



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem



THE
Tuscany Health Ecosystem

Development of multimeric radiotracers for
the design of tumor microenvironment-
selective theragnostic agents for cancer
diagnosis and therapy

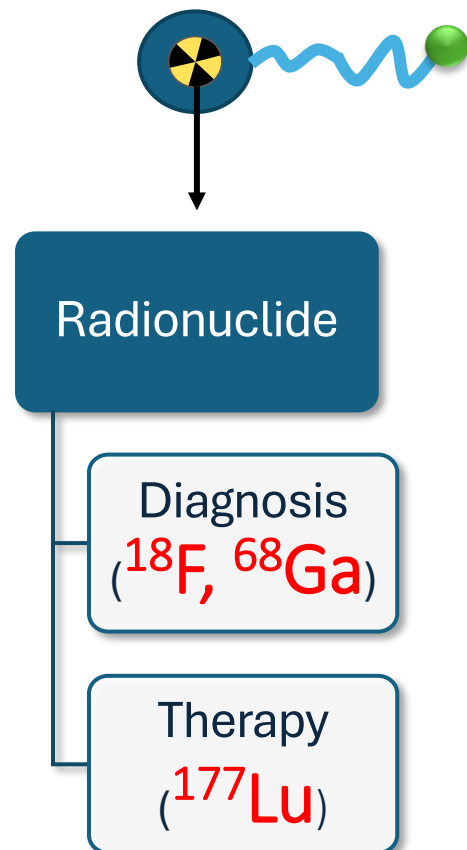


COSTANZA FABBRI

PhD student (SSSUP)

