BOOK OF ABSTRACT

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Valutazione dell'impatto nella Provincia Autonoma di Trento dell'introduzione della tecnologia emergente "Terapia con radioligandi (RLT) con [^{177}Lu]Lu-PSMA-617" nel trattamento del carcinoma prostatico

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Lo scopo dell'Health Technology Assessment (HTA) è quello di definire, tramite un approccio multidisciplinare, l'impatto clinico, economico, organizzativo, etico e sociale dell'introduzione, dell'implementazione o della dismissione di una specifica tecnologia. La definizione del valore di una tecnologia sanitaria è un'attività cruciale che consente un migliore utilizzo delle risorse disponibili e una consapevole programmazione degli interventi necessari ad una corretta gestione della stessa. L'HTA rappresenta, tenendo in considerazione tutte le proprie peculiarità metodologiche, un processo sistematico e riproducibile di valutazione, in grado di creare un "ponte" tra il mondo scientifico e quello politico-decisionale; i decisori politici richiedono infatti di conoscere le potenzialità, i vantaggi e gli svantaggi dell'utilizzo delle diverse tecnologie sanitarie al fine di poter valutare il beneficio derivante dal loro utilizzo e programmare l'offerta di salute nel rispetto del principio di efficacia ed efficienza, non dimenticando le aspettative del paziente [1] [2] [3]. L'oggetto della presente valutazione è il [^{177}Lu]Lu-PSMA-617, prima terapia con radioligandi (RLT) ad essere utilizzata nel trattamento dei pazienti con carcinoma prostatico resistente alla castrazione in progressione (mCRPC) esprimente l'antigene di membrana specifico della prostata (PSMA), che, in aggiunta allo *standard* di cura, si è dimostrata in grado di aumentare la sopravvivenza globale e la progressione libera da malattia, con un profilo rischio/beneficio favorevole [4].

Il radiofarmaco, attraverso un meccanismo target-specifico, si localizza con elevata specificità nel tessuto neoplastico prostatico esprimente l'antigene PSMA dove esercita l'effetto citotossico attraverso l'emissione di radiazioni β-, con un ridotto range tissutale ed un effetto minimo sui tessuti sani circostanti. L'approccio attraverso la RLT è una novità assoluta ed una tecnologia emergente nello scenario terapeutico del mCRPC.

Il radiofarmaco, che ha recentemente ottenuto l'approvazione EMA ed AIFA, è già inserito in varie linee guida internazionali ed ha ottenuto il punteggio massimo nella valutazione del beneficio clinico di ESMO (ESMO-MCBS v1.1, Scorecard version:1; Form 2a; last update 11/1/2023).

Il processo della valutazione di HTA oggetto di questo report si è sviluppato attraverso la creazione di un gruppo multidisciplinare che ha analizzato ed elaborato le informazioni disponibili sul radiofarmaco considerando globalmente l'impatto clinico, economico, organizzativo ed etico correlato alla sua introduzione ed al suo utilizzo. L'obiettivo è stato quello di creare una valutazione onnicomprensiva sulla tecnologia che ne consentisse una visione globale in tutte le sue dimensioni. Le caratteristiche legate all'utilizzo di [^{177}Lu]Lu-PSMA-617 hanno infatti permesso di toccare diversi punti inerenti alle problematiche cliniche del paziente con mCRPC, al ruolo del radiofarmaco in uno scenario terapeutico che è notevolmente mutato nel corso degli ultimi anni, a tutti gli aspetti organizzativi, gestionali e normativi associati all'utilizzo di un radiofarmaco, fino a toccare tematiche legate alla sostenibilità economica e di accesso alle cure, di importanza cruciale nel momento in cui una nuova tecnologia si apre al mercato e viene messa a disposizione dei pazienti.

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Production of Copper-61 from $^{nat}Zn(p,\alpha)^{61}Cu$ reaction route by solid target irradiation: preliminary results

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Background
Copper-61 (Cu-61) is emerging as a promising alternative PTE radioisotope for the development of copper-6X based radiopharmaceuticals, driven by its cost-effectiveness and favorable nuclear properties, such as a lower production cost and proper nuclear characteristics (Half-live of 3.34h, β^+) [1,2].
Different production pathways are now under investigation, with one particularly promising application involving the $^{nat}Zn(p,\alpha)^{61}Cu$ route by using medical cyclotrons. Alves [2] conducted a study utilizing a liquid target for this nuclear reaction, yielding positive results. The process involved initiating the reaction from a nitric zinc solution, showcasing the viability of such a method.
That being stated, we have explored the feasibility of applying the same pathway to a solid target instead, by using two different zinc-based materials: ^{nat}Zn and ^{nat}ZnO. The study aims at assessing and overcoming the material's limits posed by the low melting temperature of the zinc element, which is approximately 420°C. This temperature could present a potential issue with the increasing beam current, and the study seeks to address and mitigate any challenges associated with that.

Materials and methods
The ACSI TR19/300 proton cyclotron, located at the Radiopharmacutical department of IRCCS Sacro Cuore Hospital (Verona, Italy) with variable beam energy and equipped with a vertical solid target station was used to irradiate two different coin-shaped targets configurations: foil disc of ^{nat}Zn metal and sintered ^{nat}ZnO pellet

- Foil disc of 99.99% ^{nat}Zn 0.5 mm thickness.
- Pellet of 99.9% ^{nat}ZnO around 0.6 mm thickness and density around 99%.

A sandwich target configuration was explored about the disc foil configuration, incorporating a Nb backing target and an Al frontal foil, while for the pellet target one, we have innovated by developing a magnetic closing target capsule made of aluminum alloy.

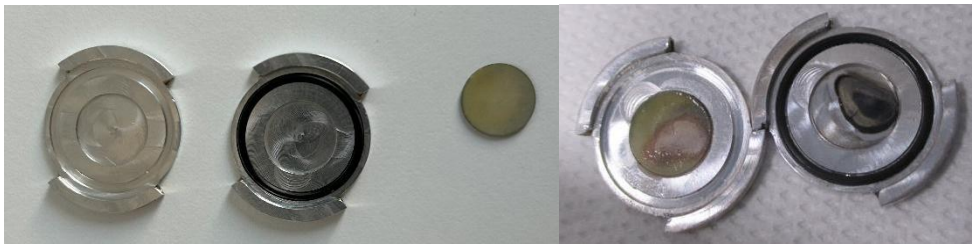

Figure 1. left, target capsule and ^{nat}ZnO pellet before beam and (right) after beam of 30 uA.

Results

This study involved target irradiations with increasing beam currents, starting from 5 µA, to assess the mechanical and thermal resistance of the ^{nat}Zn foil as well as ^{nat}ZnO pellet. The pellet successfully withstood the challenging conditions even at 30 µA without breaking (Figure 1 and Table 1). Various dissolution strategies, coupled with purification procedures, are currently being tested. Commercial resins, including Tk201, CU, and TBP, are being utilized in these tests.

Table 1. Irradiation parameters used for ^{nat}Zn foils and ^{nat}ZnO pellets.

Target	Beam E (MeV)	Beam on target (MeV)	Current (µA)	Time (min)
^{nat}Zn foil 1	18.6 MeV	14 MeV	5 µA	5 min
^{nat}Zn foil 2	18.6 MeV	14 MeV	15 µA	5min
^{nat}ZnO pellet 1	18.6 MeV	14 MeV	15 µA	5min
^{nat}ZnO pellet 2	18.6 MeV	14 MeV	30 µA	5min

Conclusion

The zinc oxide targets have withstood a 30 µA beam at an energy of 14 MeV, demonstrating their suitability for the production of copper-61.

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An assessment of the effects of autoclave sterilization on the stability of O-(2- [^{18}F]fluoroethyl)-L-tyrosine

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Background

O-(2-[18F]fluoroethyl)-L-tyrosine ([^{18}F]FET) is one of the first and most successful 18F-labeled amino acids for imaging amino acid metabolism in tumors [1]. The negative effect of heat sterilization on amino acids has been well documented in multiple studies, and in different fields of research, over the last decades [2, 3]: both essential and not essential amino acids seem to be similarly affected by autoclaving. In this study, a comparison between sterilized and non-sterilized samples of [18F]FET was carried out over seven hours; the samples were evaluated at three different times: right after the end of synthesis and dispensation (t0), four (t4), and seven (t7) hours

later. The study aimed to investigate the effects of heat treatments on [18F]FET, as well as executing an assessment of the stability of the diagnostic radiopharmaceutical.

Materials and methods

The synthesis of [^{18}F]FET was accomplished directly, using an appropriately protected derivative of tyrosine, in about 50 minutes. The radiochemical yield was about 23,3%. Autoclave sterilization was carried out at temperatures between 36°C and 137°C over about 8 minutes. Analysis of the sterilized and non-sterilized [^{18}F]FET was performed according to the fluoroethyl-L-tyrosine (18F) injection monograph, included in the European Pharmacopoeia 11.0 (EP) [4]. The following tests were performed at t0: pH, impurity A spot test, fluoroethyl-L-tyrosine and related substances, ethanol, residual solvents, bacterial endotoxins, radiochemical purity, and identification tests A, B, and C. Impurities C and D were detected via liquid chromatography, with a spectrophotometer at 225nm and a radioactivity detector connected in series. The tests for pH, fluoroethyl-L-tyrosine and related substances, ethanol, residual solvents, and for radiochemical purity were repeated at t4 and t7.

Results

Both sterilized (AC) and non-sterilized (NA) samples were compliant with EP specifications at t0, t4, and t7. A slight difference in the quantification of L-FET was observed in the test for chemical purity (Table 1).

Time	L-FET Area (AC)	L-FET Area (NA)	Variation (%)
t0	0,066	0,074	12,12
t4	0,064	0,072	12,50
t7	0,063	0,068	7,94

Table 1: Areas of L-FET observed via HPLC-UV, and variation between AC and NA samples. A similar pattern was noted in the test for enantiomeric purity, which also showed rising levels of D-FET for both AC and NA samples from t0 to t7 (Table 2).

Time	EtOH Area (AC)	EtOH Area (NA)	Variation (%)	AcN Area (AC)	AcN Area (NA)	Variation (%)
t0	370,937	419,865	13,19	0,689	0,806	16,98
t4	372,713	419,150	12,46	0,698	0,790	13,18
t7	368,720	417,943	13,35	0,659	0,773	17,30

Table 2: Areas of L-FET and D-FET observed via HPLC-UV, and variation between AC and NA samples. The tests for ethanol and residual solvents produced interesting results, as a modest variation in the content of ethanol and acetonitrile between AC and NA samples was observed throughout the study (Table 3).

Time	L-FET Area (AC)	L-FET Area (NA)	Variation (%)	D-FET Area (AC)	D- FET Area (NA)
t0	15,21615	16,35004	7,45	0,49607	0,55854
t4	13,99706	15,23518	8,85	1,26402	0,90409
t7	13,46965	15,01595	11,28	1,37409	0,73304

Table 3: Areas of solvents evaluated via GC-FID, and variation between AC and NA samples. DISCUSSION The high temperature achieved during the sterilization caused a mild L-FET degradation, which was observed via HPLC-UV in both the chemical and the enantiomeric purity tests. Interestingly, the radiochemical purity is not affected by the process: values higher than 99% were maintained throughout the study, as shown in the Supporting Information.
13% to 17% reduction of ethanol and acetonitrile suggests a significant influence of heat treatments on the evaporation of these two solvents. Although further experiments are needed to clarify the meanings and possible hazards of the rising levels of D-FET observed in the latter stages of our research, autoclave sterilization has proven to be easily applied in the routine preparation of a promising 18F-labeled amino acid for imaging cerebral and, possibly, peripheral tumors [5].

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High-purity ^{155}Tb production by hospital-cyclotrons: enriched ^{155}Gd targets at comparison

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Background

The γ-emitter ^{155}Tb (87 keV (32%) and 105 keV (25%)) is a good candidate for SPECT imaging and its long half-life allows the biodistribution of radiopharmaceuticals to be studied over several days. The interest in its production is on the rise, thanks also to the possibility to pair it with other Tb radionuclides for theranostic purposes [1]. However, to find feasible production routes for medical applications is still an open issue. This work focuses on the ^{155}Gd(p,n)^{155}Tb reaction. The challenge is to minimize the co-production of ^{156}Tb, with a half-life similar to ^{155}Tb, since it compromises the image quality and increases the patient absorbed dose due to its high-energy γ emissions [2].

Materials and Methods

The theoretical analysis is essential to identify the optimal irradiation conditions that maximize the production, limiting the harmful contaminants. Since the target purity is crucial for the production of high-quality ^{155}Tb as safe imaging agent, different levels of ^{155}Gd enrichment have been compared, namely 91.9%, 98%, 99%, and 100%. The theoretical cross sections have been calculated

with the TALYS code [3] and compared with the data available in the Literature [4,5]. Thick-target yields and radionuclidic purity (RNP) were obtained and dosimetric evaluations were accomplished using the OLINDA software [6], considering an injection of Tb-cm09 [1]. In addition, the dose increase (DI) was determined by combining the yield of all Tb radioisotopes produced with the dosimetric outcomes. Finally, to evaluate the quality of the ^{155}Tb-images, the Compton-to-peak ratio that expresses the noise contribution of high-energy γ-rays emitted by Tb-contaminants was assessed.

Results

The presence of ^{156}Gd as impurity of the enriched ^{155}Gd target may increase the production of the contaminant ^{156}Tb. The assessment of the RNP and DI illustrate that a 2% content of ^{156}Gd in the target is the maximum limit that still guarantees a safe clinical application. For the specific case the RNP of reference has yet to be established, however a 98% RNP value combined with a DI lower than 10% indicates a promising outcome. In addition, the comparison between the imaging properties of ^{155}Tb and ^{111}In (currently used in clinics) reveals comparable quality of the SPECT images.

Conclusions

This work identifies the adequate level of ^{155}Gd-enrichment of the target for the production of high-purity ^{155}Tb by using low-energy proton beams, suitable for hospital-cyclotrons. Its safe use in clinics as imaging agent requires at least a 98% enriched ^{155}Gd target. This is alternative to the use of a post-production mass spectrometry purification proposed in the Literature [4].

