In vitro and in vivo evaluation of two enantiomers of Nanocyclix® EGFR targeted PET radiotracer

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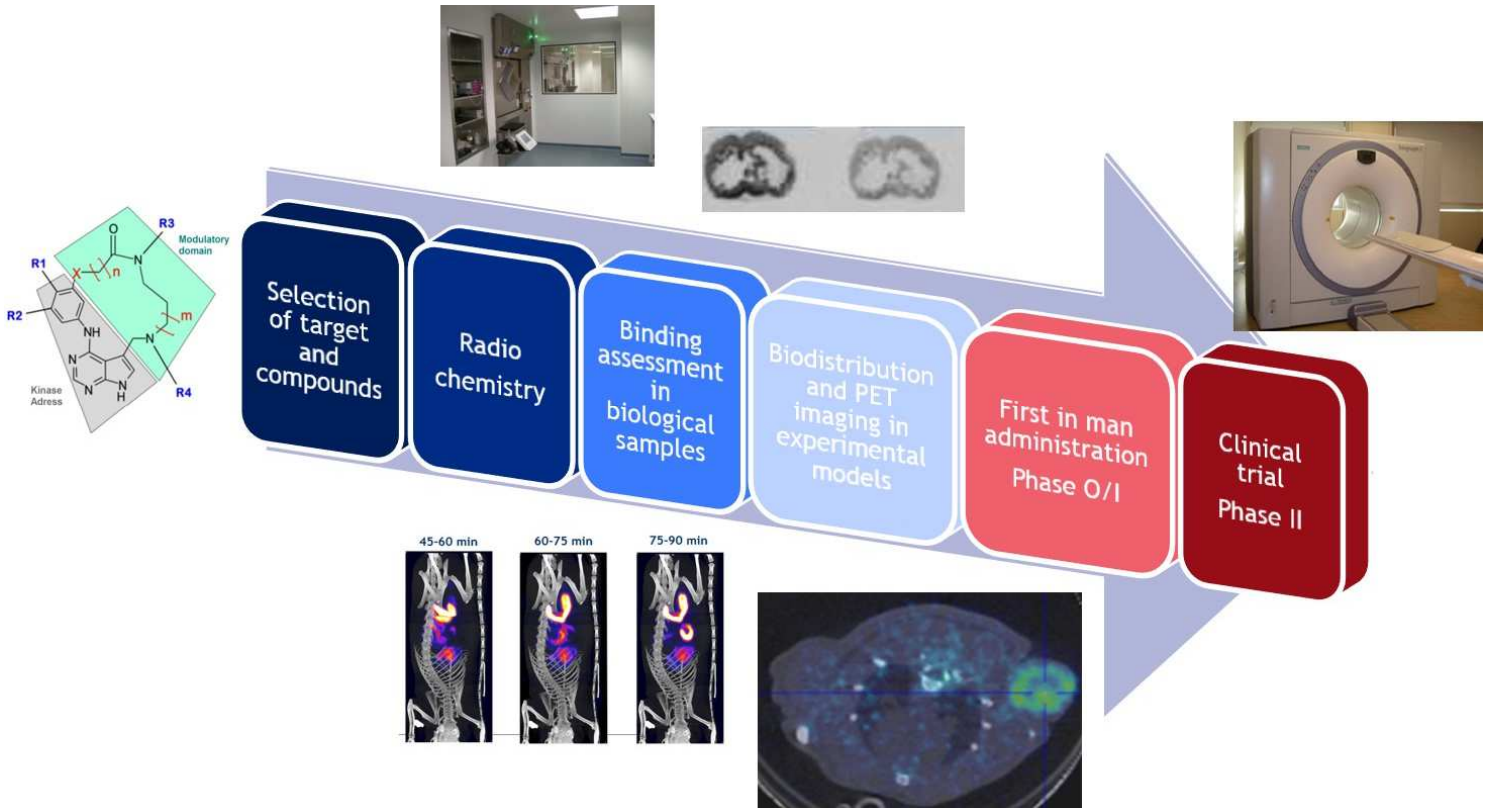
Concept of IMAkinib® Project