costanza.fabbri@santannapisa.it

Theragnostic Radiotracers



Monomeric
Radiotracers

Multimeric
Radiotracers

excellent for
imaging

short
retention
time

tumor uptake

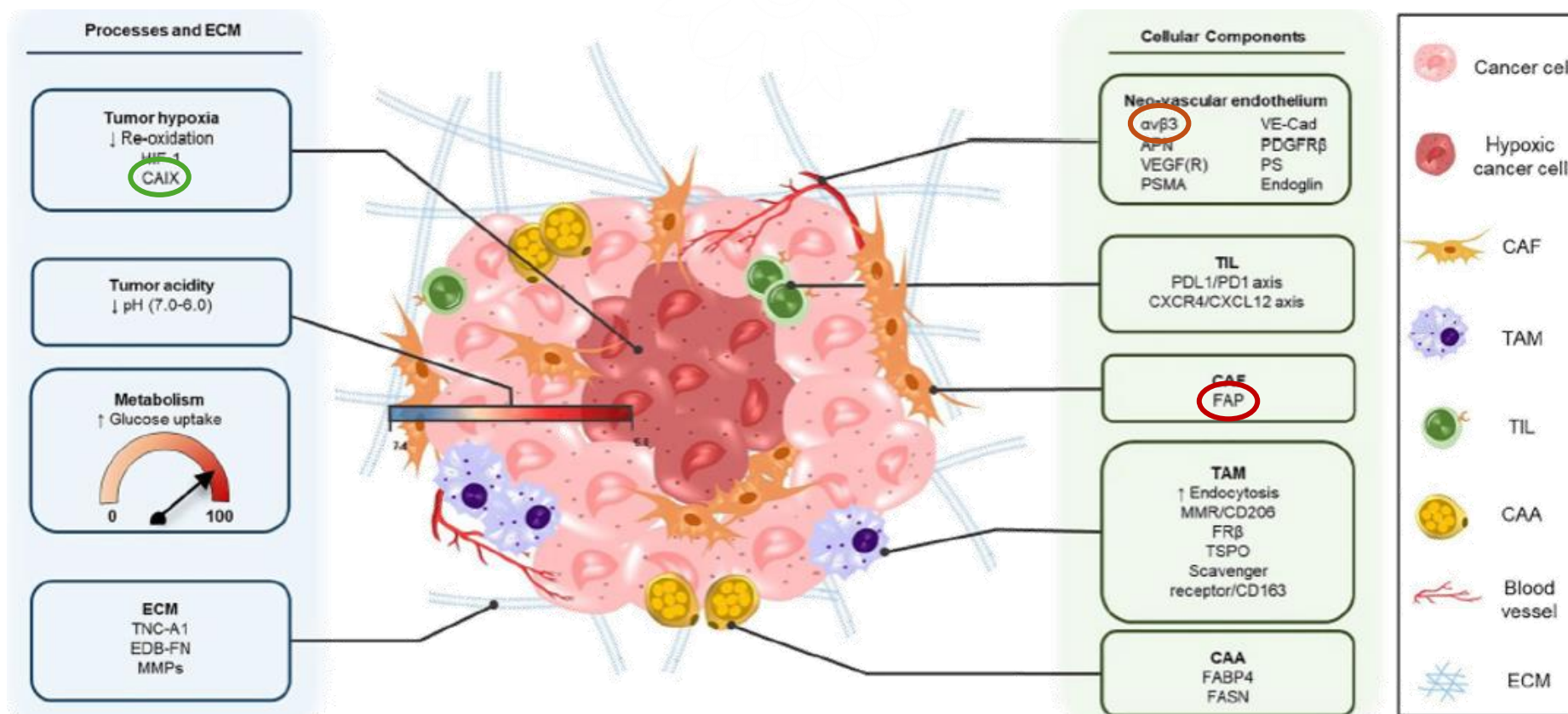
sensitivity

specificity

retention
time

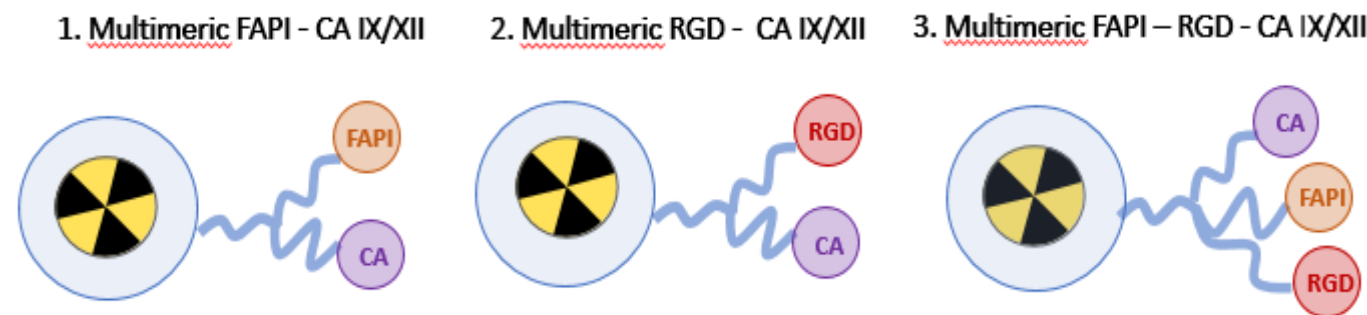
Tumor Microenvironment (TME) targets that can be used for nuclear imaging or/and therapy

CA IX/XII PET
Radiotracers

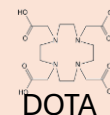


C. D. van der Heide and S. U. Dalm, *Eur. J. Nucl. Med. Mol. Imaging*, Nov. 2022, doi: 10.1007/s00259-022-05870-1

Development of novel multimeric radiotracers for the design of tumor microenvironment-selective theragnostic agents for cancer diagnosis and therapy



Chelator =
NOTA Or
DOTA



Radioisotope = $[^{18}\text{F}]\text{AlF}_2^+$ or ^{177}Lu

CANCER DIAGNOSIS



-Radiolabeling with $[^{18}\text{F}]\text{AlF}_2^+$

- In vivo PET imaging studies for the evaluation of biodistribution and uptake in Melanoma models

CANCER THERAPY



umcg
University Medical
Center Groningen

-Radiolabeling with ^{177}Lu

-Evaluation of cellular uptake, affinity, specificity and antitumor activity in vitro





Workflow



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem

Loading...

Chemical synthesis of
multimeric **Precursors**

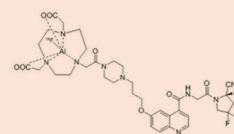
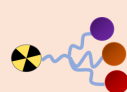


UNIVERSITÀ
DEGLI STUDI
FIRENZE

NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE
PSICOLOGIA, AREA DEL FARMACO E
SALUTE DEL BAMBINO

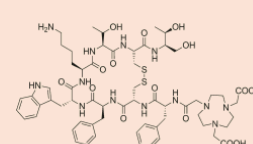
Radiolabeling with $[^{18}\text{F}]\text{AlF}^{2+}$ of radiotracers:

Mutimeric

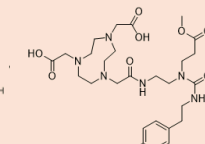


$[^{18}\text{F}]\text{AlF-NOTA-FAPI-04}$

Monomeric



$[^{18}\text{F}]\text{AlF-NOTA-RGDfK}$



$[^{18}\text{F}]\text{AlF-NOTA-CAX}$

**in vivo PET imaging
studies**

To evaluate the uptake and
biodistribution of novel
multimeric versus monomeric
radiotracers in
B16-F10 Melanoma syngeneic
murine model

The protocol for animal
use has been approved by
the Ministry of Health



<https://doi.org/10.1007/s00259-023-06587-5>

Workflow



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem

Loading...

