

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Background and aims: Fibrosis, the excessive deposition of collagen/ extracellular matrix, is an important feature in Metabolic Associated Fatty Liver Disease (MAFLD), including Non-Alcoholic SteatoHepatitis (NASH). Activated hepatic stellate cells (HSCs), a key mediator of collagen production in the liver, express Platelet-derived growth factor receptor b (PDGFRβ) on the cellular surface. The treatment and monitoring of hepatic fibrosis is hampered by the lack of suitable methods for detecting and diagnosing the disease. Therefore, it is of crucial importance to develop non-invasive methods in order to diagnose and stage fibrosis grade in patients. Herein, we report fluoride-18-labeled Affibody molecule Z09591, a novel PET probe for imaging of PDGFRβ and activated HSCs in liver fibrosis.

Method: Affibody molecule Z09591, which binds to human PDGFRβ with picomolar affinity, was labelled by fluoride-18-tetrazine to a trans-cyclooctene group at the C terminal cysteine. Binding of 6-[18F]Fluoro-N-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzyl)nicotinamide-Z09591 (^{18}F-TZ-Z09591) was investigated in hepatic tissue sections from human individuals diagnosed with MAFLD/ NASH. In vivo binding of ^{18}F-TZ-Z09591 to fibrotic lesions was evaluated in a CCl_4 mouse model of liver fibrosis vs controls, where hepatic update of the tracer was evaluated by *ex vivo* biodistribution and autoradiography and correlated to *post mortem* histology. Specificity of binding was evaluated by pretreatment with 1mg/kg unlabeled Z09591 Affibody molecule.

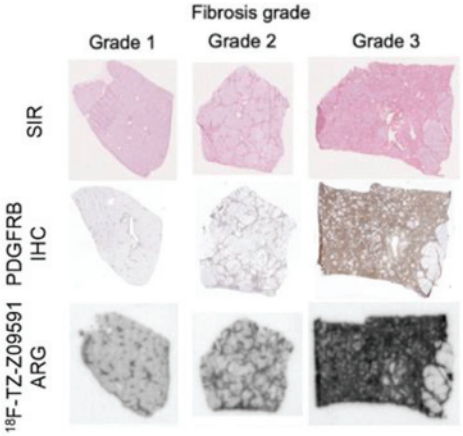

Figure 1. Human liver biopsies stained with Sirius RED (SIR), or with an antibody anti-PDGFRB, and autoradiography using ^{18}F-TZ-Z09591.

Results: Affibody molecule Z09591 was successfully radiolabelled with fluoride-18 with high purity and reproducible yield. *In vitro* autoradiography of human liver sections demonstrated high signal intensity in PDGFRβ positive hepatic sinusoids affected by fibrosis, as confirmed by Sirius red staining and IHC (Figure 1). Preclinical PET studies demonstrated that liver uptake of ^{18}F-TZ-Z09591 was higher in mice with induced hepatic fibrosis compare to healthy liver. The liver binding could also be blocked by pretreatment with Z09591 in excess, indicating receptor specific binding.

Conclusion: PDGFRβ targeting Affibody molecule ^{18}F-TZ-Z09591 is a promising tool for PET imaging of fibrotic lesions in MAFLD.

O-68
Development and evaluation of new small molecule PET-radiotracers for the molecular imaging of inflammatory processes targeting S100A9

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Objectives: Released during tissue-damage and cellular stress or phagocyte activation, the alarmin heterocomplex S100A8/A9 is involved as an early amplifier of inflammatory processes. Its local and massive secretion by phagocytes at the site of inflammation makes S100A8/A9 a promising biomarker for inflammatory disorders associated with phagocyte activation.(1) In inflammation imaging, S100A9 has been successfully targeted by a fluorescent probe, based on the S100A9 inhibitor Paquinimod.(2,3) Since the underlying quinoline carboxamide structure is characterized by a complex synthesis and thermal instability the new lead structure based on 2-amino benzimidazoles can overcome these drawbacks featuring an efficient synthesis, structural variability and thermally robust core structure with high potential for PET-imaging.(4)

Methods: Based on the 2-amino benzimidazole lead structure, an optical reference compound Cy5.5-**SST110** and three PET-tracers applying an aromatic substitution reaction at 160°C for [^{18}F]**SST034** and "click-chemistry" with [^{18}F]Fluoroethyl azide as a prostetic group for tracers [^{18}F]**SST096** and [^{18}F]**SST120** were established (Figure). Cy5.5-**SST110** serves as a reference in a newly established S100A9 Fluorescence Polarization (FP) binding assay. For an *in vivo* proof of concept, the fluorescent probe was applied in an irritant contact dermatitis (ICD) mouse model and the biodistribution behavior of all three radiotracers were investigated.