Preclinical proof of concept for the first [18F]-Nanocyclix® TKI-PET radiotracer targeting activated EGFR positive lung tumors

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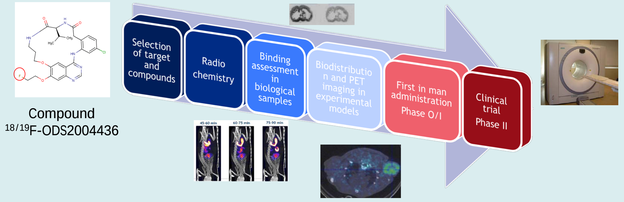
INTRODUCTION

Concept of IMAkinib® project

Flowchart of radiotracer development

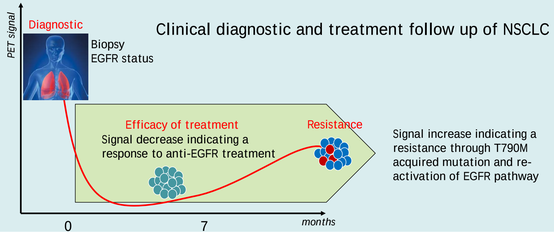


Compound
$^{18/19}$F-ODS2004436

IMAkinib® program is an **innovative approach, based on Nanocyclix® chemistry technology**, which aims to develop new Tyrosine Kinase Inhibitors (TKIs) radiotracers used for Positron Emission Tomography (PET).

The epidermal growth factor receptor (EGFR) is an **established target for the treatment of advanced non-small cell lung cancer (NSCLC)**. TKIs targeting EGFR are standard treatment of tumors harboring EGFR mutation (ie: L858R), unfortunately, the majority of patients develop a **resistance to the TKI within 1 year**, which is for most of them (>50%) related to an acquired T790M mutation of EGFR.

TKI PET-imaging can provide a diagnostic tool to determine and predict the activity of EGFR and the responsiveness to EGFR TKI.



Clinical diagnostic and treatment follow up of NSCLC

Signal increase indicating a resistance through T790M acquired mutation and re-activation of EGFR pathway

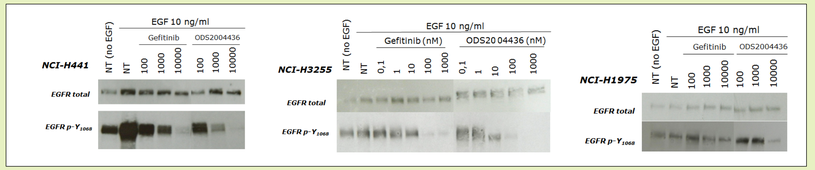
RESULTS

Properties of the compound

		ODS2004436 (^{19}F/^{18}F)	Gefitinib
Biochemical IC_{50} (nM)	EGFR_ WT	4.6	1.4
	EGFR_ L858R	5	1.8
	EGFR_ L858R/ T790M	536	1350
Cellular IC_{50} (µM)	NCI-H441	42 ± 27	14.8 ± 1.7
	NCI-H3255	0.005 ± 0.002	0.017± 0.003
	NCI-H1975	4.8 ± 0.7	18 ± 8.7
IC_{50} Auto phosphorylation (µM)	NCI-H441	≈ 1	≈ 5
	NCI-H3255	0.05	0.05
	NCI-H1975	≈ 5	> 10
Kinetic solubility at pH 7.4 (µM)		88	nd
Radiochemical stability (h)		> 20	nd
Plasmatic stability (h)		> 4	nd
Human microsomal stability	CLint (µL/min/mg prot.)	7.76 ± 2.53	nd
	t1/2 (min)	179	nd
Rat microsomal stability	CLint (µL/min/mg prot.)	37.9 ± 1.89	nd
	t1/2 (min)	36.5	nd

Selectivity of the compound ODS2004436 at 100nM against a panel of 92 kinases

Cellular activity



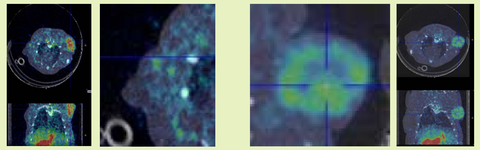
Effect of gefitinib and ODS2004436 on pY1068 EGFR phosphorylation on NCI-H3255, NCI-H441 and NCI-H1975 cell lines

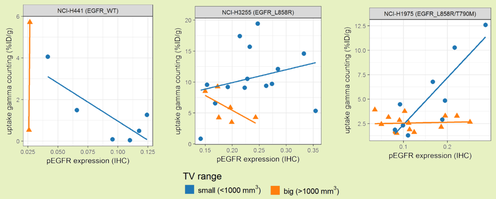
The biochemical profile of ODS2004436 is comparable to gefitinib on EGFR_WT and activated EGFR_L858R mutant (see table properties of the molecule). The cellular cytotoxic activities suggest that our compound might inhibit to a lesser extent non activated WT-EGFR, but improved inhibition is observed on EGFR double mutant (L858R/T790M) and NCI-H1975 compared to gefitinib.