- 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)
- TKI PET-imaging can provide a diagnostic tool to determine and predict the activity of kinases and the responsiveness to TKIs
- 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
- Our program aims to develop an EGFR targeted PET radiotracer for clinical use in diagnosis and treatment follow-up

Flowchart of radiotracer development

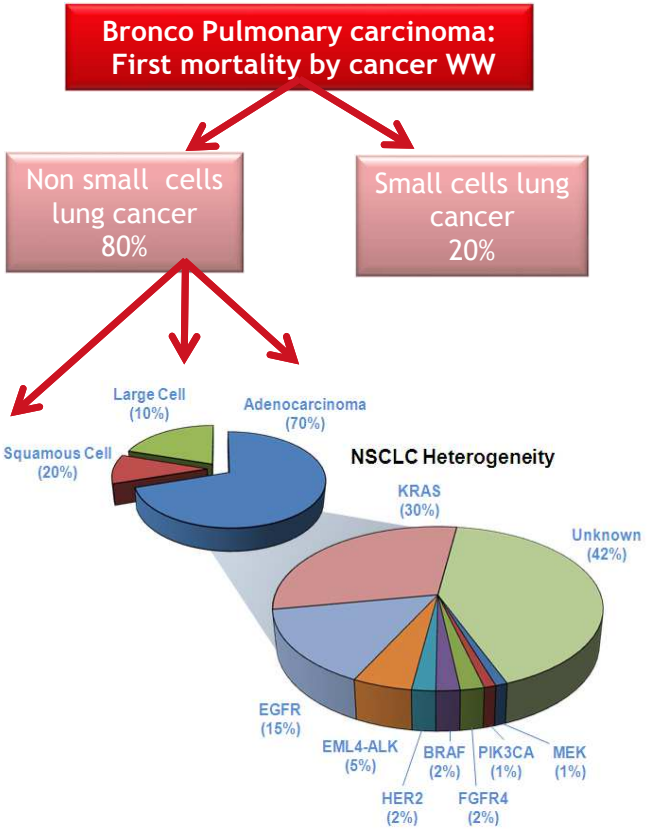
- Starting from our library of highly potent and selective Nanocyclix kinase inhibitors, targets of clinical interest are selected based on overexpression/ activity in tumors

- Compounds are radiolabeled with fluor-18 and characterized in vitro and in vivo