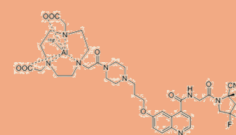
Chemical synthesis of
multimeric **Precursors**



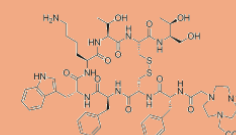
UNIVERSITÀ
DEGLI STUDI
FIRENZE

NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE
PSICOLOGIA, AREA DEL FARMACO E
SALUTE DEL BAMBINO

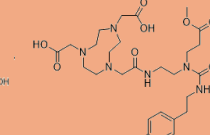
Radiolabeling with $[^{18}\text{F}]\text{AlF}^{2+}$ of radiotracers:
Mutimeric **Monomeric**



$[^{18}\text{F}]\text{AlF-NOTA-FAPI-04}$



$[^{18}\text{F}]\text{AlF-NOTA-RGDfK}$



$[^{18}\text{F}]\text{AlF-NOTA-CAX}$

**in vivo PET imaging
studies**

To evaluate the uptake and
biodistribution of novel
multimeric versus **monomeric**
radiotracers in
B16-F10 Melanoma syngeneic
murine model

The protocol for animal
use has been approved by
the Ministry of Health



<https://doi.org/10.1007/s00259-023-06587-5>

Methods

Optimization of a radiolabeling conditions with $[^{18}\text{F}]\text{AlF}^{2+}$



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca

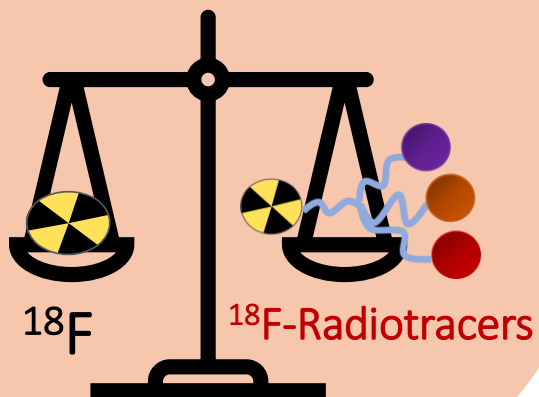


Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem

RCY
Radiochemical Yield (%)

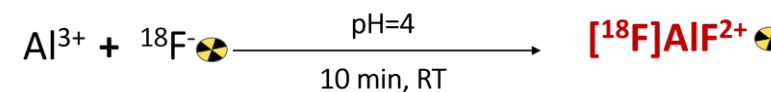


Quality Control

Radio-TLC
Radio-HPLC

1 Formation of the $[^{18}\text{F}]\text{AlF}^{2+}$ labeling agent

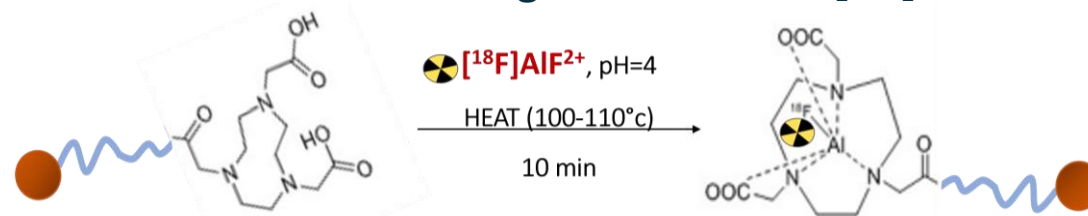
- concentration of AlCl_3

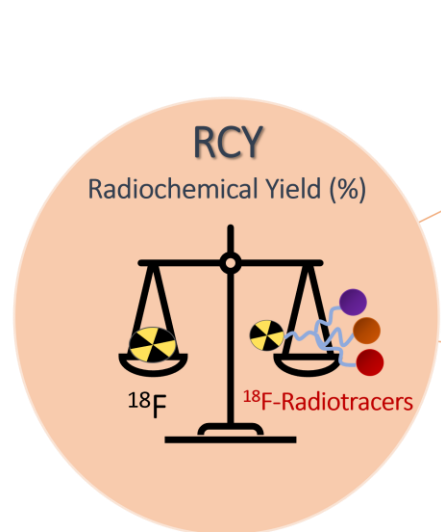


2 Optimization of chelation reaction

- Amount of precursor
- Effect of % cosolvent (v/v)

