# Application of the Nanocyclix® technology to identify clinically relevant PET tracers
# The first [$^{18}$F]-Nanocyclix® TKI-PET radiotracer targeting activated EGFR positive lung tumors

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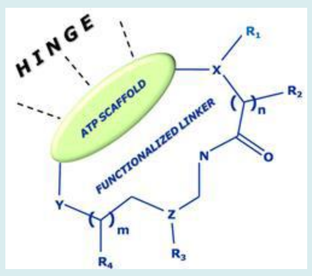
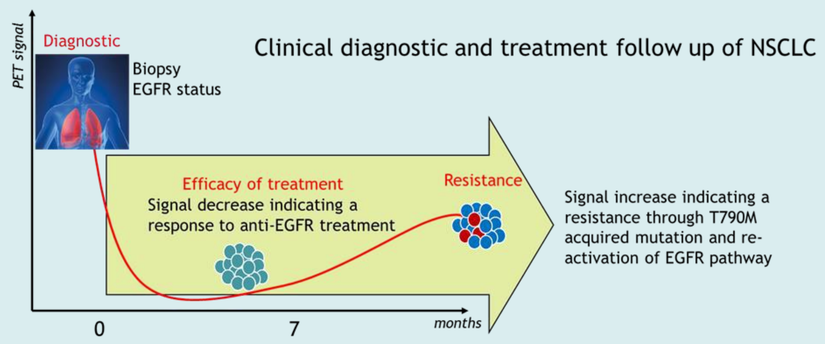
## Concept of the Nanocyclix chemistry technology and of the IMAkinib project




The Nanocyclix® chemistry technology comprises the generation of compounds through a macrocyclisation process which results in small, low MW kinase inhibitors with a unique binding mode and mode of action compared to the kinase inhibitors described in literature. The shape complementarity between the inhibitor and the active site of the kinase is believed to be the main driver for high potency and high selectivity. Not only the Nanocyclix® technology is used in the search for therapeutically active agents, but also in the identification of novel clinically relevant Positron Emission Tomography (PET) tracers.

Within the IMAkinib® program, a new macrocyclic PET tracer has been identified for the epidermal growth factor receptor (EGFR). 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.




## METHODS

### Strategy applied for the identification of ODS2004436

A small set of non-fluorinated compounds was selected from the Nanocyclix® library which showed highly selective activity on EGFR_WT and activated EGFR_L858R mutant. Different activity profiles have been noted on the double mutant EGFR L858R/T790M.

| Compound ID | EGFR WT $IC_{50}$ (nM) | EGFR L858R $IC_{50}$ (nM) | EGFR L858R/T790M $IC_{50}$ (nM) | %kinases inhibited >80% (tested kinases) |
|---|---|---|---|---|
| ODS2000001-1 | 4 | 2 | 33 | 10% (77) |
| ODS2000019-1 | 5 | 4 | > 1000 | 1% (107) |
| ODS2000075-1 | 4 | 1 | 57 | 3% (130) |
| ODS2000114-1 | 7 | 1 | 37 | 4% (130) |
| ODS2000204-1 | 3 | 0.7 | 232 | 5% (130) |
| ODS2000252-1 | 5 | 1 | 79 | 11% (19) |
| ODS2000284-1 | 3 | 0.8 | 2 | 11% (131) |
| ODS2000336-1 | 6 | 1 | 53 | 1% (124) |
| ODS2000374-1 | 9 | 1 | 10 | 20% (41) |
| ODS2000724-1 | 4 | 1 | 44 | 9% (188) |

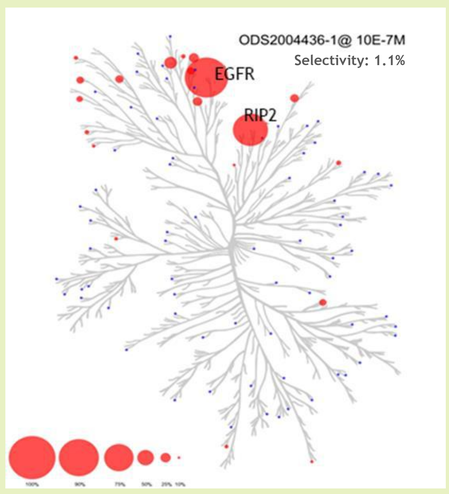
**Biochemical activities ($IC_{50}$) and selectivity of non-fluorinated compounds**

The following cut-offs were applied:
- $IC_{50}$ < 100 nM; selectivity < 5%
- 100 nM > < 1000 nM; 5% > < 10%
- > 1000 nM; > 10%

### Properties of the compound ODS2004436

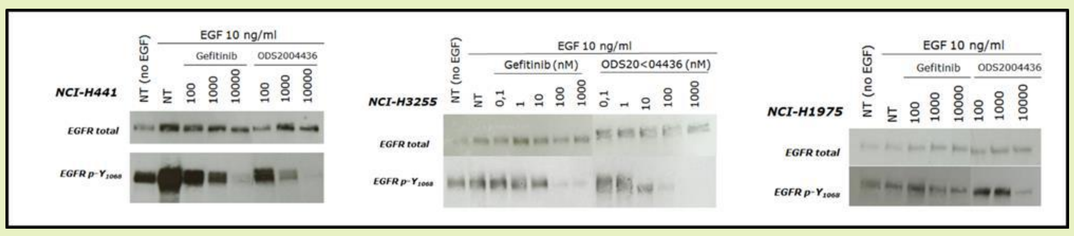
Based on the above small set of compounds, analogues have been designed and synthesized in which a $^{19}$F-containing group was incorporated. The cold fluorinated compounds were then profiled for activity, selectivity and eADME properties. Compound ODS2004436 was selected for its *in-vitro* activity on EGFR and metabolism favorable to develop a radiotracer. Radiolabeling with [$^{18}$F] has been developed.