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Development of [^{99m}Tc][Tc(N)(PNP)]-based PSMA targeting agents: the PNP$_3$OH experience

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Introduction

[^{99m}Tc][Tc(N)(PNP)]-approach has long been used to label biomolecules. The most important advantage of this technology is its high chemical flexibility that allows for a fine modulation of the chemicalphysical properties of the corresponding tagged targeting vector; nevertheless, the usage of traditional alkoxyalkyl PNPs leads to the need for heating overextended times to attain high radiochemical yields. These conditions are not suitable for labeling temperature-sensitive biomolecules. Water-soluble phosphines are an attractive class of oxidatively stable ligands that generate stable hydrophilic chelates with good pharmacokinetics. Modification of the substituents on the P atoms induces the variation of electronic and sterical properties of the ligand, which affect its reactivity for the metal ions, influencing the reaction rate, and the stereochemistry of the final complex. Hence, the water-soluble (ws) PNP_3OH, $\{[(OHCH_2)_2PCH_2CH_2]_2NCH_2CH_2OCH_3\}$, was designed and the effect of the substituents on the corresponding ws-$[Tc(N)(PNP_3OH)]^{2+}$- framework was investigated for the preparation of target specific compounds at room temperature and mild reaction conditions. Within, we reported our experience in the usage of [^{99}mTc][Tc(N)(PNP_3OH)]-framework to label PSMA targeting molecules, including a small molecular weight PSMA inhibitor and the fragment scFvD2B for PSMA imaging.

Materials and Methods

PNP3OH was synthesized and characterized. The biomolecules mentioned above were conjugated with a terminal cysteine residue, cys~, to allow the coordination of the ws[^{99m}Tc][Tc(N)(PNP_3OH)]-synthon. scFvD2B does not contain reactive Cys residues; hence, it was derivatized via a site-specific enzymatic reaction catalyzed by transglutaminase (TGase), with the H-Cys-GlyLys-Gly-OH tetrapeptide (H3Cys). Radiosyntheses were efficiently performed using a two-step reaction. The receptor specificity of the radiolabeled biomolecules was assessed in-vitro in pertinent cell lines.

Results

The insertion of water-soluble groups on PNP actually improves the reactivity of ws[^{99m}Tc][Tc(N)(PNP_3OH)]-framework towards cys~. Radiosyntheses were performed efficiently under physiological conditions at RT in 30 min, using a concentration range of 10-5 -10-6 M of cys-conjugated biomolecules. Data from in vitro studies clearly show that PSMA targeting molecules labeled with [^{99m}Tc][Tc(N)(PNP_3OH)]-synthon preserve their receptor targeting ability with high level of cellular uptake and internalization. In vivo studies are in progress.

Conclusions

Data support the effective application of [^{99m}Tc(N)(PNP_3OH)]-technology to labeling molecular effectors including temperature-sensitive protein derivatives. Acknowledgment to Bracco Imaging and AIRC (IG-2020 ID 24528) for financial support.

Automated Production of [^{68}Ga]FAPI-46: Evaluation of Process Performance Over 3 Years of Clinical Use

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Background

The fibroblast activation protein (FAP) is a type 2 transmenbrane serine protease that is selectively overexpressed on the so-called cancer associated fibroblasts (CAFs) in the stroma of many

malignant neoplasms. [1-3] [^{68}Ga]FAPI-46 is one of the most clinically investigated FAP-targeting tracers, with a widespread application in the diagnosis by PET/CT of a large spectrum of tumor types. [4-6]

Aim

Our group recently developed an automated synthesis method able to provide [^{68}Ga]FAPI-46 with high radiochemical purity (RCP) and good yield for PET/CT imaging within two prospective monocentric investigational trials (EudraCT:2021-006570-23 and EudraCT:2020-005549-17). Therefore, the aim of this work was to assess the long-term performance of [^{68}Ga]FAPI-46 production in our hospital setting over the 3 years period of the research studies (from 2021 to 2023).

Materials and Methods

The radiosynthesis of [^{68}Ga]FAPI-46 was performed in a fully-automated synthesis module (PharmTracer, Eckert & Ziegler) using the eluates of 2 GalliaPharm generators (1850 MBq, Eckert & Ziegler). Radiolabeling was carried out by heating to 95°C a mixture of FAPI-46 precursor, ascorbic acid, $^{68}GaCl_3$ and acetate buffer for 15 minutes. The resulting product was purified via a C18 SPE cartridge, eluted with EtOH and saline in a vial containing Vitamin C as stabilizer, and sterilised by passing through a 0.22-µm filter before dispensing. To assess process efficacy along time we measured labeling yield, RCPs, and number of injected patients per batch.

Results

Over a 30 months period, 43 synthesis of [^{68}Ga]FAPI-46 for clinical purpose were carried out, and the mean decay-corrected yield was 91,1±0.8% (range 85,8-97,6%), with minimum fluctuations even if batch activity decreased proportionally to generators shelf-life (Figure 1). Mean RCP value as determined by RP-HPLC was 99.5±0.1% and the quantity of colloids from radio TLC was 0,3±0.1%. pH, ^{68}Ge content, endotoxins and sterility were in accordance with the European Pharmacopoeia. 65 patients with different lung, ovarian, head, neck, breast cancers and sarcomas (EudraCT:2020-005549-17) and 63 patients with lung cancers (EudraCT:2021-006570-23) were subjected to [^{68}Ga]FAPI-46 PET/CT, with up to 7 injected patients per batch imaged by 4 PET/CT scanners acquiring simultaneously.

Conclusions

In this study, the performance of synthesis process for [^{68}Ga]FAPI-46 was evaluated, demonstrating quantitative labeling yields along with high reproducibility over a wide range of theoretical activities of eluted $^{68}GaCl_3$ (range 1522-2598 MBq, Figure 1). Moreover, the developed synthesis method allowed the daily production for multiple patients' PET imaging of [^{68}Ga]FAPI-46 with highly stable RCP values and radiopharmaceutical quality during the time, thus assessing great process robustness. In conclusion, our results suggest that the developed process can provide high throughput clinical activity along with the possibility of extending the fields of application of FAPI-PET modality to new clinical protocols.

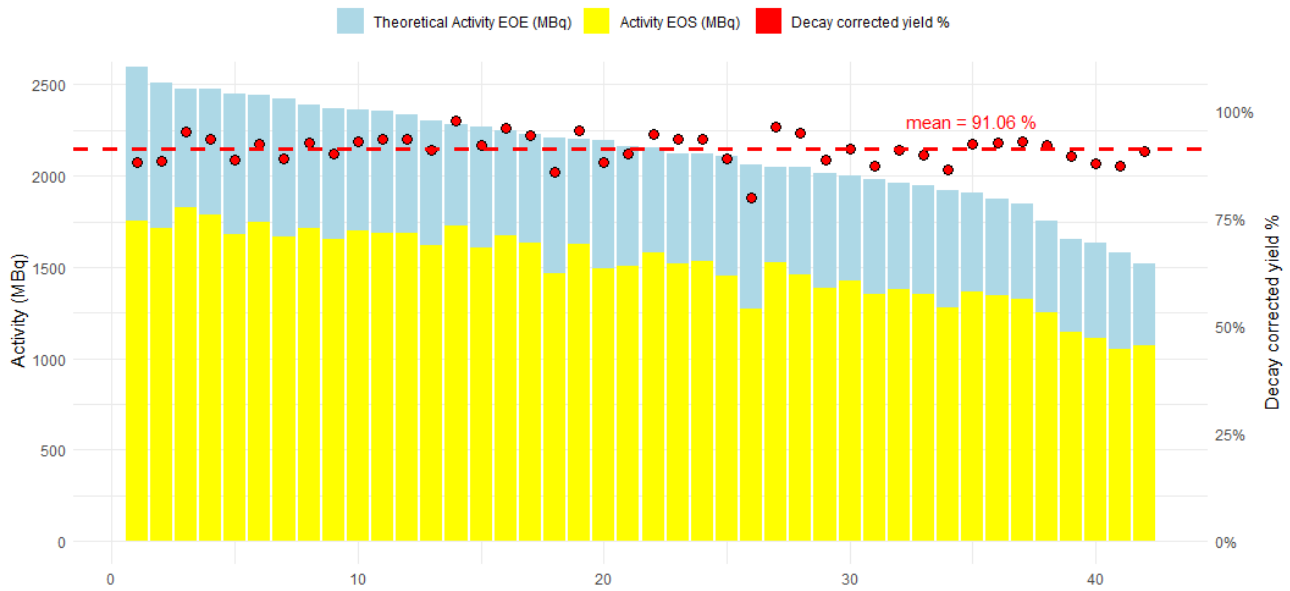

Figure 1: Performance of synthesis process for [^{68}Ga]FAPI-46

The batches were sorted in descending order of theoretical EOE activity, calculated according to decay laws, generators shelf-lives and elution yields. Labeling yields were corrected for the decay in 25 minutes. EOE = end of elution; EOS = end of synthesis.

Acknowledgement

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Development of a topical application device for non-melanoma skin cancer therapy

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Background

Non-melanoma skin cancers (NMSC), known as keratinocyte cancers, are most common malignancy worldwide. Incidence rate is high in Europe with 98% person-years. Basal cell carcinoma (BCC) and

squamous cell carcinoma (SCC) of the skin make up 99% of all NMSC. These cancers can usually be eradicated by surgery and local radiation treatment. A new method using topical application of radionuclides can been introduced as an effective alternative. The use of such radioactive skin patches involves individualized preparation and application to correlate with the patient-specific anatomical requirements. The topical use of radioisotopes offers an effective, simple approach and expected cost-effective technology in many cases. The objective of this work is to determine the ideal materials with which to formulate a patch ensuring a homogeneous distribution of the radioisotope, good radiological safety handling with minimal contamination of patients and staff. We have focused on the use of alpha-emitting patches because their high linear energy transfer (LET) results in the release of large amounts of energy over very short distances.

Materials and methods

We utilized the radioisotope Ra-223, as RaCl solution, impregnated in a scaffold, paper, or mixed with a resin material to form a uniform thickness layer. A homogeneous radioactivity distribution was determinate by phosphorescence imaging using Cyclone, to create a standard that allows us to calculate the activity present in each prepared device. As a basic material to form the scaffold or device we used polymeric type mixtures with cornstarch dispersions at concentrations between 10-30%, polyvinylpyrrolidone solution at 15%, gelatin solutions at concentrations between 20-30% and hydroxypropyl methylcellulose dispersions at different concentrations 2-30% with the inclusion of 10% glycerol in all case, as a plasticizing agent. In each case, rheological characteristics were observed at the time of making the patch, and the distributions of the Ra-223 activity were determined, using for this the calibration curve elaborated.

Results

For scaffold made with gelatin and polyvinylpyrrolidone solutions, viscosity was insufficient to maintain the dispersion loaded with the radioisotope, on the polymeric base, which makes it impossible to avoid contamination of the patient. On case of cornstarch, activity distribution was not uniform. Meanwhile HPMC dispersion at 30% with glycerin 10% showed best viscosity characteristics and most uniform distribution of activity.

Conclusions

HPMC dispersion was, of those studied, the one that showed the best results in applicability and distribution of activity. It is necessary to optimize the manufacturing process, and later test the operation in cell culture.

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Feasibility of LASER-assisted radiolabelling: the case of [^{68}Ga]Ga-MAA

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Background

^{68}Ga radiolabelling of commercially available Macro-Aggregate of Albumin (MAA) kits has recently gained interest, due to the availability of ^{68}Ge/^{68}Ga generators and the enhanced spatial resolution offered by PET imaging, with superior imaging characteristics and quantification capabilities. There are numerous clinical investigations focusing on the evaluation of [^{68}Ga]Ga-MAA, but setting up a standardized and effective production procedure remains crucial.. Conventionally, a heating

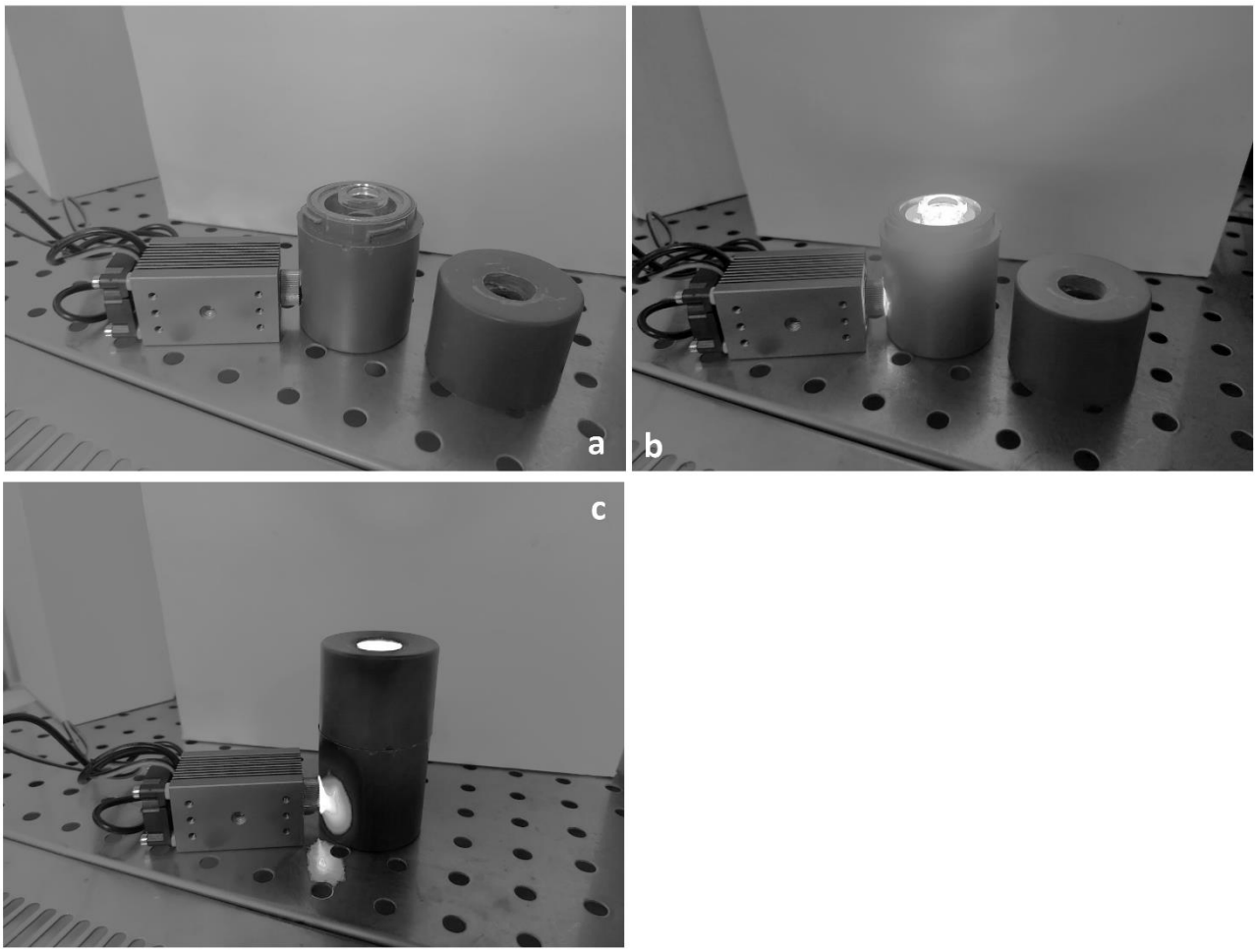
procedure up to 90°C is employed. Nevertheless, this method is associated with temperature-induced partial disintegration of MAA particles, leading to the need for an additional step of purification.