Standard radiolabeling conditions with $[^{18}\text{F}]\text{AlF}^{2+}$





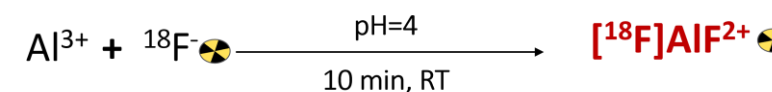
Formation of
the
 $[^{18}\text{F}]\text{AlF}_2^+$
labeling agent

2
Optimization
of chelation
reaction

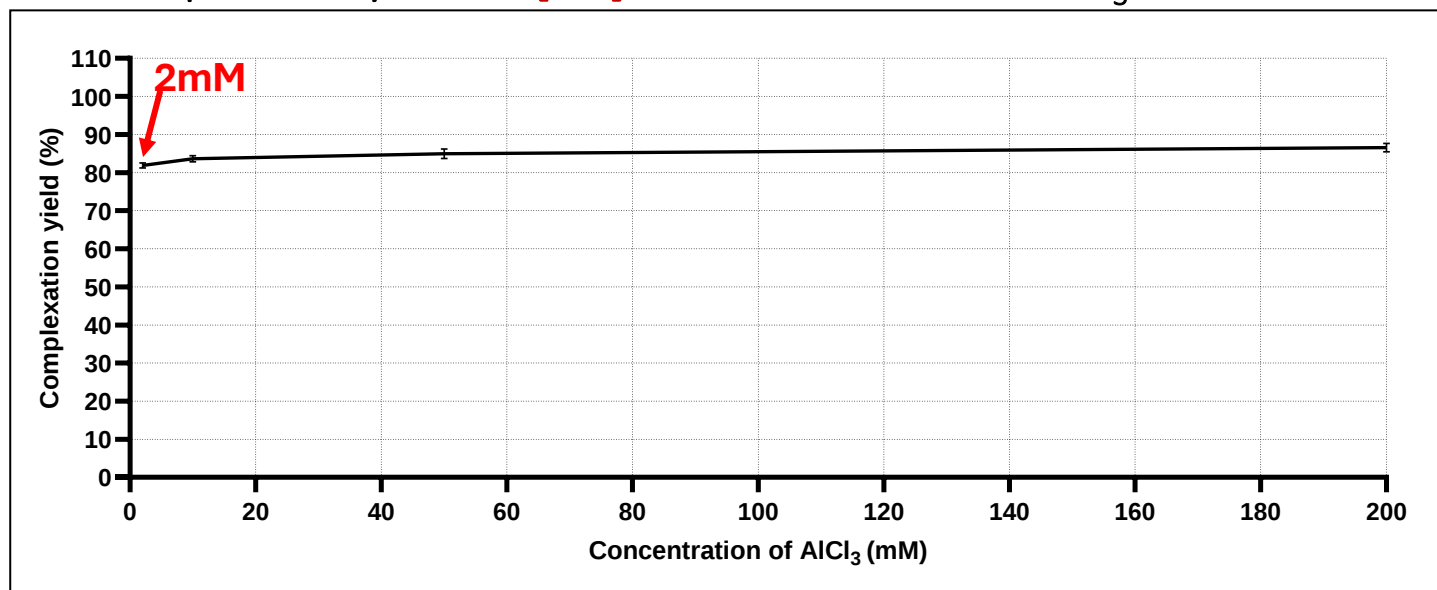
- amount of precursor
- Effect of % cosolvent (v/v)

• Concentration of AlCl_3

Reaction conditions



Complexation yields of $[^{18}\text{F}]\text{AlF}_2^+$ as a function of AlCl_3 concentration



Quality Control

Cation exchanger
(Maxi-Clean™ SPE
0,5ml IC-H, Alltech)

Results



Finanziato
dall'Unione europea
NextGenerationEU



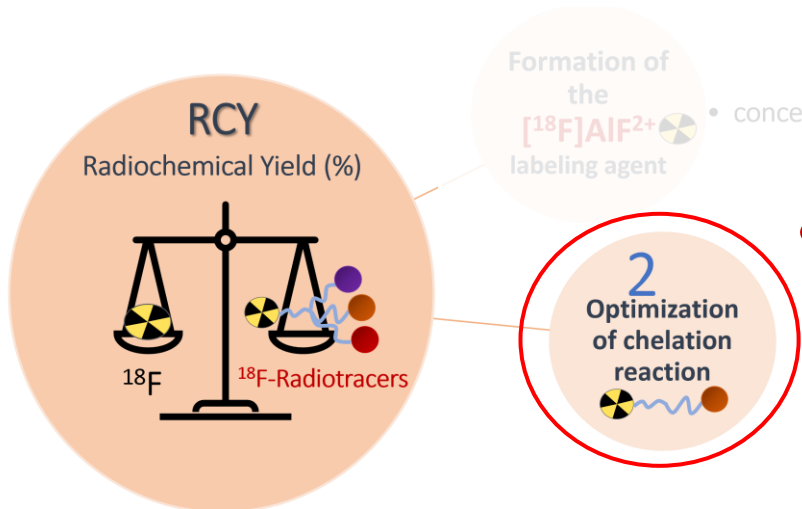
Ministero
dell'Università
e della Ricerca



