Radiosynthesis of the first [^{18}F]-Nanocyclix® TKI-PET radiotracer targeting activated EGFR positive lung tumors

Dr. C. Mothes[1,2,3], Dr. G. Viot[3], Dr. C. Vala[1,3], Dr. O. Raguin[2,4], Dr. P. Provent[4], Prof. D. Guilloteau[1,5], Dr. J. Hoflack[4], Dr. C. Berthet[2,4], Dr. P. Blom[4], Dr. J. Vercouillie[1,5]

[1]U930, CERRP - Tours, France, [2]Pharmimage - Dijon, France, [3]Laboratoires Cyclopharma - Saint Beauzire, France, [4]Oncodesign - Dijon, France, [5]Tours Hospital - Tours, France

Introduction

IMA*kinib*® program is an innovative approach to develop new Tyrosine Kinase Inhibitors (TKIs) as potential radiotracers for positron emission tomography (PET) imaging. Nanocyclix® technology allow to provide potent and selective macrocyclic compounds for this program.

The epidermal growth factor receptor (EGFR) is an established target for the treatment of advanced non-small cell lung cancer (NSCLC). Four TKIs targeting EGFR have already been approved for treatment of NSCLC: Gefitinib (Iressa®), Erlotinib (Tarceva®) Afatinib (Giotrif®) and Osimertinib (Tagrisso®). Unfortunately, the majority of patients develop a resistance to the TKI in the long term (6-12 months) which is for most of them (> 50%) related to an acquired T790M mutation of EGFR. Thus, **PET imaging with radiolabeled TKIs can provide a diagnostic tool to determine and predict the activity of EGFR and the responsiveness to EGFR TKI.**

Starting from Nanocyclix® library of kinase inhibitors, a new compound targeting specifically EGFR mutated, **ODS2004436** (Fig. 1), was selected for its biological *in vitro* characteristics and its favorable metabolism, to be radiolabeled with fluorine-18 **([^{18}F]-ODS2004436)** and then, evaluated *in vivo*. **In order to determine if [^{18}F]-ODS2004436 is a good candidate to predict the activity of EGFR, correlated with its mutational status, the objective of this work was to developed a fully automated radiosynthesis of [^{18}F]-ODS2004436 for preclinical and clinical PET studies, on two commercial radiosynthesis modules (GE Tracerlab FX N Pro and Trasis AllinOne (AIO)).**

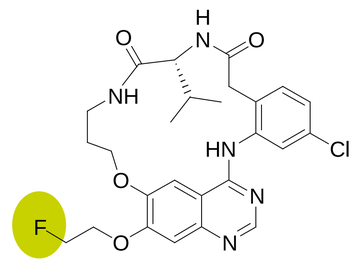


Fig. 1: ODS2004436

Materials & Methods

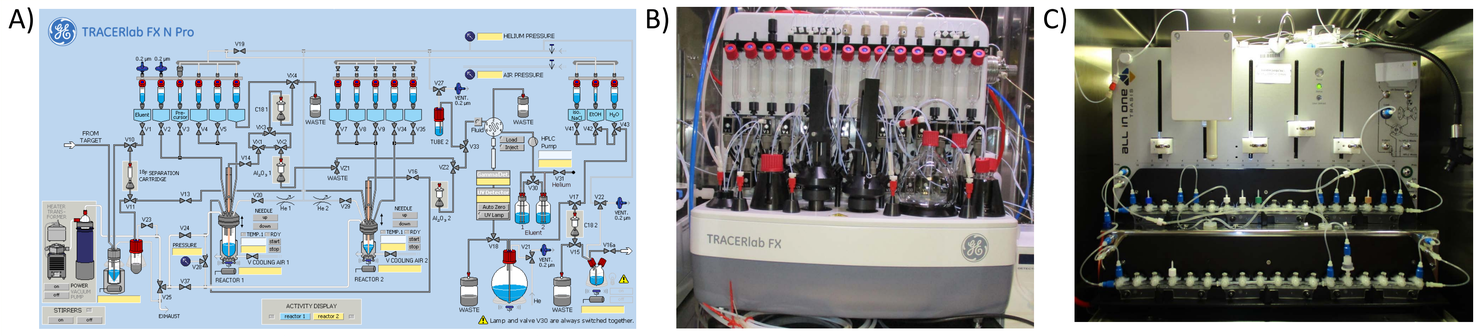


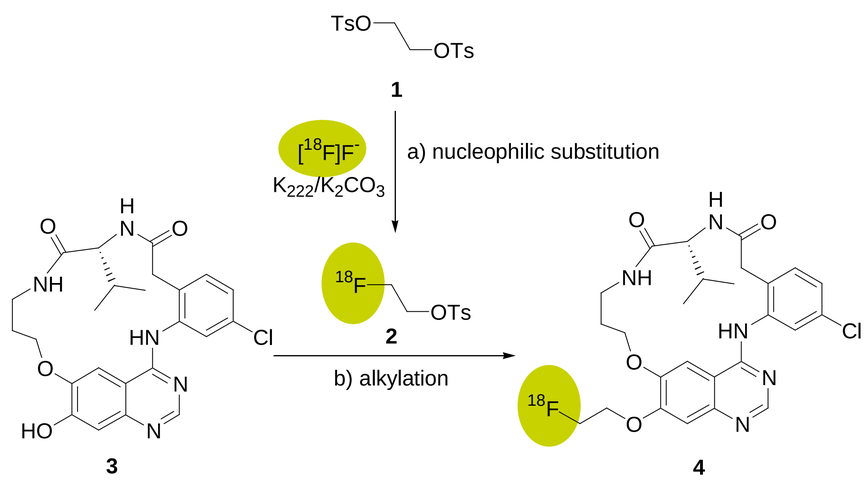
Fig. 2: A) FX-FN-Pro layout; B) Module Tracerlab; C) Module AIO with disposable cassette

In order to evaluate [^{18}F]-ODS2004436 in preclinical imaging studies, an automated synthesis was first developed on a GE Tracerlab FX N Pro module regarding its flexibility (Fig2B). This module with two reactor heaters was used in its basic configuration without modifications (Fig2A). Due to the encouraging preclinical results, the automation of [^{18}F]-ODS2004436 was rapidly considered on Trasis AIO module for further clinical application, by using single use cassettes regarding pharmaceutical processes (Fig2C).