In this study, we present the outcomes of a novel radiolabelling LASER-assisted method for [^{68}Ga]Ga-MAA preparation

Materials and Methods

Three vials of MAA (Pulmocis ®) were reconstituted with 4 ml of saline each. To each vial, 200 MBq of buffered ^{68}Ga solution in 1.25 mL were added. Vial-1 was kept at room temperature (RT) for 15 min, Vial-2 was heated at 75 °C for 15 minutes, and Vial-3 underwent irradiation with a blue LASER (Techhodd PWM/TTL Blue laser OEM Module, China) (wavelength 450 nm, power 7 W, frequency 30 Hz) for 15 minutes in a custom-built facility designed to shield irradiation. The temperature of Vial-3 was monitored during irradiation using a thermocouple thermometer.

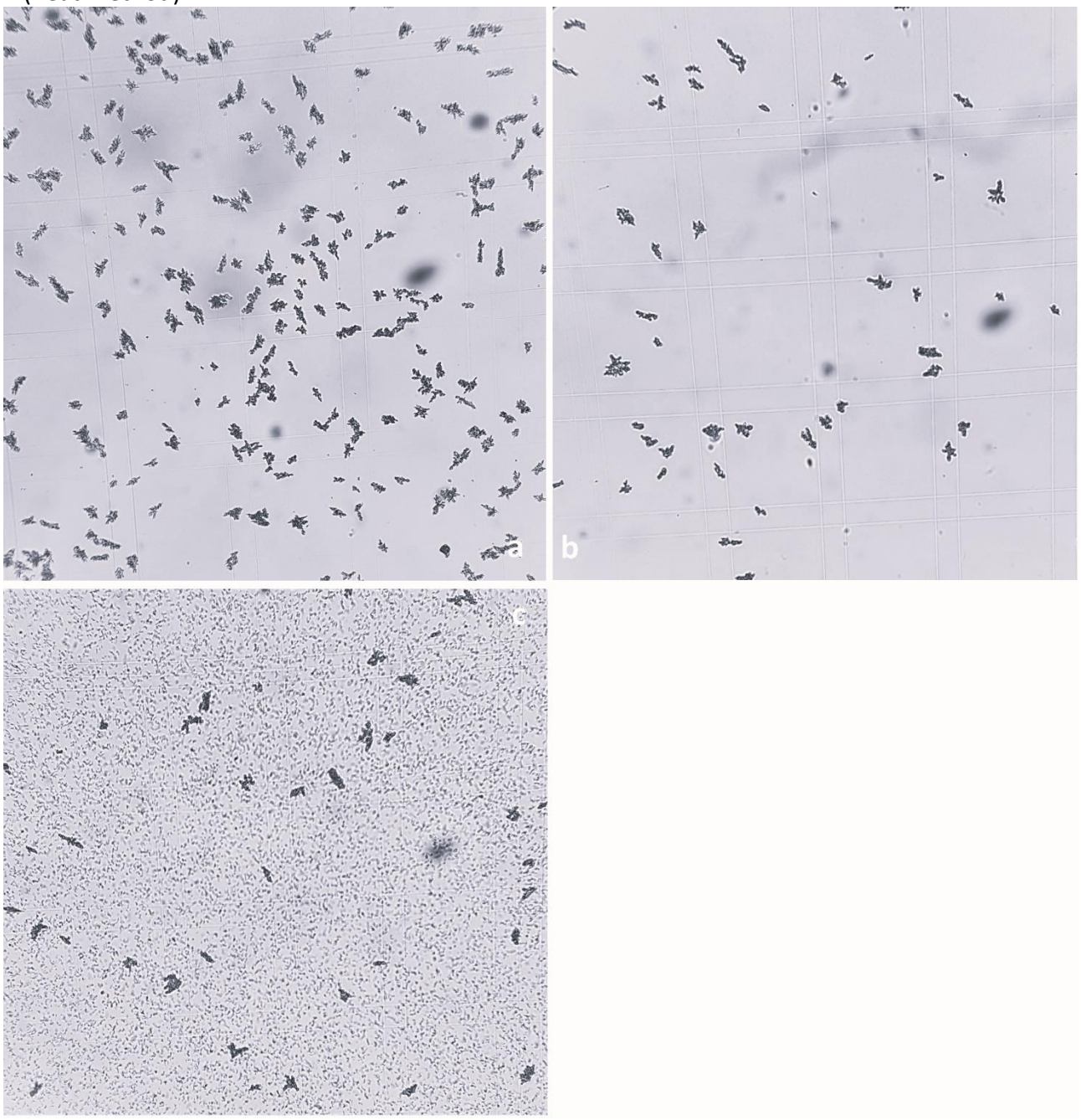
The Labelling Yield was assessed after the labelling procedure using thin-layer chromatography, with ITLC-SG and as mobile phase 0.1 M tribasic citrate solution. Stability tests in serum were also conducted. Particle dimensions were measured using a series of two polycarbonate membrane filters (3 µm and 400 nm pores) and observed in Burker chamber. The particle sizes were then compared to those obtained after standard ^{99m}Tc labelling.



Extremely simple equipment used for LASER-assisted radiolabelling: LASER, standard lead shielded containers for vials with a drill hole in the lead armor of 1 cm at the base and the MAA kit vial (a). (b) and (c) show how it can be easily opened and closed in LASER-operating mode.

Results

Labelling Yield (LY) of [^{68}Ga]Ga-MAA obtained in Vial-1 was very low (38%), while reached 88 % in Vial-2 (heat method) and to > 95% in Vial-3 (LASER method). In vial-3 only a minimum increase of temperature, up to 38°C, was reported. LY was stable after serum incubation. Moreover, the percentage of particles with size <400 nm was negligible in Vial-1 and Vial-3 (about 1%), similar to the results of routine ^{99m}Tc labelling procedure, while it was dramatically higher, up to 68%, in Vial-2 (heat method).



[^{68}Ga]Ga-MAA at room temperature (a), LASER (b) and 75° C (c) radiolabelling in Burker chamber in comparison.

Conclusion

LASER assisted radiolabelling of [^{68}Ga]Ga-MAA assures a labelling yield higher than the usual heating method and does not affect the MAA size, thus avoiding the need of further purification steps necessary for the methods proposed so far. LASER Induced chemical reaction is an interesting

approach under development, with no previous applications in the field of radiopharmaceuticals production.

In vitro evaluation of [^{18}F]NaF as potential radiopharmaceutical in imaging of cardiac amyloidosis vs [^{99m}Tc]Tc-DPD

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Background Cardiac amyloidosis (CA) consists of a group of diseases characterized by a deposition of insoluble and misfolded proteins in various organs. Cardiac involvement is responsible of survival and quality of life. Until now, different Technetium labelled bone seeking agents such as DPD, PYP and HMP are recognized as highly accurate for the non-invasive diagnosis of transthyretin (ATTR) cardiac amyloidosis
^{18}F-fluoride ([^{18}F]NaF) is a radionuclide approved for bone metabolism studies in Positron Emission Tomography (PET), that raised our interest as a potential alternative for ATTR diagnosis.
Compared to ^{99m}Tc bone-seeking agents, commonly employed for CA-ATTR diagnosis, the positron emitter [^{18}F]NaF benefits from the improved spatial resolution of PET imaging, offering superior imaging characteristics and quantification capabilities. These features could potentially lead to better diagnostic performance, especially in the early stages of the disease.
To date, few data are available about the actual affinity of [^{18}F]NaF for fibrils. The aim of this study was to evaluate, as an essential preliminary step for planning a future clinical study, the *in vitro* affinity of [^{18}F]NaF for synthetic fibrils, in comparison with [^{99m}Tc]Tc-DPD.
Materials and Methods
Amyloid fibrils were generated dissolving 10 mg of insulin in an aqueous solution of HCl (pH 2.0) and incubating the solution at 55°C to induce fibril formation.
Ten vials were prepared, each with 120 uL of fibril preparation. In five vials, increasing amounts of [^{18}F]NaF (50, 100, 200, 500, 1000 kBq) were added and brought to a volume of 2 mL with saline solution. In other five vials, increasing amounts of [^{99m}Tc]Tc-DPD (50, 100, 200, 500, 1000 kBq) were added and brought to a volume of 2 mL with saline solution. As per protocol for bone acquisition, the [^{18}F]NaF vials were incubated for 60 min, while the [^{99m}Tc]Tc-DPD vials were incubated up to 180 min. Labelling yield was subsequently evaluated using ITLC-SG and a mobile phase consisting of acetonitrile and water (9:1) for [^{18}F]NaF, and TLC silica gel and acetone and methanol (1:1) for [^{99m}Tc]Tc-DPD.
Results
The binding kinetics of both [^{99m}Tc]Tc-DPD and [^{18}F]NaF followed a specific-type pattern even if at different concentration, characterized by a sigmoidal shape suggestive of a BET isotherm model. Notably, [^{18}F]NaF exhibited a higher affinity than [^{99m}Tc]Tc-DPD.
Conclusion
Our findings suggest that [^{18}F]NaF may possess superior binding ability for amyloid fibrils compared to [^{99m}Tc]Tc-DPD. Potentially, it could provide to be a more effective tracer for cardiac amyloidosis imaging. Further clinical investigations are needed to validate the diagnostic utility of [^{18}F]NaF in comparison to [^{99m}Tc]Tc-DPD and to elucidate its role in clinical practice.

^{68}Ga-Labelling of the potential theragnostic agent NOTA-R54 on an E&Z ModularLab PharmTracer

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Introduction

CXCR-4 is a receptor abundantly expressed in different human cancers and involved in tumor growth, invasion, angiogenesis and metastasis. Its crucial role in tumor progression has made it an interesting target for the development of new theragnostic radiopharmaceuticals. Recently a new family of low-molecular weight cyclic peptides has been designed by engineering the N-terminal region of CXCL12, the endogenous ligand, with a chelating agent (NOTA) using an amide linkage (AMBHA).[1] By so doing the resulting molecule, named NOTA-R54, can be labelled with either ^{68}Ga for PET diagnosis and with appropriate alpha/beta emitters for therapy. We are currently investigating this opportunity in a PNRR-funded study (PNRR-POC-2022-12376329) led by IRCCS "Fondazione G. Pascale" (Naples) in collaboration with University "Federico II" (Naples).

Material and Methods

The precursor NOTA-R54 was aliquoted in H_2O TraceSELECT™ and kept for months at -20°C. Radiolabelling with ^{68}Ga was carried out on an E&Z ModularLab PharmTracer fully automated cassette module, using an E&Z GalliaPharm ^{68}Ga generator . A pre-purification of the generator eluate on a SCX cartridge and a final purification on a C18 SepPak were applied. Different reaction times, temperatures, pHs and precursor amount were tested in order to optimize both radiochemical yield and radiochemical purity of the radiolabelled peptide. The latter was investigated by means of two differents radioTLC methods (iTLC-SG with MeOH/$AcONH_4$ (0.77g/l) 1:1 or Na citrate (pH 5) as mobile phase). Gradient HPLC analyses (eluent A: H_2O with 0.1% TFA; eluent B: MeCN with 0.1% TFA) were carried out on a Kinetex EVO 150 x 4.6 mm, 5 µm (0-5 min 10% B, 5-25 min 10→50% B; flow rate 1 ml/min) and on a ACE C18 150 x 3 mm, 3 µm (0-0.5 min 5% B, 0.5-10 min 5→40% B, 10-12 min 40% B; flow rate 0,6 ml/min). A UV detector set at 220 nm and a Raytest detector Gabi Star were placed at the columns exit. In a preliminary study, the Kinetex-EVO column was associated to a Diode array to determine the correct absorption wavelength of the peptide (228 nm).

Results

Radiochemical yields above 80% (d.c. at SOS) were obtained in ca. 16 min by using 50 µg precursor dissolved in 600 µL of acetate buffer at pH 4 and a 90°C x 240 s reaction. The iTLCs showed the absence of free or colloidal ^{68}Ga, while on radioHPLC the radiochemical purity was 95-96%. Any peak observed on the UV chromatograms was always of much lower intensity than the precursor one.

Discussion

The 95% radiochemical purity observed can probably be further improved in view of the fact that the old batch of precursor in our hands showed only an 80% purity on the HPLC. However, these impurities were almost entirely removed by the final C18 purification. Thus, apart from applying new reaction conditions, additional tests will be carried out starting from a new, fresh batch.

Finally, tests of the radiotracer on NSCLC-PDX models are expected to start soon.