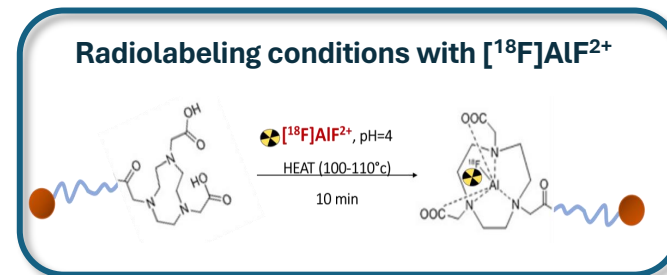
Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



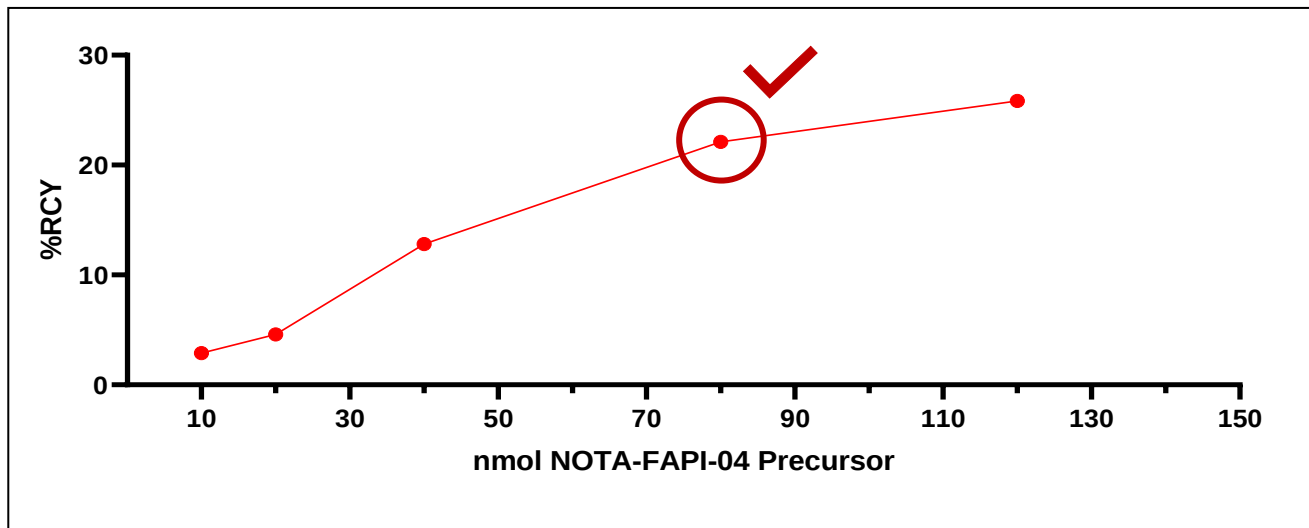
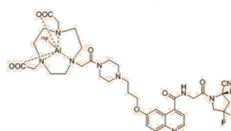
THE
Tuscany Health Ecosystem



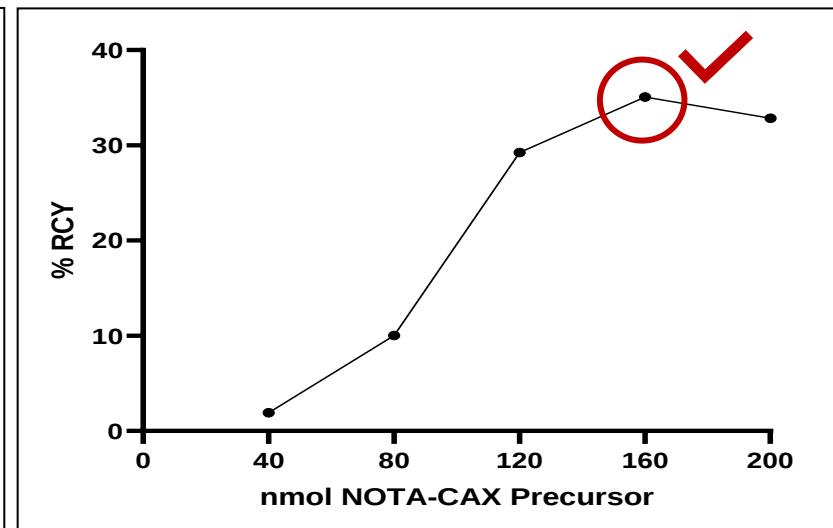
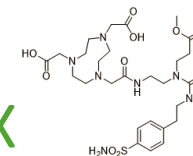
• Effect of precursor amount