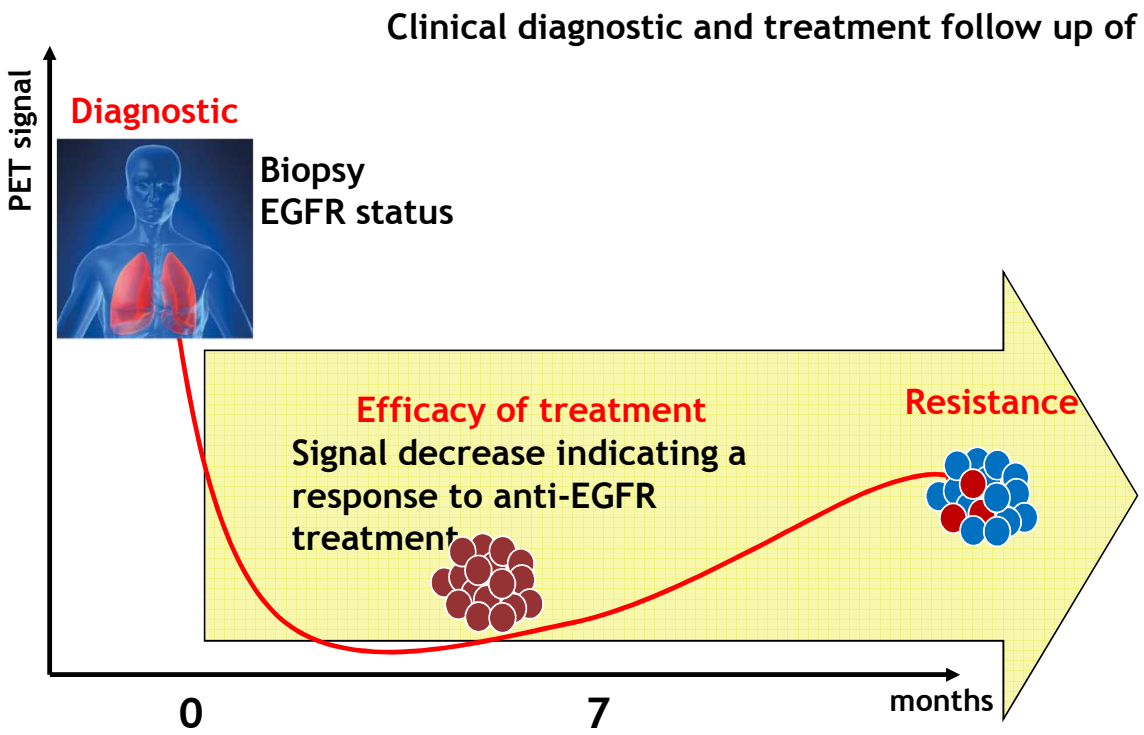


EGFR mutation: Biomarker in lung cancer

- Clinical diagnostic biomarker are associated with activating EGFR mutation in lung cancer
  - *Activating EGFR mutations is a criteria of patient selection for EGFR targeted therapies in NSCLC (ex: Iressa, Tarceva)*
  - *EGFR expression itself is not well correlated with clinical patient outcome*
- 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
- The EGFR radiotracer must be a marker of EGFR kinase activity, correlated with its mutational status
- EGFR activity under treatment can be monitored by the radiotracer to propose alternative therapies



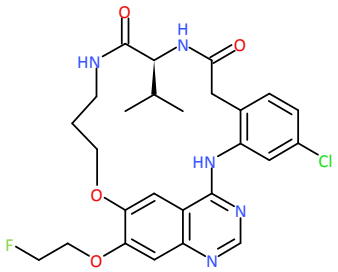
Hypothesis for diagnostic application of the EGFR radiotracer



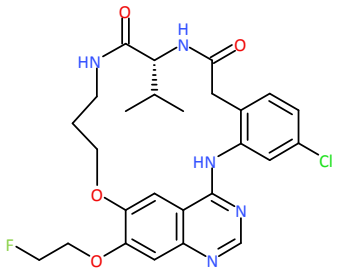
Signal increase indicating a resistance through T790M acquired mutation and re-activation of EGFR pathway

Overview all enantiomers biochemical and cellular activities

- An initial proof-of-concept was done on ODS2004436 compound, and we evaluated here each of the 2 enantiomers obtained after chiral purification



S-enantiomer



R-enantiomer

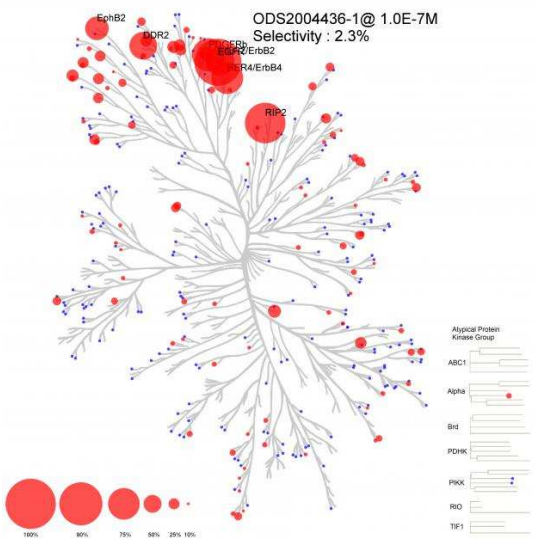
Compound	EGFR WT	EGFR L858R	EGFR d752-759	EGFR G719S	EGFR L861Q	EGFR T790M	EGFR T790M/ L858R
ODS2004436 (~70% S/30% R)	4.1	4.3	7.0	5.1	3.5	209	220
R-enantiomer	3.1	2.7	5.0	2.5	2.9	55	78
S-Enantiomer	5.9	6.0	11	5.8	6.2	1011	924
Gefitinib	1.4	1.8					1350