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Sustainable production of ^{67}Cu with medical cyclotrons: target manufacturing technology development and recovery process optimization

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Introduction

Radiopharmaceuticals labelled with Cu-radioisotopes have shown great potential to advance the theranostic approach in nuclear medicine [1]. ^{67}Cu is witnessing growing attention due to its favourable physical properties. However, its production in high yield is still limited. A multilayer target composed by ^{68}Zn and ^{70}Zn is promising for its production with high-energy proton cyclotron [2]. Moreover, ^{70}Zn(p,α)^{67}Cu nuclear reaction could be used exploiting low-medium energy medical cyclotron. In both cases, the expensive material ^{70}Zn-enriched, is anyway needed.

The LARAMED group at INFN-LNL [3] has started R&D activity on target manufacturing and material recycling, considering ZnO as the starting material (CUPRUM_TTD project). In collaboration with Padova and Ferrara Universities, and IRCCS Sacro Cuore Don Calabria Hospital (SCDCH), target manufacturing and recovery process have been developed to demonstrate the feasibility of reusing the costly isotope-enriched material for further targets productions. These are important steps in closing the technology loop for an economically sustainable ^{67}Cu production with medical cyclotron. Irradiation tests and material analysis have guided the optimization processes of target manufacturing and recycling.

Materials and methods

^{nat}ZnO powder was sintered using the Spark Plasma Sintering technique with the TT_Sinter machine [4]. Two target configurations were tested: the pellet, closed on an Al-holder, and the pellet bonded to a backing disc (Au-Nb).

The powder, pellets and targets were analysed by SEM and XRD. Irradiation tests were performed using the ACSI TR19-300 cyclotron at SCDCH at increasing proton beam current, to prove the thermal stability of the targets.

Two different recovery processes were tested, both on ^{nat}ZnO under powder and pellets form. The first one relies on zinc precipitation with ammonium oxalate, while the other with sodium hydroxide. In both procedures, the precipitate is treated in a muffle furnace at 300°C for conversion to ZnO.

Results

The manufacturing technology for the realization of ZnO pellets by SPS was established. The ZnO pellets had high density (95% with respect to bulk density) and are strong enough to withstand loading/unloading at the cyclotron target station and the irradiation at 50 μA, 19 MeV in both configurations. The ZnO pellet and the backing disc are well attached, as confirmed by SEM cross-sectional images and the visual inspection after the irradiation.
The zinc precipitation in the form of oxalate yields a recovery rate of ~65%, whereas the precipitation as zinc hydroxide, of 95%. In this latter case, the particle size distribution of the recovered powder is comparable to that of the commercial powder. Indeed, it was successfully reused for manufacturing new pellets and target (ZnO/Au-Nb) with the same parameters. The characteristics of the rec-pellets were comparable with the pellet obtained with the commercial powder.

Conclusions

The possibility of producing ^{67}Cu radioisotope in hospital is of great interest in the implementation of routine supply for preclinical studies. In this work, we have developed ZnO targets capable of withstand 1 kW/cm^2 thermal power and the material recycling process to make this production route economically viable. The next step will be the use of ^{70}ZnO materials for the first batch of ^{67}Cu for radiopharmaceutical studies.

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Development of a facile radiosynthesis procedure for [^{99m}Tc]Tc-PSMA I&S production employed for-SPECT Imaging and Radioguided Surgery

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Introduction

Prostate Specific Membrane Antigen Radio-Guided Surgery (PSMA-RGS) with [^{99m}Tc]Tc-PSMA I&S holds potential for identifying lymph node invasion in both primary and salvage extended pelvic lymph node dissections. A procedure for preparation and quality control of [^{99m}Tc]Tc-PSMA I&S was developed and an IMPD was prepared for authorities approval.
Radiosynthesis proceeds through the intermediate formation of a weak complex of technetium with tartrate followed by transchelation with the chelating group of PSMA to give the more stable final complex. The process was optimized in order to minimize the formation of radiochemical impurities, including diastereoisomers that could occur due to the presence of the Tc=O group.

Material and methods

The influence of different parameters as temperature, reaction time, pH and disodium tartrate quantity was investigated to optimize the method. In particular, time and temperature was found to have a great impact on the formation of the two diastereoisomers of the metallic complex, while tartrate quantity affected the formation of hydrolysed compounds of technetium. The analytical HPLC method developed allowed to discriminate each impurity, including diastereoisomers. Given the absence of reference standards to identify the diasteroisomers and the lack of biologic data on difference of behaviour in vivo, we have chosen to optimize the process in order to obtain only the more thermodynamically stable compound.

[^{99m}Tc]Tc-PSMA I&S was prepared starting with 40 µg of PSMA I&S in presence of disodium tartrate, stannous chloride and ascorbic acid in phosphate buffer at pH 7. After addition of pertechnetate, the mixture was reacted at 110°C for 20 minutes. Purification was performed after cooling down at room temperature by solid phase extraction on C18 light cartridge. Product was eluted with EtOH/NaCl 0.9% 1:1 and diluted with NaCl 0.9% before filtering on 0.22 µm-sterile filter. Specific analytical procedures were developed for the quality control of the radiopharmaceutical; in particular radiochemical purity was determined by HPLC coupled with radioTLC to quantify all potential rardiochemical impurities. Moreover, quality control involves other test for radionuclidic identification, chemical identification, pH, aspect and filter integrity.

Results

Synthesis is completed in about 40-45 minutes and yields is 84,8 ± 4,4%. 52 productions were made until now for patient administration and we registered only one failure due to an operator error. Analytical chromatographic method is reliable and able to individuate radiochemical impurities that radioTLC alone or other literature HPLC method cannot appreciate. Radiochemical purity was 96,6 ± 1,0% and the diastereoisomeric impurity was always below 3%.

Conclusion

The availability of a GMP precursor and the absence of a commercial kit spurred us to develop a simple and reproducible method for the production of [^{99m}Tc]Tc-PSMA I&S for use in clinical settings. The method developed achieved the proposed objectives and is currently employed in a clinical trial.

Production and Quality Control of [^{68}Ga]GaFAPI46: Development of an Investigational Medicinal Product Dossier for Clinical Trials for a multicentric clinical trial

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Introduction

FAPI-46 is a quinoline-structured inhibitor of Fibroblast Activating Protein (FAP) a type II membrane serine protease overexpressed by tumour stroma-associated fibroblasts (CAFs). FAP is also associated with a poor prognosis in cancer patients and it’s involved in biological mechanisms of tumour development, for this reason actually FAP is an ideal target for diagnostic and therapeutic

radiopharmaceuticals. [^{68}Ga]GaFAPI-46 is an experimental drug useful for PET imaging of tumour tissues that overexpresses FAP.
The aim of this abstractis to describe the structure of an investigational medicinal product dossier (IMPD) for a multicenter clinical trial.

Materials and methods

Our clinical trial invlolves two clinical centers. The drug substances described in the IMPD are precursor FAPI-46 (Sofie) and Gallium-68 obtained by Ge-68/Ga-68 generator GalliaPharm (Eckert Ziegler) with marketing authorization. The radiolabelling of [^{68}Ga]GaFAPI46 is carried out by two different synthesis modules palced in the two clinical centers, in particular Eazy (Eckert Ziegler) and MiniAIO (Trasis). Consumables and the reagent kit are different in the two centers based on the different manufacturing process. The quality control equipments are different, but the release specifications are the same.

Results

The IMP produced in the two sites consists in a multidose solution of [68Ga]Ga-FAPI-46 with a radioactive concentration between 50-70 MBq/ml at the End of Synthesis (EOS) that is considered ART. Acceptance criteria, specifications, and release timing are the same for both centers and were chosen in compliance with the general texts and monographs of the current European Pharmacopoeia. All the tests, except sterility are carried out before the release. The sterility test are performed by the same external Laboratory. The validation of the analytical procedures, the acceptance limits, and the parameters considered (specificity, linearity, range, accuracy, precision, quantification, and detection limit) were carried out by the two centers according to the ICH guideline Q2(R1). Both sites performed process validation by three different batches of [^{68}Ga]GaFAPI-46. Each batch was fully characterized from the analytical point of view, to confirm the compliance with the established acceptance criteria. The acceptance criteria were verified also to verify the two-hour stability at room temperature for all three validation batches.

Conclusion

This work demonstrates that [^{68}Ga]Ga-FAPI-46 can be prepared as an IMP by different centers involved in the same clinical trials.In this case Regulatory Agency requires a single integrated IMPD detailing both manufacturing processes. The center applicant need to demonstrate the consistency of radiopharmaceuticals produced at the different sites, justifying and detailing any differences in manufacturing processes, controls, and/or specifications. The dossier should report the process validation obtained for each site, while information common to both sites should be reported only once.

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[^{18}F]F-JKPSMA7 from HPLC to SPE purification.

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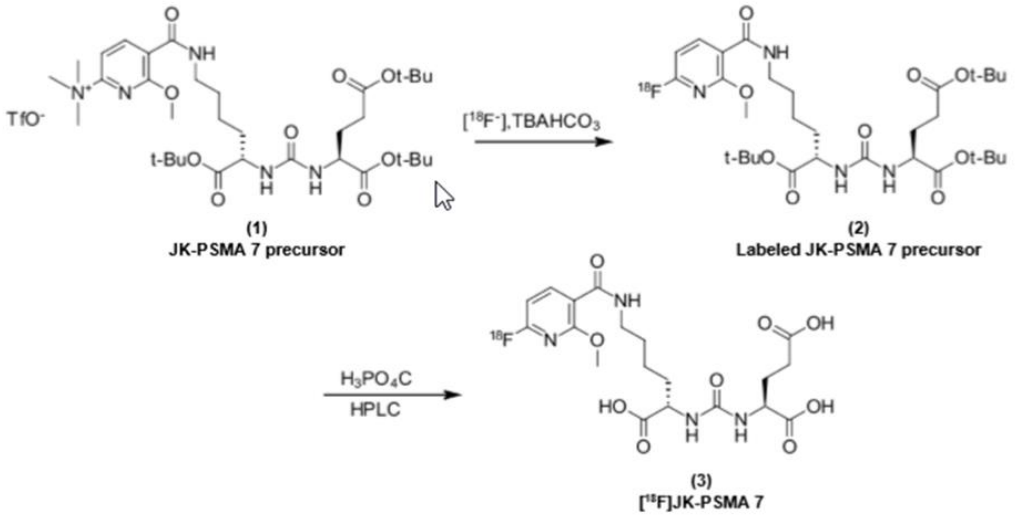
Background

18F-JK-PSMA-7 is a new promising PSMA specific tracer under clinical evaluation to evaluate the prostatic malignancy recurrency in oncologic patients. [1] The radiopharmaceutical is produced in a Trasis synthesis module through nucleophilic aromatic substitutions followed by acid deprotection and purified by HPLC. HPLC purification is a bottleneck step on Rph production due to the limited numbers of synthesis modules equipment with HPLC and due to the multiples possible failure caused by this technique.



To overcome these limitations, we had to study a possible purification pathway to reduce the synthesis steps and time and make synthesis stability and easiness.

Materials and methods

The [^{18}F]F-JKPMSA7 was produced on miniAIO TRASIS synthesis module, the reaction was copied from the AIO procedure up to crude reactor step. On this step the reaction was quenched by an appropriate solution and then transferred out to the module.
The crude reaction solution was diluted with different solvent like pure distilled water or physiological solution. The final dilutes solutions were divided on multiple samples to assess different SPE purification methods.
SPE cartridges tested were supplied by:

- C18, C18 Long, OASIS, HLB, Al, QMA were supplied by Waters GmbH.
- C18 ec was supplied by Mackerey-Nagle.

Starting from the different solutions with a radioactive HPLC profile shown in figure 1, we would like to obtain a similar qualitative HPLC profile recorded from [^{18}F]F-JKPMSA7 purified through HPLC.

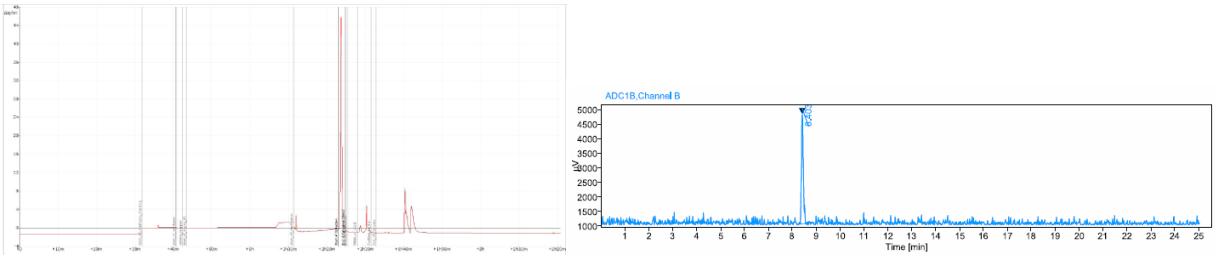


Figure 1. Crude reaction solution (up) and HPLC purified [^{18}F]F-JKPMSA7 (down).

Results

Evaluating the profile of the reactor solution we identify some impurity like free fluorine-18, partially deprotected moieties and the product. C18 trapping was evaluated in different sorbent materials and amounts to better understand the best trapping conditions. Water wash allows to remove all the inorganic impurities and left the product on cartridge. fractionated elution with different ethanol concentrations allowed to find the best ratio to use, final Aluminum cartridge was used to completely remove the free fluorine-18.

Conclusion

The SPE purification applied to radiopharmaceutical preparation simplifies the operation and increases the yield reducing synthesis time. The quality control profile is to comply with the [^{18}F]F-JKPMSA7 monograph draft published in Pharmeuropa .

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^{177}Lu and ^{161}Tb: comparison of cell damage produced by somatostatin analog radiopharmaceuticals

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Introduction

^{177}Lu-radiopharmaceuticals (RPs) are currently the most widely used for targeted radionuclide therapy (TRT), as they have demonstrated favorable safety and good response rates to treatment [1], but the worldwide ^{177}Lu availability is limited [2]. To overcome this problem, and being the decay properties of ^{161}Tb quite similar to those of ^{177}Lu, its use has been proposed for TRT [3]. Similarly to ^{177}Lu, ^{161}Tb emits relatively low-energy β^-particles (E_{avg}=154 KeV) and low-energy photons (48.9 keV (17%) and 74.6 keV (10%)), useful for SPECT imaging. In addition it also emits a significant number of internal conversion (IE) and Auger electrons (AE) with energies ≤ 40 keV, which could be advantageous for improving therapeutic efficacy [4]. The aim of this study was to evaluate and compare the biological damage produced by ^{161}Tb-somatostatin (SST) and ^{177}Lu-SST analog RPs localized in different regions within pancreatic tumor AR42J cells [5].