Effect of both compounds was also compared on pY1068 EGFR phosphorylation showing similar profile than cytotoxic activity on all 3 cell lines respectively.

Lung cancer cell lines	EGFR status
NCI-H441	EGFR_WT
NCI-H3255	EGFR_L858R
NCI-H1975	EGFR_L858R/T790M

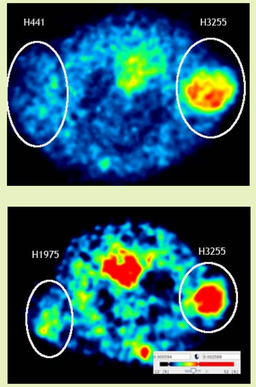
In-vivo PET





A good correlation was observed between the radiotracer uptake in the tumors and pEGFR immunostaining, suggesting that [18F]-ODS2004436 is a good biomarker of activated EGFR, regardless of the mutation.

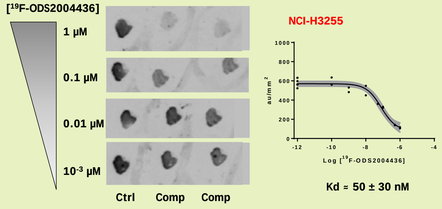
[18F]-ODS2004436 was evaluated *in-vivo* in 3 clinically relevant lung cancer cell lines (NCI-H441; NCI-H3255; NCI-H1975) xenografted in nude rats.



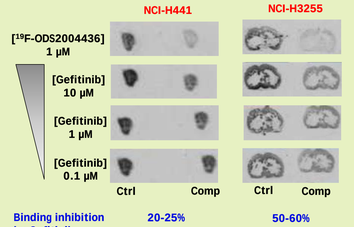
Ex-vivo binding

Ex-vivo binding experiments showed that [18F]-ODS2004436 specifically bound to cell line tumor samples expressing EGFR.

Competition with excess of [19F]-ODS2004436



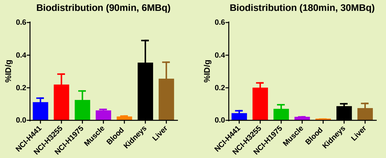
Competition with excess of Gefitinib



Binding inhibition by Gefitinib: 20-25% | 50-60%

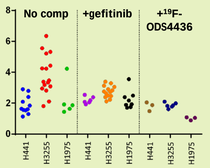
Ctrl: Control binding ([18F]-ODS2004436 alone)
Comp: Competition binding ([18F]-ODS2004436 + increasing concentrations of [19F]-ODS2004436) or Gefitinib

Biodistribution & competition



Biodistribution of the radiotracer in the main organs (in %ID/g)

The *in-vivo* experiments in rats showed that [18F]-ODS2004436 was rapidly cleared from the blood. Nevertheless the tumor uptake is stable overtime (up to 180 min) with a mean tumor/muscle ratio > 4 in NCI-H3255 and > 2 in NCI-H1975 at 90 min instead no specific binding was observed in the WT tumor, NCI-H441.



Ratio of tumor over muscle normalized uptakes with or without competition with either gefitinib or [19F]-ODS2004436

CONCLUSIONS

- **Within the IMAkinib® program, a new TKI-PET radiotracer targeting EGFR has been developed based on the Nanocyclix technology.**
- **The EGFR radiotracer development was performed in collaboration with the CERRP and CEA-Cyceron academic institutions. In the mean time, the whole development flowchart, from radiochemistry to clinical PET imaging, is manageable in Pharmimage platforms for academic and industrial projects.**
- ***In-vitro* ODS2004436 compound showed a biochemical profile comparable to gefitinib on WT EGFR or L858R mutated EGFR whereas improved activity is observed on L858R/T790M EGFR.**
- ***In-vivo* studies suggested that the radiotracer [18F]-ODS2004436 binds selectively to activated EGFR, and is a good candidate to evaluate the EGFR activity in NLCSC.**
- **Clinical evaluation of this novel radiotracer is ongoing (first in-man phase 0/I clinical trial NCT02847377).**

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