$[^{18}\text{F}]\text{AlF}$ -NOTA-FAPI-04



$[^{18}\text{F}]\text{AlF}$ -NOTA-CAX



Quality Control
Radio-TLC

Results



Finanziato
dall'Unione europea
NextGenerationEU



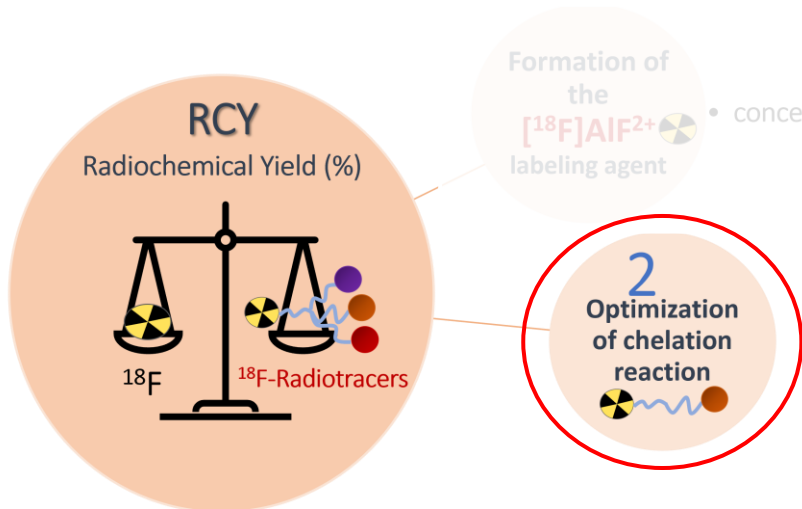
Ministero
dell'Università
e della Ricerca



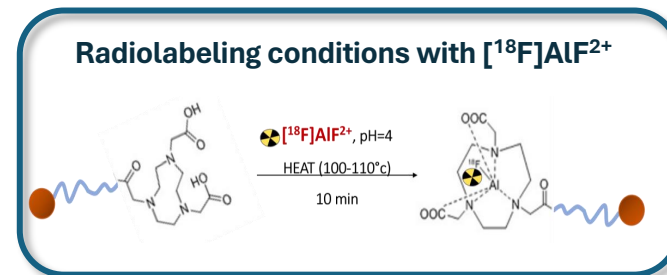
Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



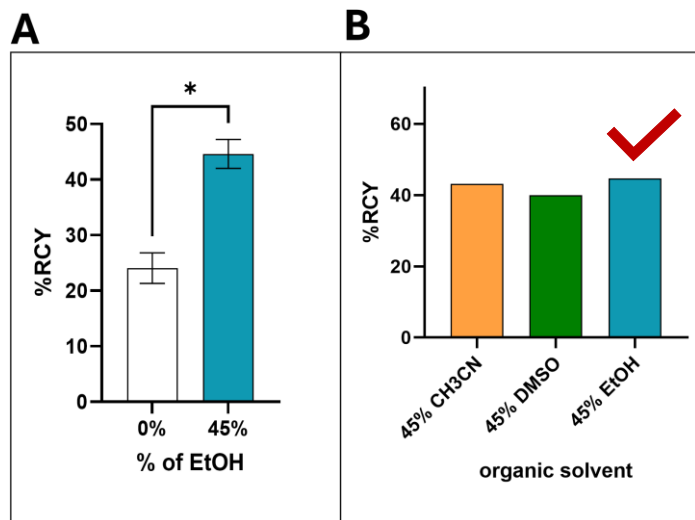
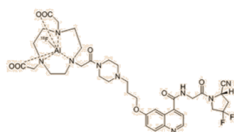
THE
Tuscany Health Ecosystem