Materials and methods

The biological damage caused to AR42J cell clusters of different sizes by three different SST analog RPs, labeled with ^{161}Tb or ^{177}Lu and located in different regions within the cells, was obtained with the MIRDcell code [6] by evaluating the absorbed dose (AD) to the cell nuclei and the cell survival fraction (Sf). The Sf for each treatment was evaluated using the linear quadratic model equation:

$$Sf = e^{-\alpha_{self} D_{self} - \beta_{self} D^2_{self}} \times e^{-\alpha_{cross} D_{cross} - \beta_{cross} D^2_{cross}}$$

taking into account the AD generated by the radiation emitted within the same cell (D_{self}) and the radiation emitted by neighboring cells (D_{cross}). The α and β parameter for AR42J cells were determined experimentally.

Results

Dosimetric evaluations show that, for a given cluster size, ^{177}Lu-RPs localization inside the cells only slightly affects the AD and the biological damage generated. In contrast, ^{161}Tb-RPs localization causes larger differences in AD due to the IE and AE emitted by ^{161}Tb, but the consequences in terms of Sf differences are also negligible. However, for the same number of disintegrations, the AD and the biological damage generated by ^{166}Tb-RPs are larger compared to ^{177}Lu-RPs.

Conclusions

For both ^{177}Lu-RPs and ^{161}Tb-RPs the main factors affecting the biological outcome are the dimensions of the cell cluster and the fraction of labelled cells inside the cluster. For a fixed cluster size and % of labelled cells, the localization of the RP inside the different cell compartments has a minimal influence on the AD to the cell nuclei and cell survival.

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Radiolabelling of different quantities of PSMA-617 with ^{225}Ac

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Introduction

As novel cytotoxic agents for Targeted Alpha Therapy (TAT), alpha-emitters are receiving a great deal of interest nowadays. These radionuclides enable the release of α-particles with a short path length and high linear energy transfer (LET) in tissue, leading to a higher double strain breaks probability in DNA. However, among α-particle emitters, only a few nuclides have gained a great interest for TAT. Actinium-225 (^{225}Ac) with a half-life of 9.92 days, decays through six short-lived daughter radionuclides, releasing 4 α-particles, 2 β-particles and minor γ-co-emissions.

Recently, PSMA-617 has been approved for treating prostate cancer, after being radiolabelled with ^{177}Lu.

The aim was to determine the optimal quantity of peptide required in a standardized method for producing [^{225}Ac]Ac-PSMA-617.

Materials and Methods

It was investigated two different quantities of PSMA-617: 50 or 100 μg of PSMA-617 dissolved in water/DMSO 40%. The peptide was added to 350 μL of gentisic buffer (gentisic acid 0,25M and sodium acetate 0,35M) pH 5.5. This solution was added to 100 μL of $AcCl_3$ (~8 MBq) and heated at 97±2°C for 30 minutes. [^{225}Ac]Ac-PSMA-617 was monitored via iTLC and HPLC analyses. The firs analysis was performed using Silica gel as stationary phase and, Sodium Citrate 0,1M pH 5 and Acetonitrile/Water 1:1 as mobile phase. The iTLC strips were acquired post-synthesis and reacquired at 3 hours, when ^{221}Fr and ^{213}Bi were in Secular Equilibrium with ^{225}Ac[1], to establish with accuracy the radiochemical purity (RCP) of radiopharmaceutical. The HPLC analysis was performed using a C18-column and ACN/TFA 0,1% - Water/TFA 0,1% as mobile phase on gradient concentration and a flow rate of 1mL/min.

Results

The RCP post-synthesis (Time 0) of [^{225}Ac]Ac-PSMA-617 was more high for the syntheses performed starting from 50μg of PSMA-617 (Table 1). However, reacquiring the same iTLC strips after 3 hours (Time 0 R 3h), the values have come closer together (89,87±1,48% in Sodium Citrate and 96,38±1,39% in Acetonitrile/Water). HPLC analysis results were in agreement with iTLC data.

Sinthesys	PSMA-617 [μg]	Sodium Citrate		Acetonitrile/Water	
		Time 0	Time 0 R 3h	Time 0	Time 0 R 3h
A	50	74,7	88,8	92,8	96,3
B		75,0	91,0	85,8	95,3
C		74,9	89,9	88,8	95,9
D	100	50,3	92,0	83,4	94,8
E		56,2	89,6	88,2	98,5
F		55,3	87,9	86,2	97,5

Table 1: Percentage of RCP obtained by the iTLC analysis

Conclusion

The use of different quantities of peptide for [^{225}Ac]Ac-PSMA-617 synthesis does not affect the RCP. However, it is necessary to improve the method of radiolabelling because in both cases the RCP values do not to satisfy the QC guideline. On this basis, small quantity of peptide is the optimal

choice because high specific activity results in fewer receptors being occupied by unlabelled PSMA in vivo.

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A Roadmap to the Rational Development of Radium-223 and Barium-131/135m Chelators for Targeted Theranostics

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Introduction

Radium-223 (^{223}Ra, $t_{1/2}$ = 11.43 d) is an α-emitter suitable for the treatment of metastatic tumors, currently approved as [^{223}Ra]$RaCl_2$ (Xofigo®) for the palliative treatment of bone metastases in castration-resistant prostate cancer.[1] Barium-131 (^{131}Ba, $t_{1/2}$ = 11.50 d) and barium-135m (^{135m}Ba, $t_{1/2}$ = 28.7 h) are γ-emitters and could serve as ^{223}Ra surrogates for SPECT imaging, allowing a ^{223}Ra/^{131,135m}Ba-based theranostic approach.[2]

To enlarge the plethora of treatable tumors, overcoming the spontaneous accumulation of these metals in the bones is crucial. This can be achieved by stably complexing Ra^{2+}/Ba^{2+} through a chelator, in turn conjugated to a targeting vector to direct radiation to the tumor site.[3] However, the scarcity of chelating agents capable to firmly trap these radionuclides *in vivo* has been a limiting factor, hindering their utilization to date. The poorly explored fundamental coordination chemistry of Ba^{2+} and Ra^{2+} has further complicated the rational design of proper chelators.[4] Herein we delve into the coordination preferences of Ra^{2+} and Ba^{2+}, assessing their affinity to different donor groups. This exploration lays the groundwork for the subsequent design of tailored chelators, crucial for the development of ^{223}Ra/$^{131/135m}$Ba-based radiopharmaceuticals.

Materials and methods

Various monodentate and bidentate ligands bearing different donor groups were considered. To compare the behavior of Ba^{2+} and Ra^{2+}, the electronic binding energies (Δ*E*) for the reaction $M^{2+} + L^{n-} \rightarrow [ML]^{(2-n)+}$ in water (M^{2+} = Ba^{2+} or Ra^{2+}, L = ligand, and *n* = its charge when fully deprotonated) were calculated by Density Functional Theory (DFT, COSMO(water)-ZORA-PBE-D3/TZ2P level of theory).[5] The thermodynamic stability (log*β*) of $[BaL]^{(2-n)+}$ complexes was determined by titrations of $Ba(ClO_4)_2$-ligand mixtures in aqueous solution followed by potentiometry, ^{1}H-NMR or UV-Visible spectroscopy at 25°C. Conditional stability constants (log*β*') at pH 4 and 7.4 were derived from the log*β* to consider both the metal-ligand affinity and the protonation state of the ligand under radiochemically relevant conditions.

Results

DFT calculations unveiled striking similarities in the behavior of Ba^{2+} and Ra^{2+}, as evidenced by their nearly identical Δ*E* values across all the investigated ligands (Figure 1). The correlation between

computed Δ*E* and experimental log*β* of Ba^{2+} complexes is shown in Figure 1. Both methods converge in revealing the preference of Ra^{2+} and Ba^{2+} for more negatively charged ligands (*e.g.* 2− phosphonates > 1− carboxylates > DMSO) and for oxygen rather than nitrogen or sulfur donors (*e.g.* compare O with S in phenolate *vs* thiophenolate, and N,O with O,O in 2-aminobenzoate *vs* salicylate).

To design chelators for radiopharmaceutical applications, log*β*' values should be considered because they represent the effective stability of the complexes at a given pH. At pH 4 and 7.4 (both common in radiolabeling experiments and 7.4 also being the physiological pH) picolinate, 2-hydroxypyridine 1-oxide (1,2-HOPO), and malonate provide the most stable $[BaL]^{(2-n)+}$ complexes among those investigated (Figure 2).

Conclusion

We are currently developing multidentate chelators for Ra^{2+} and Ba^{2+} utilizing the most promising building blocks identified through this research. This endeavor aims to pave the way for the creation of cutting-edge $^{223}Ra/^{131,135m}Ba$-based radiopharmaceuticals for targeted theranostics of cancer.

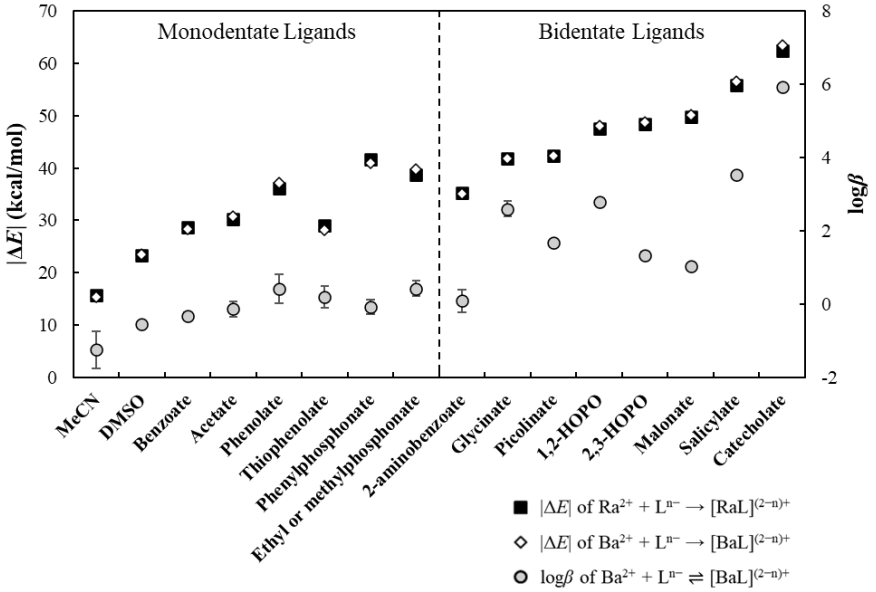

Figure 1. Electronic binding energies (|Δ*E*|) for selected monodentate and bidentate ligands to Ra^{2+} and Ba^{2+} calculated *in silico*, together with thermodynamic stability constants (log*β*) of $[BaL]^{(2-n)+}$ complexes. Level of theory: COSMO-ZORA-PBE-D3/TZ2P.

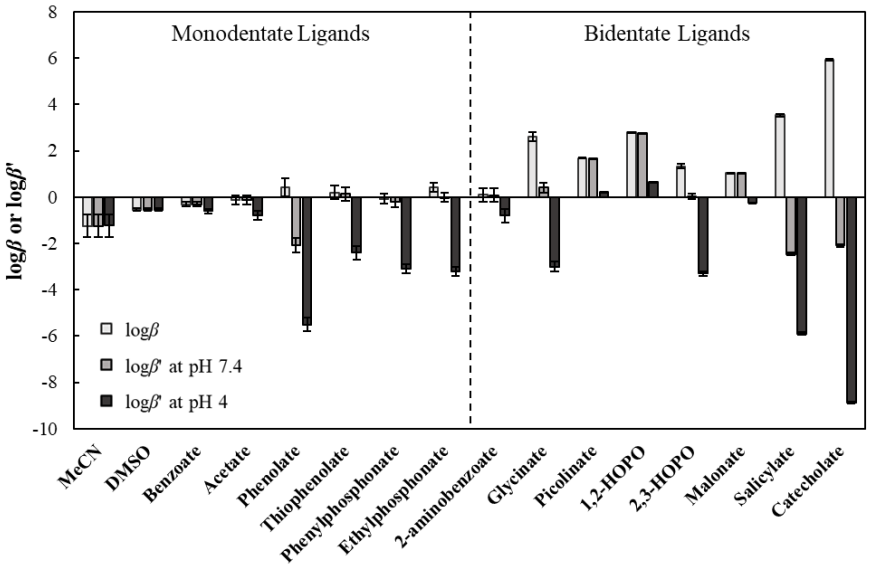

Figure 2. Thermodynamic (logβ) and conditional (logβ') stability constants at pH 7.4 and 4 of the experimentally investigated $[BaL]^{(2-n)+}$ complexes.

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Novel PET radiotracers for measuring P-glycoprotein function in neural disorders.

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Introduction

P-glycoprotein (P-gp, Mdr1) is an ATPase protein, belonging to the ATP-Binding Cassette (ABC) transporter and is involved in the absorption and elimination of xenobiotics. P-gp is localized at the apical side of cell membranes of the same organs such as the liver, kidney, and gut, and in some barriers such as the Blood-Brain Barrier.[1] Changes in the P-gp expression and function are involved in several neurological disorders such as Alzheimer's and Parkinson's disease. For this reason, the development of PET radioligands to detect P-gp activity can be useful in the clinical setting of neurodegenerative disorders.[2] This study relates to a novel PET-radiotracer [^{18}F]MC225 (Fig.1), patented by our group,[3] with high affinity and selectivity towards P-gp and its potential for the in vivo evaluation of P-gp role in neural disorders.