- Non significant biochemical activity difference between the 2 enantiomers on EGFR wild-type (WT) and all activated EGFR mutants
- Enantiomer R is more active than enantiomer S notably on T790M EGFR mutant proteins and NCI-H1975 cell line
- Cellular activity is correlated with biochemical activities, Enantiomer R being more active on NCI-H1975 cells

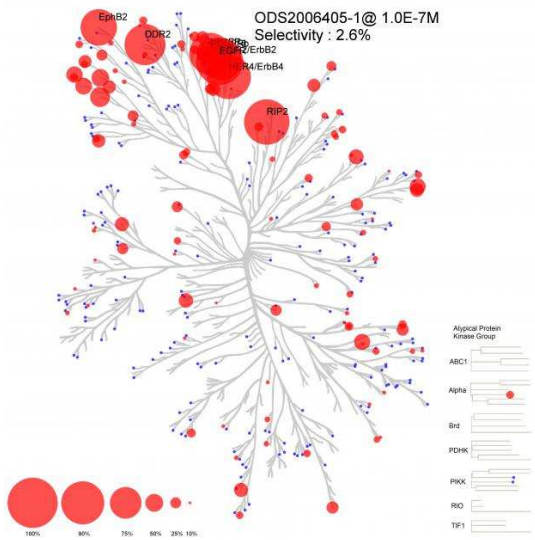
Compound	GI50 (nM)	
	NCI-H3255 (EGFR L858R)	NCI-H1975 (EGFR T790M/ L858R)
ODS2004436 (~70% S/30% R)	1.6	4 633
R-enantiomer	0.4	3 600
S-enantiomer	8.6	12 866
Gefitinib	17	> 25 000

Biochemical Selectivity @ 100 nM

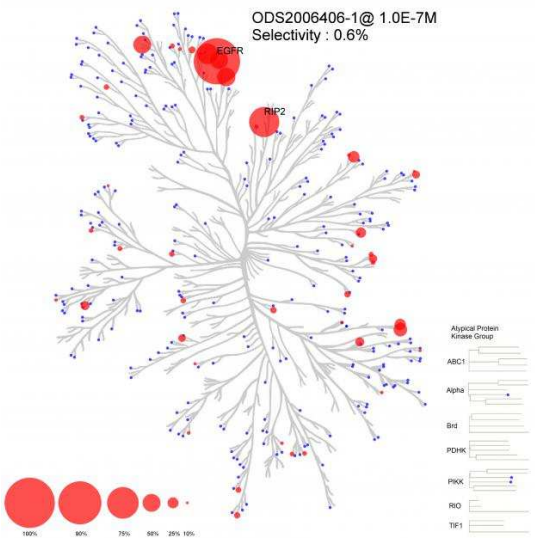
- Enantiomer S is more selective than Enantiomer R
- Identified off targets: RIPK2, DDR2, EPHB2, ERB2/4, PDGFRβ with a shift estimated around 5-15x
- Profile of ODS2004436 at 100 nM (~20x IC50) is coherent with a mix profile of R/S-enantiomer