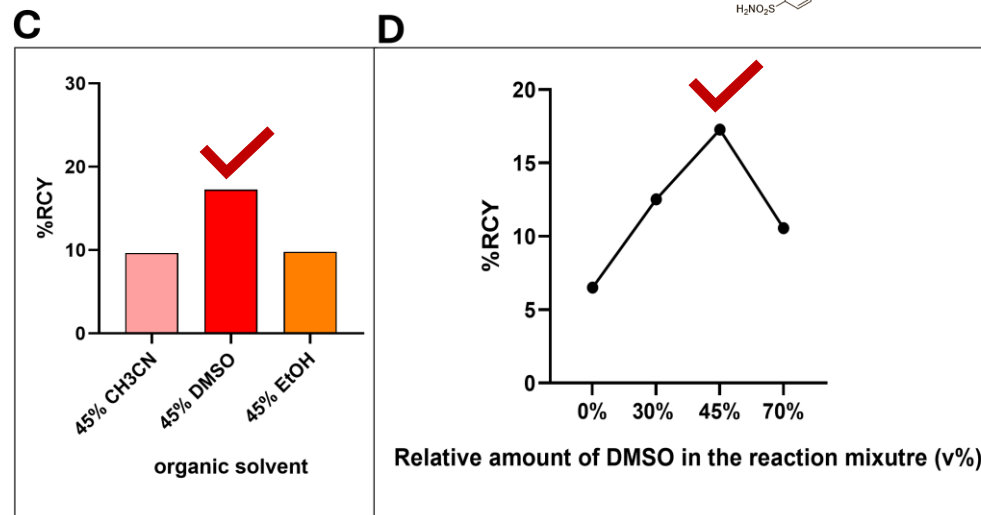
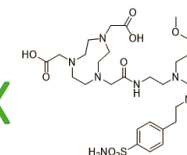
• Effect of % cosolvent (v/v)



$[^{18}\text{F}]\text{AlF}$ -NOTA-**FAP**I-04



$[^{18}\text{F}]\text{AlF}$ -NOTA-**CAX**



Quality Control

Radio-TLC
Radio-HPLC

Results

Radiosynthesis for in vivo experiment
and stability test



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

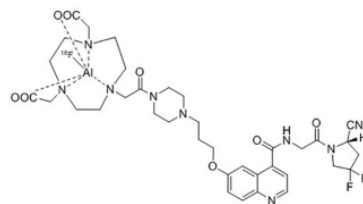


THE
Tuscany Health Ecosystem



Optimization of radiolabeling reactions following the **purification step**
lead to:

$[^{18}\text{F}]$ AlF-NOTA-FAPI-04

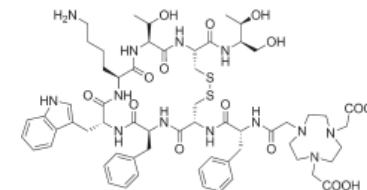


RCY= **$30.9 \pm 2.8\%$**

Additional information

(N=3) Molar Activity = $3,0 \pm 0,2$ GBq/ μmol

$[^{18}\text{F}]$ AlF-NOTA-RGDfK



RCY= **$28.3 \pm 3.0 \%$**

Additional information

(N=2) Molar Activity = 1.9 ± 0.7 GBq/ μmol



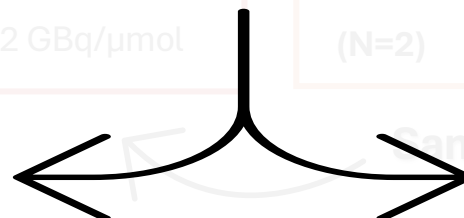
Same radiolabeling conditions of:



FOR ANIMAL USE:

1. RCP $\geq 95\%$
(Radiochemical Purity)

Purification with
solid phase extraction
(SPE) cartridge
(to remove free ^{18}F)



**2. STABILITY
TEST**

(Pure Radiotracers incubated at 37°C
for 2 hours
in **PBS** and **FBS**)



Results

Radiosynthesis for in vivo experiment
and stability test



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



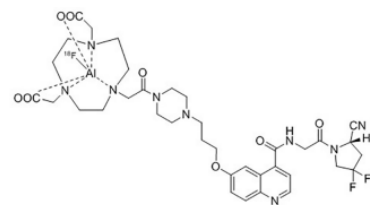
Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem

Radiochemical purity and stability test:

$[^{18}\text{F}]\text{AlF-NOTA-FAPI-04}$

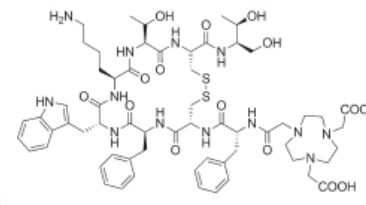


RCP=
 $\geq 98.51 \pm 0.19 \%$

Stability after 2h
RCP = $\geq 98.51\%$



$[^{18}\text{F}]\text{AlF-NOTA-RGDfK}$

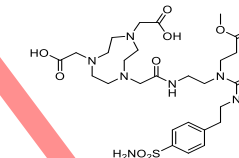


RCP=
 $\geq 97.41 \pm 0.8 \%$

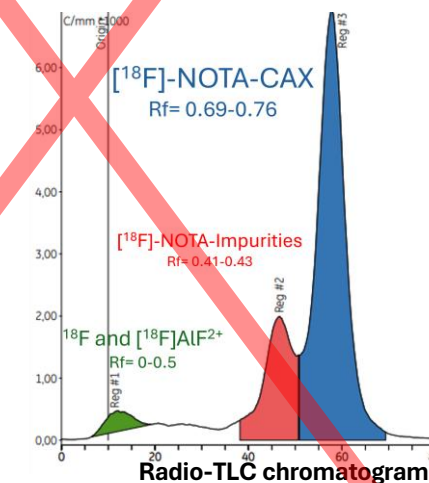
Stability after 2h
RCP = $\geq 96.61\%$



$[^{18}\text{F}]\text{AlF-NOTA-CAX}$



RCP=
 $\leq 95\%$



the NOTA-CAX precursor
will be purified by



UNIVERSITÀ
DEGLI STUDI
FIRENZE

NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE,
PSICOLOGIA, AREA DEL FARMACO E
SALUTE DEL BAMBINO

Loading...

Chemical synthesis of
multimeric Precursors

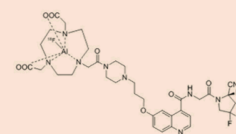
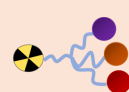


UNIVERSITÀ
DEGLI STUDI
FIRENZE

NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE
PSICOLOGIA, AREA DEL FARMACO E
SALUTE DEL BAMBINO

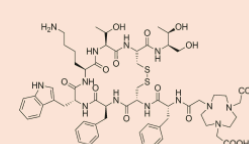
Radiolabeling with $[^{18}\text{F}]\text{AlF}^{2+}$ of radiotracers:

Mutimeric

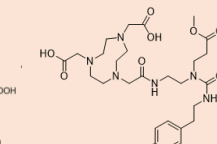


$[^{18}\text{F}]\text{AlF-NOTA-FAPI-04}$

Monomeric



$[^{18}\text{F}]\text{AlF-NOTA-RGDfK}$



$[^{18}\text{F}]\text{AlF-NOTA-CAX}$

**in vivo PET imaging
studies**

To evaluate the uptake and
biodistribution of novel
multimeric versus monomeric
radiotracers in
B16-F10 Melanoma syngeneic
murine model

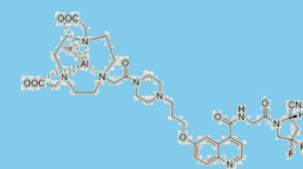
The protocol for animal
use has been approved by
the Ministry of Health



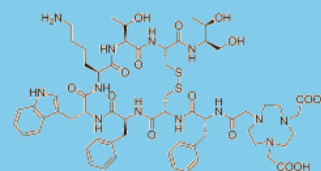
<https://doi.org/10.1007/s00259-023-06587-5>

Fine-tuning of:

1. The syngeneic mouse model of B16-F10 Melanoma
2. Imaging PET studies with Monomeric radiotracers



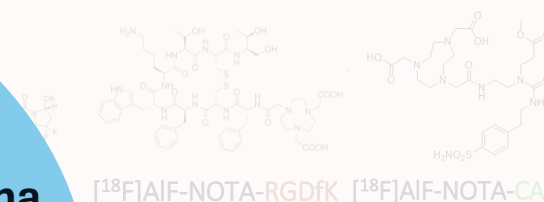
[¹⁸F]AIF-NOTA-FAPI-04



[¹⁸F]AIF-NOTA-RGDfK

Radiolabeling with [¹⁸F]AIF²⁺ of radiotracers:

Monomeric



in vivo PET imaging studies

To evaluate the uptake and biodistribution of novel multimeric versus monomeric radiotracers in B16-F10 Melanoma syngeneic murine model

The protocol for animal use has been approved by the Ministry of Health



<https://doi.org/10.1007/s00259-023-06587-5>



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



THE
Tuscany Health Ecosystem

THANKS YOU FOR YOUR ATTENTION

People involved(WP7) @CNR IFC

Luca Menichetti
Debora Petroni
Daniele Panetta
Patricia Iozzo

PhD (SSSUP)
Costanza Fabbri