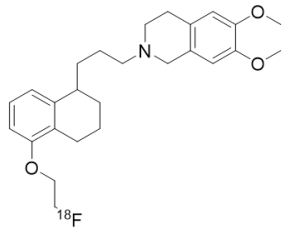
Materials and methods

For the synthesis of [^{18}F]MC225 precursor (MC226), the basic nucleus common to the best P-gp inhibitors such as Tariquidar and Elacridar (dimethoxytetrahydroisoquinoline moiety), has been functionalized with the no-basic moiety (methoxy tetraline ring) of PB28 a cyclohexylpiperazine derivative displaying P-gp modulating activity.[4] The corresponding ^{11}C-labeled compound [^{11}C]MC266 was prepared using [^{11}C]CH_3I (RY = 30%, RP > 98%, SA > 100 TBq/mmol) and tested in rats demonstrating high affinity and selectivity towards P-gp.[5]

[^{18}F]MC255 was synthesized in a two-step automated method using [^{18}F]bromoethyl fluoride (RY = 11%, RP > 95%, SA> 100 GBq/µmol). All process validation batches complied with the product specifications and have been approved for the first-in-human clinical research by the Institutional PET Drug Committee and MHLW Certified Clinical Research Review Board, Tokyo Metropolitan Geriatric Medical Center (jRCTs031190136).[6,7] To develop a more robust synthetic procedure, it was of interest to produce the radiotracer via a 1-step synthesis. However, the development of a mesylate precursor from the phenol derivative to facilitate direct ^{18}F-fluorination was challenging and the product obtained was not GMP compliant.[8]

Results

[^{11}C]MC266 and [^{18}F]MC255 are both selective substrates for P-gp with higher baseline uptake than the gold standard [^{11}C]Verapamil. These mean that an upregulation of P-gp function in response to treatment may be more detectable using [^{11}C]MC266 and [^{18}F]MC255 with respect [^{11}C]Verapamil.[9] Furthermore, the longer half-life of fluorine-18 enables the use of [^{18}F]MC225 in centers without an onsite cyclotron. No acute toxicity or mutagenic activity was observed for [^{18}F]MC225 at the dose required for adequate PET imaging.[10]



[^{18}F]MC225

5-(1-(2-[18F]fluoroethoxy))-[3-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-propyl]-5,6,7,8-tetrahydronaphthalen

Figure 1. Chemical structure of [^{18}F]MC225.

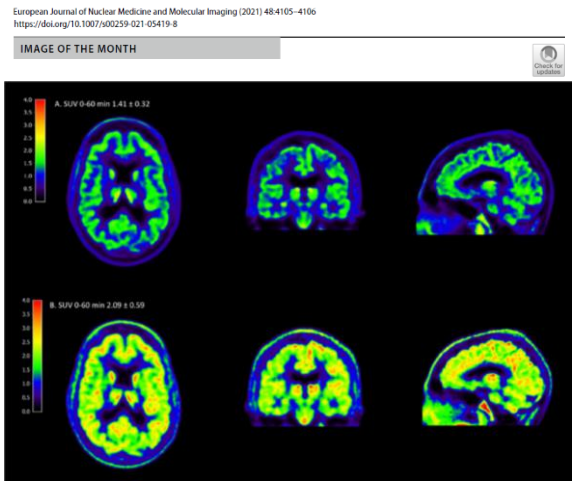


Figure 2. These standardized uptake value (SUV) images show the first [^{18}F]MC225 PET brain scans in healthy human subjects in both unblocked (A) and blocked (B) P-gp state. Blocking was achieved by continuous intravenous administration of the specific P-gp inhibitor cyclosporin (2.5 mg/kg/h), starting 30 min prior to the scan.[6]

Conclusions

The first [^{18}F]MC225 PET brain scans in a healthy human subject in both unblocked (A) and blocked (B) P-gp state (Fig.2) quantitatively show higher uptake at baseline levels (VT = 4.38) and after P-gp inhibition (VT = 5.48) with respect [^{11}C]Verapamil (VT = 1.28 at baseline, VT = 2.00 after P-gp inhibition) facilitating the measurement of P-gp function in the brain.

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SOMAKIT-TOC, PERSONALIZED DOSES AND RISK ASSESSMENT

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Introduction

The availability of authorized kits for the labelling of Gallium-68 radiopharmaceuticals also allows Hospital Radiopharmacies that do not prepare Officinal Formulae to carry out the labelling in a simple, rapid, and safe way. It is the case of SomaKit-TOC, which, after radiolabelling with a Gallium-68 chloride solution, is authorized for Positron Emission Tomography (PET) imaging of somatostatin receptor overexpression in adult patients with confirmed or suspected well-differentiated gastroenteropancreatic neuroendocrine tumors (GEP-NET) for localizing primary tumors and their metastases.

The preparation of the SomaKit-TOC requires the use of a full $^{68}Ge/^{68}Ga$ generator eluate volume (5 ml), so the available Gallium-68 activity is until 1.2-1.3 GBq for a 1.85 GBq $^{68}Ge/^{68}Ga$ generator at the calibration date. Nevertheless, the license of the kit is for a single dose, which is between 100 and 200 MBq [1, 2]. The single dose use and the time required (4h) to achieve full yield after the last elution of the $^{68}Ge/^{68}Ga$ generator make it impossible to perform more than a single PET scan on the same day, even though each kit has a high activity (MBq) of finished product potentially suitable for 3-4 patients.

The aim of this work was to identify a pharmaceutical approach, regarding technical and regulatory, for overcoming this limitation.

Materials and methods

Joint meetings between the Radiopharmacies of Modena, Ferrara, and Cesena/Meldola occurred to analyze the current pharmaceutical legislation regarding the dispensing in personalized doses, intended for the individual patient, of a drug approved for single-use according to a medical prescription and to evaluate its applicability to the SomaKit-TOC [3-5]. A risk assessment was performed by applying FMEA-FMECA analysis to the full process, including the production and dispensing of the drug. To validate the method of multiple personalized doses-dispensing of the drug, a sterility test on the residual of the SomaKit-TOC preparation was performed in triplicate (on three preparations).

Results

The only critical issue that occurred during the risk assessment of the process of production and dispensing in multiple doses of the SomaKit-TOC was the maintenance of the sterility of the preparation, performing multiple manual doses (Index Priority Risk = 11). The result of the sterility tests performed during the validation process highlighted the maintenance of the sterility.

Discussion/conclusion

We analyzed the current pharmaceutical legislation and performed the risk analysis to guarantee access to the PET imaging of somatostatin receptors for more cancer patients by dispensing in multiple personalized doses the radiopharmaceutical [^{68}Ga]Ga-DOTATOC, obtained from the SomaKit-TOC prepared according to RCP.

According to the regulatory system for drugs, the partition of medicine in personalized doses is comparable to a Magistral Formula [3-5]. This classification allows the Pharmacist to dispense the Somakit-Toc final preparation in individual doses according to a nuclear medicine physician single prescription in order to satisfy a clinical need [6, 7].

The physician's prescription ensures the safety and efficacy of dosage, but the pharmaceutical quality depends on the operator, who must apply validated procedures.

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[^{89}Zr]ZrDOTA reactivity evaluation to optimize [^{89}Zr]ZrDOTA-c(RGDfK) preparation starting from zirconium-89 produced through solid target.

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Background

Zirconium-89 is an interesting isotope to study long biological events like antibody distribution because of the nuclear properties, like half life of 78.8 h allow to acquire late imaging of the radiopharmaceuticals.

Zirconium-89 can be produced in high activity and chipper way, using solid target technology [1] the purification process it's well know on [^{89}Zr]ZrOx formulation, stable and easy to use radiopharmaceutical precursor, unlucky not all the ligands well react with this formulation. DOTA complex of zirconium-89 are stable and can be produced through [^{89}Zr]$ZrCl_4$ precursor [2], that can be produced by two different pathways, the first classic one request a previews production of oxalate formulation and then a conversion on chloridric form using a QMA SPE. The second procedure requests a different, more acid, dissolution condition and just one step of purification by TBP SPE. To develop the final [^{89}Zr]ZrDOTA- cyclo(RGDfK) complex, we have tested and optimized both reaction protocols starting with the two [^{89}Zr]$ZrCl_4$ solutions and DOTA ligand. Finally starting from the promising protocol with DOTA we have optimized the reaction condition with the ligand DOTA- cyclo(RGDfK).

Materials and methods

The ACSI TR19/300 proton cyclotron, was used in combination with yttrium coin shaped solid target to produce zirconium-89. The isotope was recovered in high yield in three different formulations based on SPE process using ZR and TBP cartridge form Triskem and QMA, C18 from waters. DOTA was provided by Macrocyclics, DOTA-cyclo(RGDfK) fromABX. All the reagents were ultrapure metal free used without future purification.

DOTA reactivity study was conducted by increasing ligand amount and activity to find the best ratio, in all the cases the solution was let react at 95 °C and 550 rpm. Reaction kinetics was evaluated at different timepoints 15, 30, 60 minutes. The reaction yield was evaluated under iTLC-SA.The [^{89}Zr]ZrDOTA-cyclo (RGDfK) was obtained in 88% of yield, under the 95 % set up a

goal, to increasing the yield a SPE based purification procedure was developed and allow to isolate the pure product as show by HPLC and iTLC-SA.

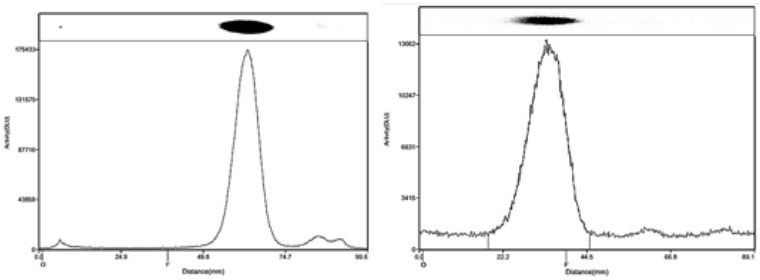

Figure 1. left,[^{89}Zr]ZrDOTA and [^{89}Zr]ZrDOTA-cyclo(RGDfK) (right) in iTLC-SA/ MeOH/H_2O(1:1)4%TFA.

Results

Table 1. Reactions conditions summary.

Reaction	Ligand (ug)	Reaction condition	yield
^{89t}ZrCl4 + DOTA	20	1h, 95°C, 550 rpm	60 %
^{89t}ZrCl4 + DOTA	40	1h, 95°C, 550 rpm	98 %
^{89t}ZrCl4 + cyclo(RGDfK)	20	1h, 95°C, 550 rpm	70 %
^{89t}ZrCl4 + cyclo(RGDfK)	40	1h, 95°C, 550 rpm	88 %

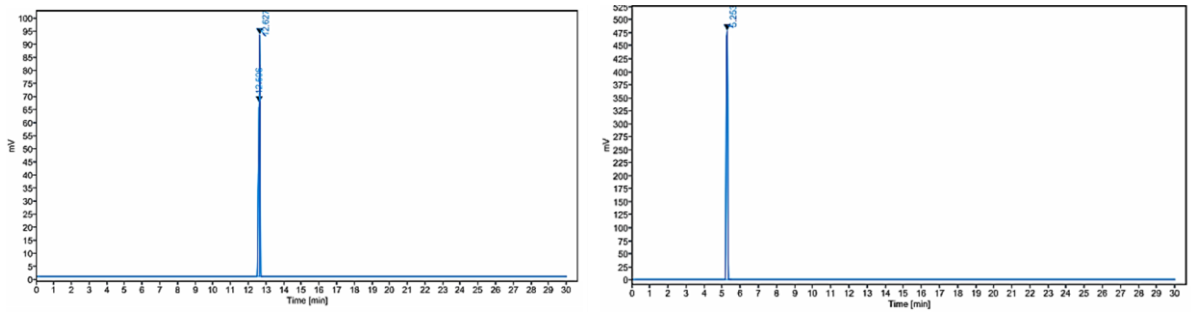

Figure 2. left,[^{89}Zr]ZrCl4 and [^{89}Zr]ZrDOTA-cyclo(RGDfK), after purification (right) HPLC profile..

Conclusion

Under developing a reaction protocol between zirconium-89 in different formulations and DOTA we identify the optimal reaction condition to conjugate DOTA ligands to zirconium-89. The application of the identity protocol on ligand DOTA-cyclo(RGDfK) gave high coordination yield over 88%. To obtain a pure radiopharmaceuticals study of purification was performed and identity to isolate a pure compound shown on HPLC and iTLC-SA.

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CCC (Coumarin Copper Complex) in Nuclear Medicine: a tool to target MCT1?

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Introduction

Coumarins are natural phyto-compounds that attracted the attention of synthetic and medicinal chemists for decades and showed a large variety of biological activities [1]. One of the recently developed MCT1 inhibitor (7ACC2) that reached clinical trials is a coumarin derivative, so this family of compounds deserves a great deal of attention [2].

MCT1 is a membrane protein responsible for the transport of lactate normally expressed in healthy tissues. However, in some cancer lines its expression results dysregulated and allows the establishment of the "Warburg effect". This mechanism explains the aggressive character of specific tumoral types and allows MCT1 to be a potential target for theranostic applications in oncological field [3]. To date, only few molecules acting as inhibitors for MCT1 are known [2], and their interactions within MCT1 pocket have been elucidated by Xray crystal data [4].

The bifunctional chelator (BFC) approach was explored to envision Copper (II) isotopes radiolabeling. This with the aim of exploiting different copper radionuclides (61-Cu, 64-Cu and 67-Cu), to reach the so called "theranostic approach".

Material and Methods

All chemicals and solvents were purchased with a highest purity grade available and used without further purification unless otherwise specified. NMR spectra were recorded by Bruker Biospin FT-NMR AVANCE III HD (600 MHz) spectrometer equipped with a CryoProbe BBO H&F 5mm and a Bruker Biospin Avance AMX (400 MHz) spectrometer with a Broad Band 5-mm probe in inverse detection. LC-MS (ESI) was performed on Agilent 6300 Ion Trap LC/MS System equipped with an electrospray ionization (ESI) interface. The compounds were separated using Agilent Zorbax SB C18 30x2.1mm, 3.5 µm. UV-visible spectra were recorded with a JASCO V-570 UV/Vis/NIR spectrophotometer at 298 K in the 250–700 nm spectral range employing quartz cells (1 cm optical path). For pKa determination, the overall protonation constants (logβqr) were evaluated from spectrophotometric data (UV-Vis spectra), as defined by the following equations:

(Eq. 1) $qL^{l^-} + rH^+ \rightleftharpoons L_qH_r^{(-lq+r)}$

(Eq. 2) $\beta_{qr} = \frac{[L_qH_r^{(-lq+r)}]}{[L^{l-}]^q \cdot [H^+]^r}$

where L is the ligand in the completely dissociated form and H is proton, data were refined by least-squares calculation, using computer program HypSpec [6].