ODS2004436



R-enantiomer R

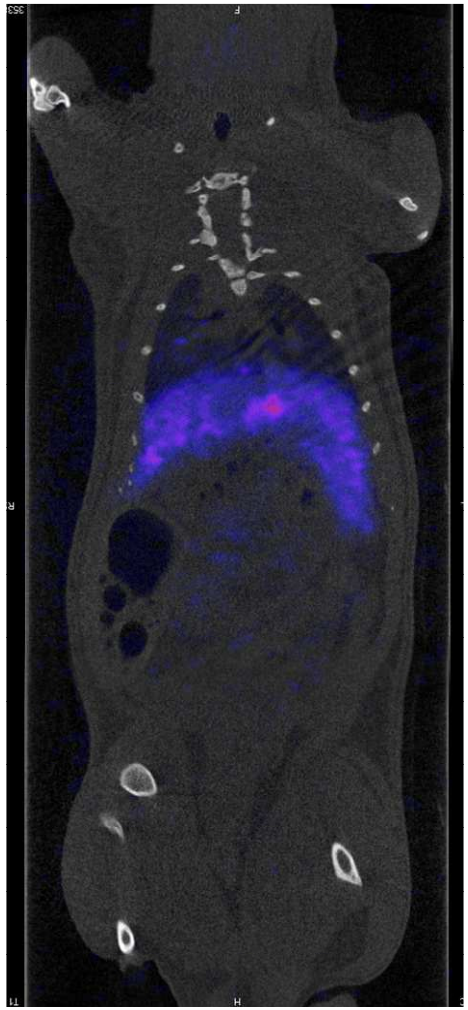


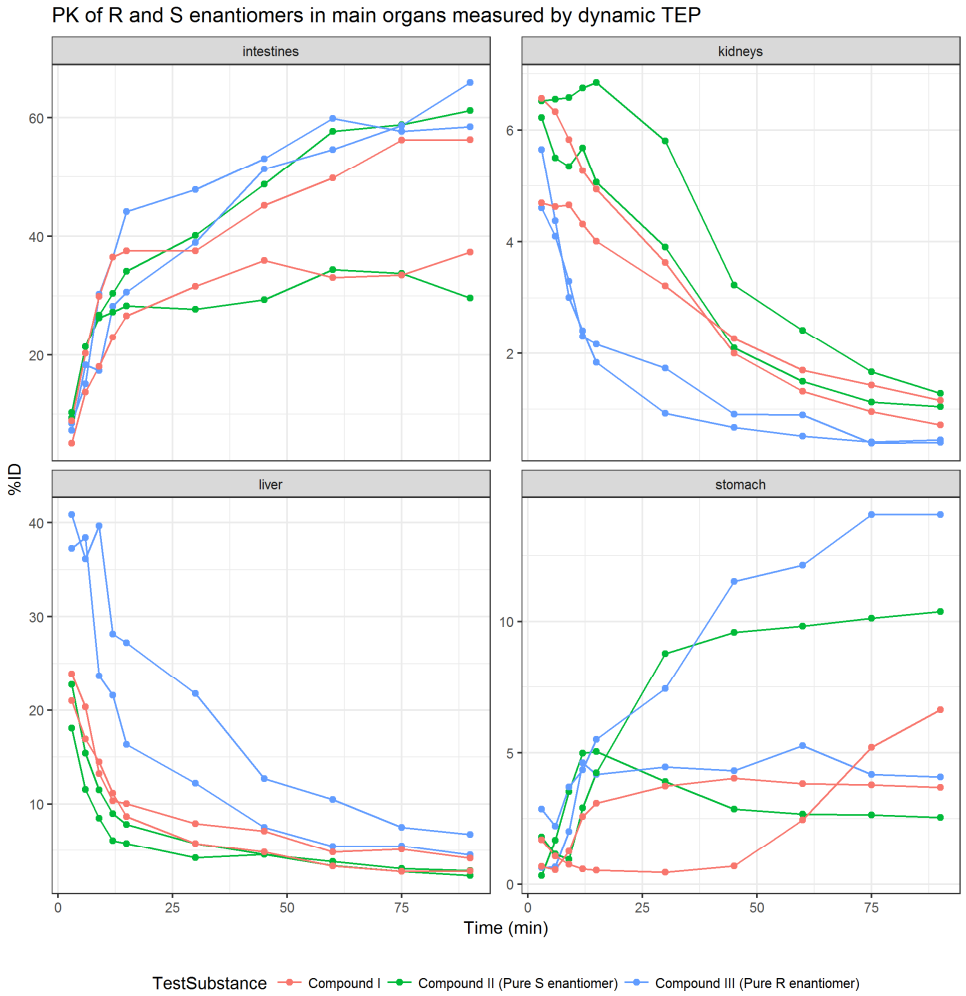
S-enantiomer

Biodistribution in rat

- Biodistribution was evaluated by dynamic PET imaging (0-90 min) in healthy rats
- No significant difference is observed between the R- (in green) and S- (in blue) enantiomers and the original ODS2004436 compound (in red)