Automation process

The whole process to prepare [^{18}F]-ODS2004436 on the both platforms (GE FX N Pro and Trasis AIO) can be divided in four parts common steps:

1) **Activation of fluoride ion ([^{18}F]F$^-$):** [^{18}F] Fluoride ion was produced with cyclotron by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. The [^{18}F] aqueous fluoride solution was transferred to synthesizer module and was passed through an a QMA cartridge. [^{18}F] fluoride ions were eluted with a mixture of K_2CO_3/K222 in water/acetonitrile in the first reactor. An azeotropic drying procedure was performed before the labelling steps.
2) **Radiofluorination:** The radiolabelling of [^{18}F]-ODS2004436 involves two steps (Scheme 1):
   a) In the first reactor, preparation of the labelling agent (2-[^{18}F]fluoroethyltosylate [^{18}F]-FETos **2** *via* nucleophilic substitution of ethylene ditosylate **1** (10 mg) in acetonitrile,
   b) In the second reactor, *O*-[^{18}F]-fluoroethylation of precursor **3** (3 mg) with [^{18}F]-FETos **2** in DMSO.
3) **HPLC purification.**
4) **Formulation** *via* solid phase extraction (SPE).



Scheme 1: Radiosynthesis of [^{18}F]-ODS2004436

Results

GE Tracerlab FX N Pro

- **Radiochemical yields: 5-25% decay corrected (n = 20)**
- Total synthesis time: 110 min (synthesis, purification and formulation)
- Injectable solution: 12.5% EtOH in 0.9% NaCl

- **Radiochemical purity: > 98%**
- Specific activity: 70-150 GBq/µmol at End Of Synthesis (EOS)

➢ [^{18}F]-ODS2004436 was prepared on the GE Tracerlab in sufficient quantity (0.6-2.8 GBq) to perform *in vitro* and animal studies .

Trasis AIO

- **Radiochemical yields: 10-25% decay corrected (n = 45)**
- Total synthesis time: 90 min (synthesis, purification and formulation)
- Injectable solution with sterile filtration: 10% EtOH in 0.9% NaCl

- **Radiochemical purity: > 98%**
- **Enantiomeric purity: > 98%**
- Specific activity: > 500 GBq/µmol at EOS
- Stability: 8h

➢ [^{18}F]-ODS2004436 (> 10 GBq per batch) was synthesized on the AIO with high reproducibility.
➢ Compliance with any QC testing required for human injection.
➢ (R)- and (S)-enantiomers can be prepared according to the same procedure with high enantiomeric purity (starting from (R)- or (S)-precursor).

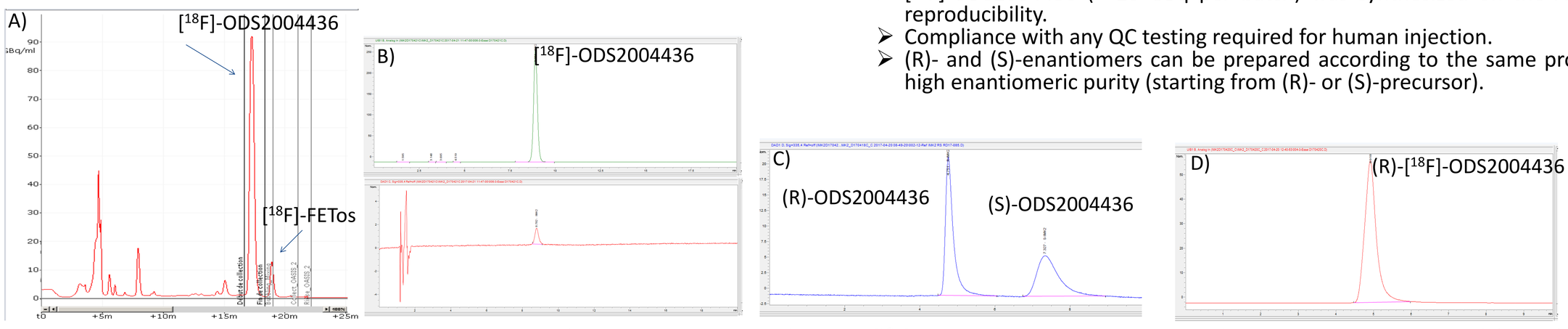


Fig. 3: A) Semi-preparative radio-HPLC with a C18 column; B) Analytical radio-HPLC of isolated [^{18}F]-ODS2004436 and UV at 335 nm; C) Analytical chiral HPLC with (R)- and (S)-enantiomers mixture; D) Analytical chiral radio-HPLC of isolated (R)-[^{18}F]-ODS2004436

Conclusions

➢ A fully automated production of [^{18}F]-ODS2004436 was proposed on two commercial platforms : GE Tracerlab FX N Pro and Trasis AIO.
➢ Reproducible and reliable production was performed on AIO in a GMP environment (yields = 18%, synthesis time = 90 min, radiochemical and enantiomeric purity > 98%).
➢ **Currently clinical evaluation of this novel radiotracer is ongoing** (first in-man phase 0/1 clinical trial NCT02847377).

Acknowledgements

This work was partly supported by a grant from BPI France and the French Government