Results

A chelating system suitable for Copper (II) must be grafted on the targeting vector (coumarin) while reducing the impact on the interaction with the target protein as much as possible. For such a goal, an already existing bifunctional polyazamacrocycle, namely no3py [5] was conjugated to the targeting vector using a PEG chain as a spacing linker. The bifunctional no3py was synthetized according to the procedure in literature [7], the ammino-acid PEG was purchased and the coumarin was synthetized according to the literature [8] and further functionalization was performed. The desired compound (reported in *figure 1*) has been successfully synthesized through amidic couplings as confirmed by NMR and HRMS analysis.

Figure 3. Target compound

Conclusions

The new synthetized compound is now under physico-chemical characterization and its ability of copper complexation are under evaluation using UV-visible spectroscopy, Cyclic Voltammetry and NMR Future radiolabeling with 64-Copper as well as hot and cold biological assays are scheduled to confirm its ability to target MCT1.

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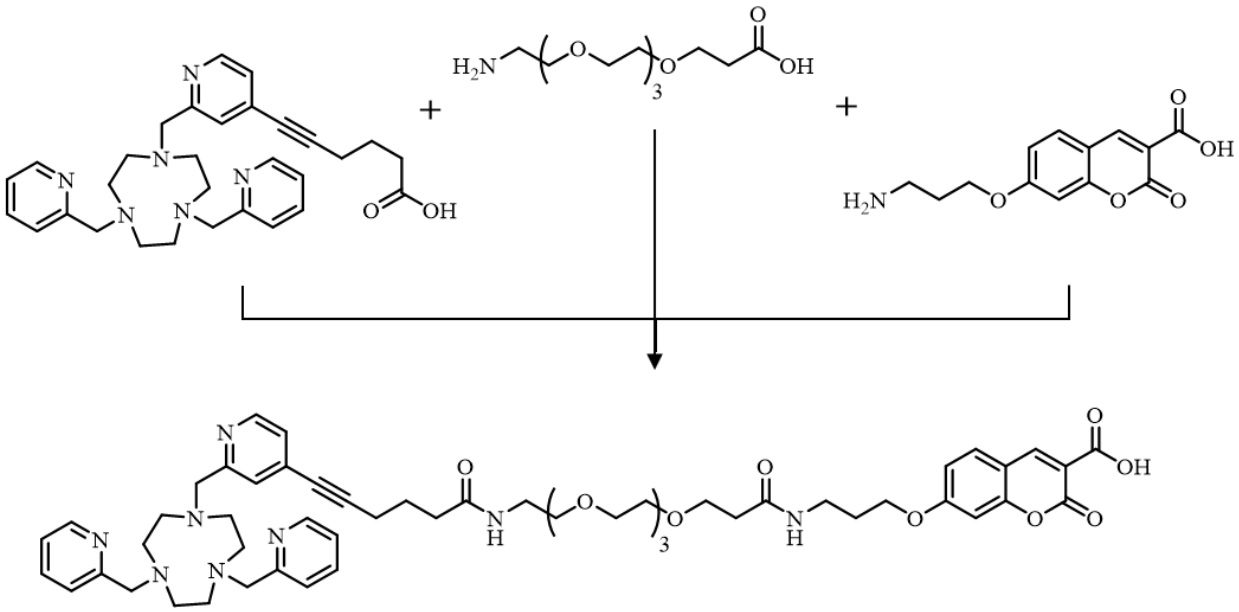

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^{99m}Tc-scFvD2B a potential theranostic pair for ^{177}Lu-scFvD2B

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Background

The ^{177}Lu-labeled single-chain variable fragment of an anti-prostate specific membrane antigen (PSMA) IgG D2B antibody (scFvD2B) penetrates solid tumors more efficiently and accesses binding sites more uniformly than the full-length antibody due to its smaller size [1]. In addition, scFvD2B allows the use of different chelating agents to label it without significantly affecting its pharmacokinetics.

Preclinical studies have demonstrated that ^{177}Lu-scFvD2B exhibits greater uptake in prostate cancer cells, resulting in an increased tumor/kidney ratio and higher tumor radiation dose when compared to both ^{177}Lu-labeled Glu-ureide-based PSMA inhibitory peptides [2] and ^{177}Lu-albumin-PSMA peptide conjugates [3]. However, no diagnostic radiopharmaceutical is currently available that can be used as a theranostic pair. The aim of this study was to synthesize and biochemically characterize a new ^{99m}Tc-scFvD2B radiotracer and assess its potential as a theranostic pair for ^{177}Lu-labeled scFvD2B.

Materials and methods

The scFvD2B-Tag and scFvD2B antibody fragments were produced in a prokaryotic system and in a eukaryotic system by ExcellGene, respectively, and purified using affinity chromatography, as reported by Frigerio et al [4]. Subsequently, two HYNIC derivative chelators, HYNIC-Gly-Gly-Cys-NH2 (HYNIC-GGC) and succinimidyl-HYNIC (S-HYNIC), were used to conjugate the scFvD2B-Tag and scFvD2B isoforms, respectively. Chemical characterization, immunoreactivity assays (affinity and specificity), radiochemical purity assays, stability studies in human serum, cellular uptake and internalization in LNCaP(PSMA+), PC3-PIP(++) or PC3(-) PCa cells of the resulting unlabeled HYNIC-scFvD2B conjugates (HscFv) and ^{99m}Tc-HscFv agents were performed.

Results

Chemical characterization of the two derivatives showed that HscFv1 (HYNIC-GGC-scFvD2B-Tag) contained an average of two to three HYNIC-GGC molecules conjugated to each scFvD2B-Tag, whereas the HscFv2 (S-HYNIC-scFVD2B) conjugates with the highest abundance were those containing one or two S-HYNIC molecules. The results showed that the incorporation of HYNIC as a chelator did not affect the affinity, specificity or stability of scFvD2B. Nevertheless, HscFv1 showed high instability under radiolabeling conditions and a very low amount of the corresponding ^{99m}Tc-HscFv1 radiotracer was obtained. After purification, the radiochemical purity of the ^{99m}Tc-HscFv radiotracers was greater than 95%. A two-sample *t*-test of ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2 uptake in PC3-PIP vs. PC3 showed a *p*-value < 0.001, indicating that the interaction between ^{99m}Tc-HscFv derivatives and the PSMA receptor was statistically significantly higher in PSMA-positive cells than in negative controls.

Conclusions

In this work, the HYNIC- scFvD2B conjugates were prepared and characterized. Both showed high in vitro stability and specific recognition for PMSA. Regarding cellular internalization, scFvD2B conjugates in PSMA+ cancer cells was >tenfold higher than that of HYNIC conjugated peptides.

However, the reduced labeling yield makes the use of ^{99m}Tc-HscFv1 unfeasible; in contrast, the labeling yields obtained for ^{99m}Tc-HscFv2 allow for further studies to optimize its production.
Nevertheless, further preclinical studies are warranted to determine whether the in vivo pharmacokinetics and tumor uptake of ^{99m}Tc-HscFv still offer sufficient advantages over HYNIC-conjugated peptides.

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Technology transfer of Iasoglio drug product on Pisa PET production site

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Introduction

Gliomas represent the most common primary brain tumors and include neoplasms ranging from low-grade to high-grade, rapidly growing and aggressive glioblastoma (GBM). More than half of gliomas are GBMs with a median survival rate of approximately 15 months and with a 5-year survival rate of approximately 5%.[1]
The diagnostic potential of O-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) positron emission tomography (PET) has already been proven in many studies, and in addition to the golden standard magnetic resonance imaging (MRI), provides important additional information for diagnosis, grading, follow-up and choice of therapy for patients suffering from brain tumors. To date, however, routine use of [^{18}F]FET is limited, due to the very low availability of radiotracer.[2]
The adoption of production processes compliant with Good Manufacturing Practice (GMP) is essential to guarantee the quality of the batches and to guarantee a large distribution.[3] At present in Italy, the [^{18}F]FET produced according to GMP is not available and its clinical use is restricted to a few nuclear medicine centers or to imported radiopharmaceuticals from abroad.
In the frame of a collaboration between the largest Italian research public institution (CNR) and one of the world leader company for the production of radiopharmaceuticals (Curium), the technological transfer process of Iasoglio ([^{18}F]FET) will be described.

Materials and Methods

The manufacturing procedure of Iasoglio, solution for injection can be summarized in 7 steps:
1)Production of [^{18}F]fluorine,
2)Recovery of [^{18}F]fluorine and evaporation of the eluent,
3)Radiolabeling of the precursor, TET (O-(2-Tosyloxyethyl)-N-trityl-L-tyrosine tertbutyl ester),
4)Deprotection of the labelled precursor,
5)Purification of the product by semipreparative HPLC,
6)Formulation and pre-filtration in dispensing cell,
7)Dilution to 2 000 MBq/mL at calibration time and dispensing after sterilizing filtration.
Some of these operations require the use of a controlled synthesis automate with preparative HPLC.

The first step towards Iasoglio commercial production on Pisa PET Production site, is the technology transfer of Iasoglio manufacturing process and Quality control from R&D department of CIS Bio international (Curium).
Technology transfer refers to the transfer of documentation, manufacturing process,
and analytical methods through different steps:

- Gap analysis: at this stage, a thorough evaluation of equipment, documentation and raw materials is carried out to plan or implement any necessary adjustments,
- Validation of the whole package of analytical methods used for the quality control analysis of Iasoglio (i.e. HPLC, TLC, GC, Endotoxins, etc.),
- Theoretical and practical staff training,
- Production and quality control analysis of 3 consecutive Iasoglio batches.

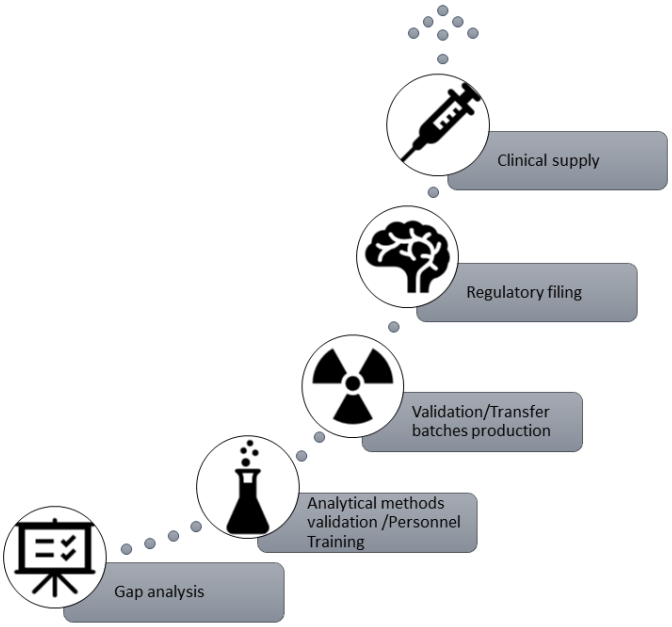

Figure 4: Technology transfer

Results/Discussion/Conclusion

The successful completion of the technology transfer should provide the documentary evidence that the process delivers a final product complying to specifications in a reliable and reproducible manner, by adhering to the process and maintaining the process parameters within the examined range. The Pisa manufacturing site will then be able to apply for a GMP certificate and subsequently be added to the current Marketing Authorization to start commercial production of Iasoglio. Hence, the availability of the drug in Italy will be ensured.

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A Template for Creating Radioimmunoconjugates

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Introduction

Immunoconjugates exploit the high affinity of monoclonal antibodies for a recognized antigen, to selectively deliver a cytotoxic payload, such as drugs or radioactive nuclides, at the site of disease. Reaction of ε-amine group of lysine residues with electrophilic reactants, such as activated esters (NHS), is the main method reported in literature as it maintains proteins in their native conformation.

Traditionally, a large excess of the activated ester is reacted to the mAb working at basic pH, generating a heterogeneous mixture of conjugates which can result in decreased target affinity. Here, we report an intradomain regioselective bioconjugation between the monoclonal antibody Trastuzumab and the Nhydroxysuccinimide ester of the DOTA chelator by a kinetically controlled reaction adding substoichiometric quantities of the activated ester to the mAb working at slightly basic pH. A new proteolysis protocol named domain mapping (patent application IT2024000001524), based on a selective domain unfolding, allowed for quantification of chemical modification at a domain level (figure 1). Data analysis based on LC–MS quantification of different analytical levels (intact, reduced chains, and domains) provided a molecular formulation of the mixture of immunoconjugates.

Materials and methods

The immunoconjugate was synthesized by adding 0.01 eq per min. of DOTA-NHS to Trastuzumab at room temperature and pH 7.2. The total time of synthesis was 500 min. Therefore, the reaction mixture was purified through size exclusion gel filtration. The resulting immunoconjugate was digested by a trypsin enzyme developing a domain mapping mass spectrometry workflow.

Results

The immunoconjugate synthesized under kinetic control showed unitary chelator to antibody ratio (CAR). Proteolysis experiments displayed that an intradomain regioselectivity was achieved, with the conjugated lysine residues not involved in the binding with the antigen. The immunoconjugate mixture was composed of 15 species, whereas up to 10^6 species are statistically possible employing traditional bioconjugations. The most abundant species in the mixture resulted in the naked Trastuzumab, with the species Trastuzumab + 1 DOTA having a relative abundance in comparison to species Trastuzumab + 2DOTA ranging from 4 to more than 20-fold (table 1).