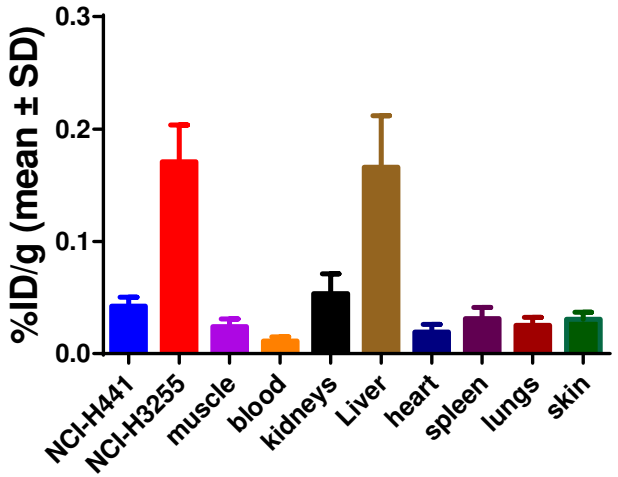
➢ **Based on favorable profile to target T790M mutation and comparable in vivo biodistribution, we selected the R-enantiomer for further preclinical characterization**





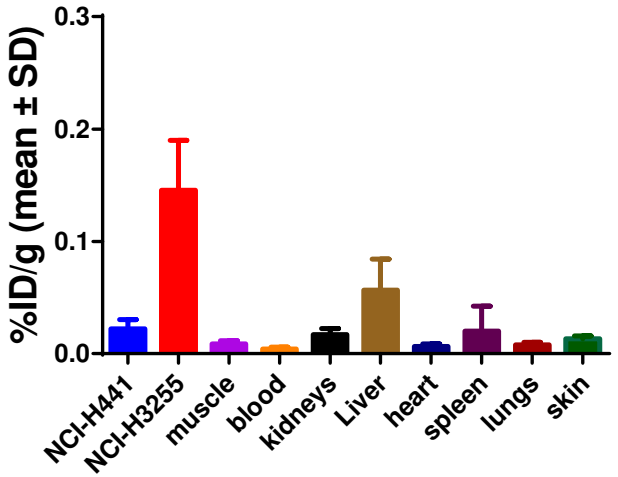
Biodistribution of the R-enantiomer in tumor bearing rats

Biodistribution (γ-counting at 90 min post-injection)



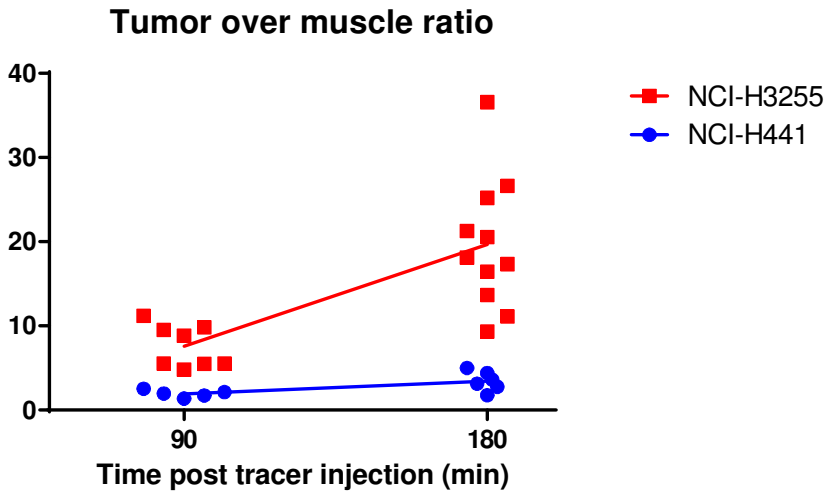
At 90 min post-injection, uptake of the R-enantiomer in tumor and liver is close to 0.2% ID/g.