| | | ODS2004436 (F) | Gefitinib |
|---|---|---|---|
| Biochemical $IC_{50}$ (nM) | EGFR_ WT | 4.6 | 1.4 |
| | EGFR_ L858R | 5 | 1.8 |
| | EGFR_ L858R/ T790M | 536 | 1350 |
| Kinase selectivity (S50 92K at 0.1 µM) | | 1.1% | 1.1% |
| 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 100 nM against a panel of 92 kinases.

### Cellular activity




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

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 cells lines respectively.

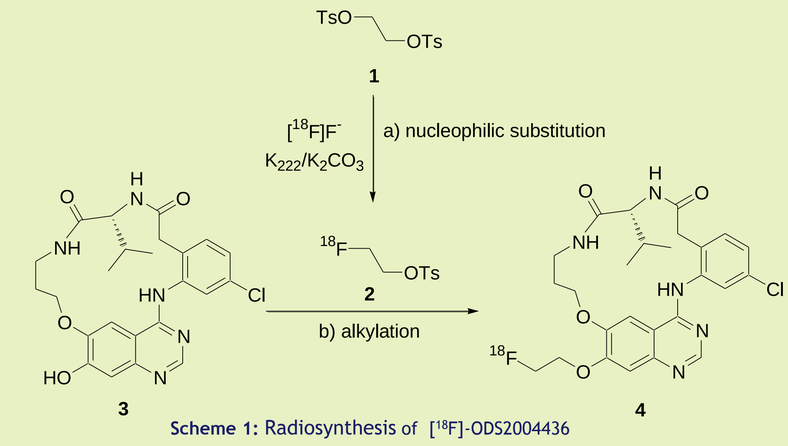
## RESULTS

### Radiochemistry of [$^{18}$F]-ODS2004436

The process to prepare [$^{18}$F]-ODS2004436 can be divided in four 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
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)** in acetonitrile,
   b. In the second reactor, O-[$^{18}$F]-fluoroethylation of precursor **(3)** with [$^{18}$F]-FETos (2) in DMSO
3. **HPLC purification**
4. **Formulation**

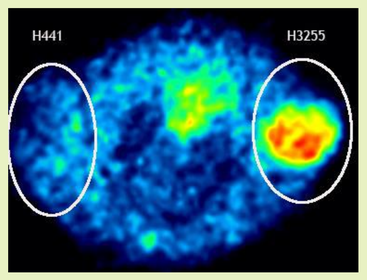
The synthesis of the cold Nanocyclix® precursor of ODS2004436, compound **(3)** has been done on gram scale. The radiosynthesis has been optimized to achieve 10-20% Decay Corrected Yield (DCY) and a radiochemical purity of more than 97%. The shelf-life of the compound is > 8 h.

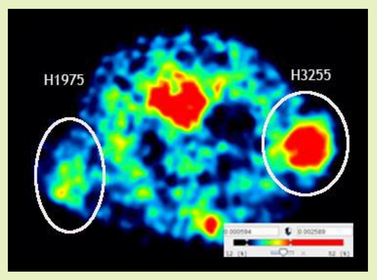



**Scheme 1:** Radiosynthesis of [$^{18}$F]-ODS2004436

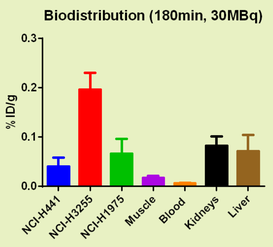
### *In-vivo* PET data

[$^{18}$F]-ODS2004436 was evaluated *in-vivo* in 3 clinically relevant lung cancer cell lines (NCI-H441; NCI-H3255; NCI-H1975) xenografted in nude rats. The *in-vivo* experiments demonstrated that [$^{18}$F]-ODS2004436 was rapidly cleared from the blood, nevertheless the tumor uptake is stable overtime (up to 180 min) with high mean tumor/muscle ratios at 90 min.




The WT tumor did not exhibit a specific binding in-vivo, which makes the compound a good candidate to evaluate the EGFR activity in NSCLC using PET imaging.




Biodistribution of the radiotracer in the main organs (in %ID/g) with a mean tumor/muscle ratio > 4 in NCI-H3255 and > 2 in NCI-H1975

## Conclusions and perspectives

- **The application of the Nanocyclix® chemistry technology within the IMAkinib® program has resulted in the design and the synthesis of a highly selective and potent [$^{18}$F]-Nanocyclix® TKI-PET radiotracer targeting activated EGFR positive lung tumors.**
- **Clinical evaluation of this novel radiotracer is ongoing (first in-man phase 0/I clinical trial NCT02847377).**

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