Biodistribution (γ-counting at 180 min post-injection)

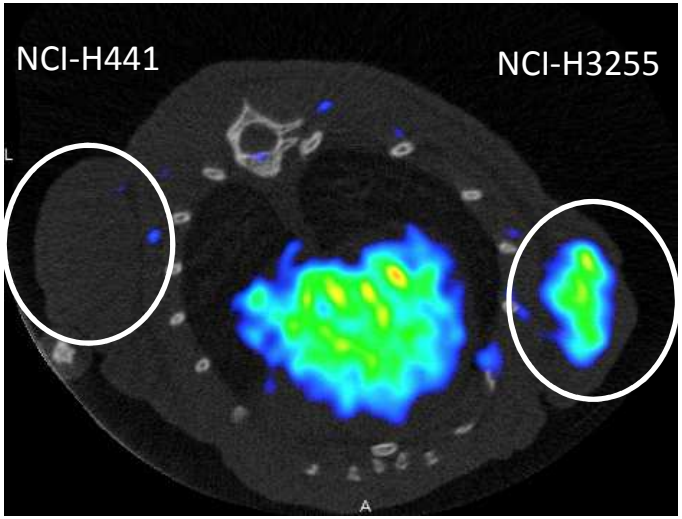


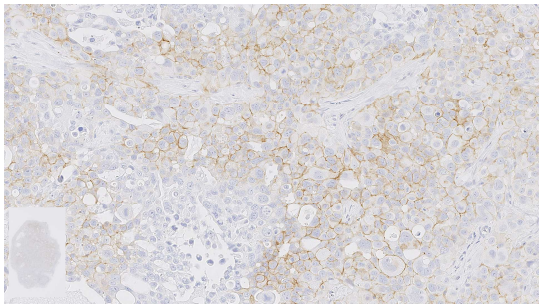
At 180 min post-injection, uptake of the R-enantiomer in tumor remains close to 0.2% ID/g while uptake in all other organs is decreased.

Biodistribution of the R-enantiomer in tumor bearing rats

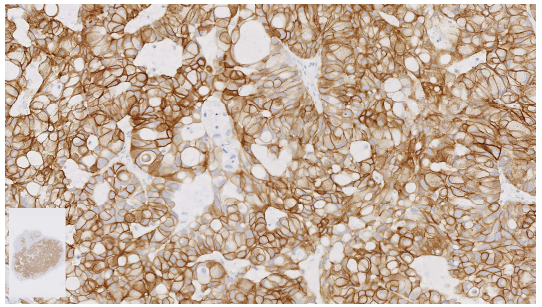


Uptake of the R-enantiomer in organs is decreased from 90 to 180 min post-injection. As it remains unchanged in tumor, tumor-to-organ ratios are improved at 180 min post-injection. PET image contrast is also improved.





NCI-H441 (EGFR WT)



NCI-H3255 (L858R EGFR mutant)

Tumor radiotracer uptake is correlated to phospho-EGFR expression

Conclusions

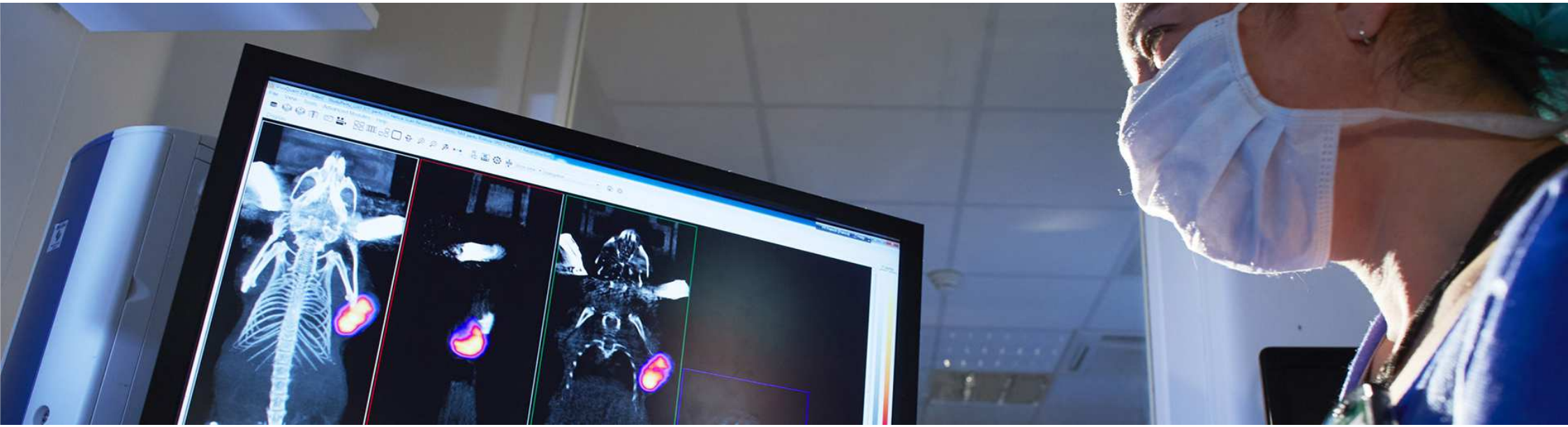
- Within the IMAkinib® program, a new TKI-PET radiotracer targeting EGFR is being developed (from radiochemistry to clinical trial) based on our Nanocyclix® technology using comprehensive Pharmimage capabilities

- In vitro the compound ODS2004436 shows a biochemical profile comparable to gefitinib on WT EGFR or L858R mutated EGFR whereas improved activity is observed on L858R/T790M EGFR, notably with the R-enantiomer

- Enantiomer R of ODS2004436 was selected for further preclinical and clinical evaluation related to its improved binding profile to T790M EGFR mutant

- In vivo studies suggests that the radiotracer [^{18}F]-R-ODS2004436 binds selectively to activated EGFR, and is a good candidate to evaluate the EGFR activity in NLCSC

- Clinical evaluation of this novel radiotracer, [^{18}F]-R-ODS2004436, is ongoing (first in-man phase 0/I clinical trial NCT02847377)



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Abstract

- **Aim:** IMAkinib® program is an innovative approach to develop new PET radiotracers, based on Nanocyclix® chemistry technology. We developed ODS2004436, a novel radiotracer to target the epidermal growth factor receptor (EGFR) and its mutated forms. One of the mutations, T790M, is involved in resistance to first-line treatment in advanced non-small cell lung cancer (NSCLC). ODS2004436 could be a valuable tool to stratify patients for EGFR activity and responsiveness to EGFR-targeted therapies. Clinical evaluation of this radiotracer is ongoing (first in-man phase 0/I clinical trial NCT02847377).

- **Material and Methods:** Two enantiomers of ODS2004436 were characterized regarding their binding capabilities for EGFR (wild-type versus mutated). Biochemical EGFR activity, kinome selectivity, cellular activity in NSCLC cell lines, efficiency in radiochemistry, in vivo biodistribution were performed to select the best enantiomer able to selectively bind to double mutant L858R/T790M EGFR. Our study was conducted on the Pharmimage radiochemistry and imaging platforms using a new PET/MRI prototype.

- **Results:** Both enantiomers displayed a similar biochemical profile on EGFR wild-type and eight mutated/deleted isoforms (i.e. L858R). Interestingly the (R)-enantiomer showed an improved activity on EGFR L858R/T790M (IC50: 55 nM (R) vs 1011 nM (S)). Selectivity score S(50%) on a panel of 320 wild-type kinases was better for (S) versus (R)-enantiomer (respectively 0.6% and 2.5%, 100 nM). In NSCLC cell lines, the (R) enantiomer was the most active as it appeared slightly more potent than the (S)-enantiomer. Both enantiomers showed a marked cytotoxic activity in NCI-H3255 cells (L858R; IC50≈10-50 nM), a weak activity in NCI-H1975 cells (L858R/T790M; IC50≈10-20µM), and no cytotoxicity in NCI-H441 cells (wild-type; IC50>25µM). F-18 radiolabeling of both enantiomers is achieved with a similar specific activity. Their biodistribution in healthy Sprague-Dawley rats was evaluated using PET imaging and ex vivo γ-counting showing a similar distribution pattern for both compounds without any preferential organ accumulation. They were rapidly cleared from blood and mainly eliminated through kidneys and liver. Based on these results, the (R)-enantiomer was selected to study the differential tumor uptake in rats and compare in vivo binding to various forms of EGFR.

- **Conclusion:** In vitro and in vivo characterization of ODS2004436 as a radiotracer targeting EGFR led to the selection of the (R)-enantiomer, with improved binding to EGFR double mutant L858R/T790M. This radiotracer is a promising tool to assess sensitivity or resistance of patients with tumors acquiring EGFR mutation upon first-line EGFR-targeted therapies.