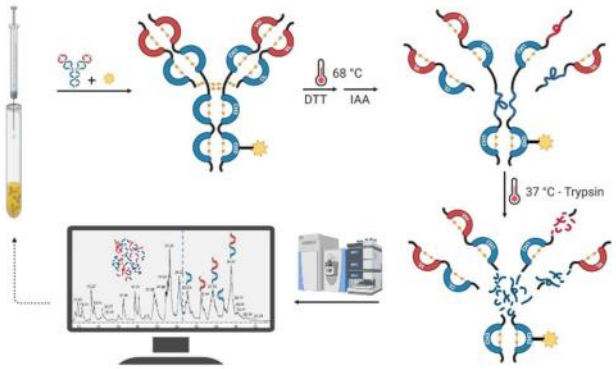

Figure 1. Schematic representation of kinetically controlled bioconjugation coupled to the domain mapping MS-workflow.

S	%
T	35
S_{CH3}	13,46
S_{CH2}	12,94
S_{VH}	11,39
S_{CL}	6,21
$S_{CH3-CH3}$	3,10
$S_{CH2-CH3}$	2,98
$S_{CH2-CH2}$	2,87
S_{VH-CH3}	2,62
S_{VH-CH2}	2,52
S_{VH-VH}	2,22
S_{CH3-CL}	1,43
S_{CH2-CL}	1,38
S_{VH-CL}	1,21
S_{CL-CL}	0,66

Table 1. Percentage composition of the immunoconjugate mixture. T indicate naked Trastuzumab; S indicate a generic species; subscripts indicate the conjugated domain.

Discussion

During the past decade, research in radioimmunoconjugates moved toward a lower CAR. Beyond limiting the degree of modification to preserve mAb immunoreactivity, reduced CAR means more radiolabeled probes for the same quantity of radioactivity or even better reduced radioactive dose to patients to obtain a tumor to background ratio similar to that of radioimmunoconjugates with higher CAR. In the current study, we demonstrate that it is possible to synthesize immunoconjugates having unitary CAR achieving an intradomain regioselectivity, through a kinetically controlled bioconjugation. The synthesized mixture should ensure improved affinity for the antigen and lower radioactive dose to patients in comparison to traditionally synthesized radioimmunoconjugates. Preclinical in vitro and in vivo studies are currently performed to demonstrate the reduced radiotoxicity. Moreover, the choice of DOTA chelator allows for theragnostic application. In conclusion, the coupling of synthesis under kinetic control with its monitoring using domain mapping could provide a model to obtain immunoconjugates which ensure a pharmaceutical quality, nowadays not achievable with traditional bioconjugation employing activated esters.

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Cytotoxic and Antiproliferative Effects of $[^{64}Cu]CuCl_2$ in Tumor Cells for Radiometabolic Therapy: a Preliminary Study

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Introduction

Peritoneal carcinomatosis (PC) represents a relatively common condition in the advanced stages of various tumors, characterized by the dissemination of malignant cells from the primary organ to the peritoneum. Its impact is global, affecting approximately 25,000 individuals in Italy and 1.4 million worldwide every year, with mostly negative outcomes [1]. The goal of this study is to delineate a therapeutic approach aimed at increasing the life expectancy of PC patients, overcoming the challenge associated with the marked genetic instability of cancer cells, which is incompatible with the receptor-targeted and antigenic therapies currently proposed [2]. In pursuit of this objective, the radionuclide copper-64, in the form of $[^{64}Cu]CuCl_2$, was employed. Recent studies have shown that copper, in its ionic form Cu^{2+}, can accumulate at significantly higher levels in cancer cells than in healthy ones, which makes its cytotoxic effect highly specific. This effect can be achieved by exploiting the nuclear decay properties of the radionuclide ^{64}Cu (^{64}Cu, $T_{1/2}$ 12.7 h; $E_{\beta+mean}$ 278 keV; $E_{\beta-mean}$ 191 keV; Auger emission) [3,4].

Materials and methods

Human tumor cell lines related to the development of peritoneal metastases (MDA-MB-231, human breast adenocarcinoma cell line; NCI-N87, human gastric carcinoma cell line) and a healthy control cell line (HEK293, human embryonic kidney cell line) were utilized in this study. These cell lines were incubated with different activities of $[^{64}Cu]CuCl_2$ (10 µCi/mL; 100 µCi/mL; 250 µCi/mL) to evaluate their uptake and the antiproliferative and cytotoxic effects. For this purpose, an analysis was conducted to evaluate the level of ^{64}Cu incorporation in the nucleus and cytoplasm. Subsequently, in vitro studies on cell viability (XTT assay), apoptosis, and necrosis (Annexin V/SYTOX assay) were conducted following 72h and 96h of treatment.

Results

This study revealed a greater uptake of the $[^{64}Cu]CuCl_2$ in the carcinoma lines than in the healthy ones and, in particular, a greater localization at the nuclear level. As a result of the exposure to different activities of $[^{64}Cu]CuCl_2$, a greater reduction of the viability in tumor lines was observed compared to the healthy control line as well as a significant increase in apoptosis in the MDA-MB-231 and NCI-N87 tumor lines.

Discussions and conclusions

Our preliminary results confirm the increased uptake of $[^{64}Cu]CuCl_2$ within the nuclear compartment of cancer cells and suggest the ability of the radiopharmaceutical to determine cell death through the induction of apoptosis. Further research to evaluate the antiproliferative and cytotoxic effects of $[^{64}Cu]CuCl_2$ using higher activities of ^{64}Cu are currently under investigation.

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^{68}Ga-FAPi-46: radiolabelling and quality control at the European Institute of Oncology

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Introduction

^{68}Ga-FAPi-46 represents a very promising new PET radiopharmaceutical for molecular imaging of various tumors types; it is considered a pan-tumor agent capable of selectively binding to the transmembrane protein FAP overexpressed by the fibroblasts in the tumor microenvironment. In particular, ^{68}Ga-FAPi-46 has been used in tumors with low ^{18}FDG avidity, such as primary liver cancer and gastro-entero-pancreatic tract, or in regions with an unfavourable ^{18}FDG tumor/background ratio.

Materials & Methods

FAPi-46 was purchased in 50□g GMP grade aliquots from Sophie Bioscience. ^{68}Ga was obtained from a pharmaceutical-grade E&Z GalliaPharm® 1.85 GBq ^{68}Ge/^{68}Ga generator. All other reagents used (water, Sodium Acetate, Sodium Ascorbate) were pharmaceutical grade with low level of metallic impurities obtained from Sigma-Aldrich. Labelling of ^{68}Ga-FAPi-46 was carried out in a "kit-like" mode optimizing volumes and reaction conditions. A mixture of 1.5M sodium acetate and 0.07M sodium ascorbate (1.5mL, pH 8) was used as reaction buffer. Two labelling methods were tested: initially FAPi-46 was dissolved in the reaction buffer and subsequently mixed with 6mL of $^{68}GaCl_3$ eluate in 0.1N HCl. Conversely, FAPi-46 was directly mixed with the $^{68}GaCl_3$ eluate and subsequently buffered. Reaction vial in both cases was heated in a digital thermoblock at 95°C up to a maximum of 15 minutes. Quality control parameters were checked: pH, chemical purity and radiochemical purity (RCP) were performed with radio-TLC and radio-HPLC. Radio-TLC was performed on ITLC-SG strips in NH_4Ac:MeOH 1:1. Radio-HPLC was performed by Reversed Phase C_{18} chromatography with a linear H_2O:CH_3CN gradient. In vitro stability was evaluated for up to 4 hours using the same chromatographic techniques; after appropriate decay, the batches were analyzed for sterility and apyrogenicity; radionuclidic purity was evaluated with HPGe □-spectrometry.

Results

Reaction mixture pH was always constant in the range 3.5-4.5. In the first method the RCP was found variable, randomly giving low values (<90%), most likely due to the relative chemical instability of FAPi-46 at the basic pH of the buffer. In the second method, where the incubation time was also increased to 15', the RCP obtained was > 99%. Radio-HPLC method was able to completely differentiate $^{68}Ga^{3+}$ (Rt=3.5') and ^{68}Ga-FAPi-46 (Rt=7') and no other impurity was detected. ^{68}Ga-FAPi-46 was stable for up to 4h while always maintaining RCP > 95%. Radionuclidic contamination was found 100-fold lower than the limit (typically 0.1Bq ^{68}Ge/MBq ^{68}Ga) and all batches were sterile and apyrogenic. In our study we labelled 50µg aliquots of FAPi-46 GMP grade with a ^{68}Ga activity up to 1200 MBq. Other published studies have highlighted how it is possible to further increase the specific activity by decreasing the quantity of peptide used, but this is to the detriment of the final radiochemical purity obtained.

Conclusion: The preparation of ^{68}Ga-FAPi-46, through the previously described labelling process, was found to be stable and reproducible with a high labelling yield and high chemical and radiochemical purity. Use in daily clinical practice confirms the results of this study.

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[^{18}F]DPA-714 synthesis, characterization and in vivo studies for the assessment of neuroinflammation in amyotrophic lateral sclerosis

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Background

The translocator protein 18 kDa (TSPO) is today a validated imaging biomarker for the evaluation of neuro-inflammation and its progression, as well as a recognized target for the development of new therapeutic agents for neurological, psychiatric disorders and oncological applications. Among several radiotracers targeting TSPO, [^{18}F]DPA-714 is nowadays largely used as a PET imaging probe in many clinical trials. Here, we report our preliminary experience in the production and quality controls of the tracer (QCs) as well as the collected data in experimental models of amyotrophic lateral sclerosis (ALS).

Materials/methods

The process was fully automated on a AllInOne 36 (Trasis) synthesizer equipped with semi-preparative HPLC using an in-house designed cassette and a dedicated sequence. [^{18}F]DPA-714 was synthesized by a one-step labelling process using 4.5-5.5 mg of Tos-DPA-714 as precursor, reacting with activated [^{18}F]Fluoride in ACN at 95°C for 10 min and followed by semi-preparative purification. The isolated product was trapped on C18 cartridge, then removed with ethanol 70% and reformulated with saline up to the volume of 15 mL and finally sterilized. QCs of the product were carried out according to the specifications reported in the general monographs of the European Pharmacopoeia and the literature.

The in vivo study included: 9 B6SJL-TgN (SOD1^{G93A})1Gur mice expressing a high number of mutant human SOD1 copies with a Gly93Ala substitution (SOD1^{G93A}) and 7 control background-matched B6SJL wild-type mice (Jackson Laboratories, Bar Harbor, ME, USA). SOD1^{G93A} mice were studied both before (60/90days) and after (120 days) the onset of motor impairment. Age matched wildtype mice were used as controls. Mice were submitted to a dedicated micro-PET imaging (Albira, Bruker, USA) whose dual ring configuration allows the acquisition of the whole mouse body by static acquisition lasted ten minutes. A series of volumes of interest (VOIs) was drawn on the obtained images for brain, eyes, thymus, lungs, kidneys and in the skeletal muscles of both

hindlimbs. The estimation of [^{18}F]DPA-714 retention was expressed as standardized uptake value (SUV).

Results

[^{18}F]DPA-714 production was easily implemented with a simple and robust process with high radiochemical yields of 17,4-22,6% (ndc, n=10), molar activities of 364-613 GBq/µmol and radiochemical purity in the range of 98,6-99% EoS. All QCs results complied with the requirements of the European Pharmacopoeia and other designed specifications, including stability studies (up to 6 hours EoS). [^{18}F]DPA-714 uptake was enhanced in the skeletal muscle and in the thymus of SOD1^{G93A} mice.

Conclusion

The developed [^{18}F]DPA-714 automated synthetic process is reliable and robust using disposable materials and standard reagents, being implementable for routine manufacturing and possibly for human use in clinical trials. Although usually applied for the study of neuro-inflammation, its distribution is abnormal in the studied model of amyotrophic lateral sclerosis suggesting the possible increase in TSPO expression in tissues different from the central nervous system.

Development of an automated radiolabelling platform and preliminary results using a new potential [^{18}F]Fluorinated-PARP10 imaging agent

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Background

Human poly-ADP-ribose polymerases (PARPs) are an enzyme family of 17 members that catalyses the covalent attachment of poly- or mono- ADP-ribose units post-translationally on a variety of amino acid residues of target proteins. While the research of new PARP 1 and 2 inhibitors is widely pursued thanks to their use in clinic as anticancer agent, the development of mono-ADP ribose transferases inhibitors is still in the early stages. In this context, PARP10 role is not completely elucidated yet, however, its overexpression in various cancer cell lines led to the hypothesis that this enzyme promotes cancer proliferation and acts as an oncogene. Recent studies have described that PARP10 suppressions, by knockout cell lines or use of a selective inhibitor, had a huge impact in tumour progression, showing PARP10 as promising druggable target. Based on previous work about PARP10 inhibitors (PARP10i), our goal is to develop a new PET imaging agent for studying this enzyme role in different cancer models.

Methods

A flexible radiolabelling protocol was developed on a AllInOne36 synthesizer platform (Trasis, Belgium) able to carry out Cu-mediated [^{18}F]-fluorodeboronation starting from different boronic pinacol ester precursors, including deprotection and semipreparative purification before reformulation. Variations in the labeling protocol were performed in order to optimize labeling yields by changing solvents, catalysts type and amount of precursors. The best resulting protocol involved the use of DMI and precursors protected with SEM (2-(Trimethylsilyl)ethoxy]methyl), followed by cleavage with HCl. Biological assays of [^{18}F]-PARP10i tracer were carried out and

analysed in two different cell lines (MCF7, HT-29) expressing PARP10s using a real-time monitoring equipment (LigandTracer, Sweden).

Results/Discussion

The automated radiosynthesis on AllInOne synthesizer allowed to reliable produce the first promising imaging candidate [^{18}F]-PARP10i with high chemical and radiochemical purity, suitable for in vitro and in vivo experiments, in about 90 min including HPLC post purification and final reformulation. From 1,75 to 4,42 GBq were produced with an activity yield (AY) of 10,2-24,0 % (ndc) and a molar activity (Am) in the range of 75-222,9 GBq/µmol. [^{18}F]-PARP10i was finally tested in MCF7 and in HT-29 tumor cell lines respectively showing a moderate and fast uptake.

Conclusion

The preliminary results showed the potential of [^{18}F]-PARP10i as new PET tracer for PARP10 imaging in cancer. Further experiments are in progress on tumor cell lines with and without PARP10 expression to confirm the potential of this new PET diagnostic agent.

WHICH PSMA-BASED RADIPHARMACEUTICALS FOR PROSTATIC CANCER?

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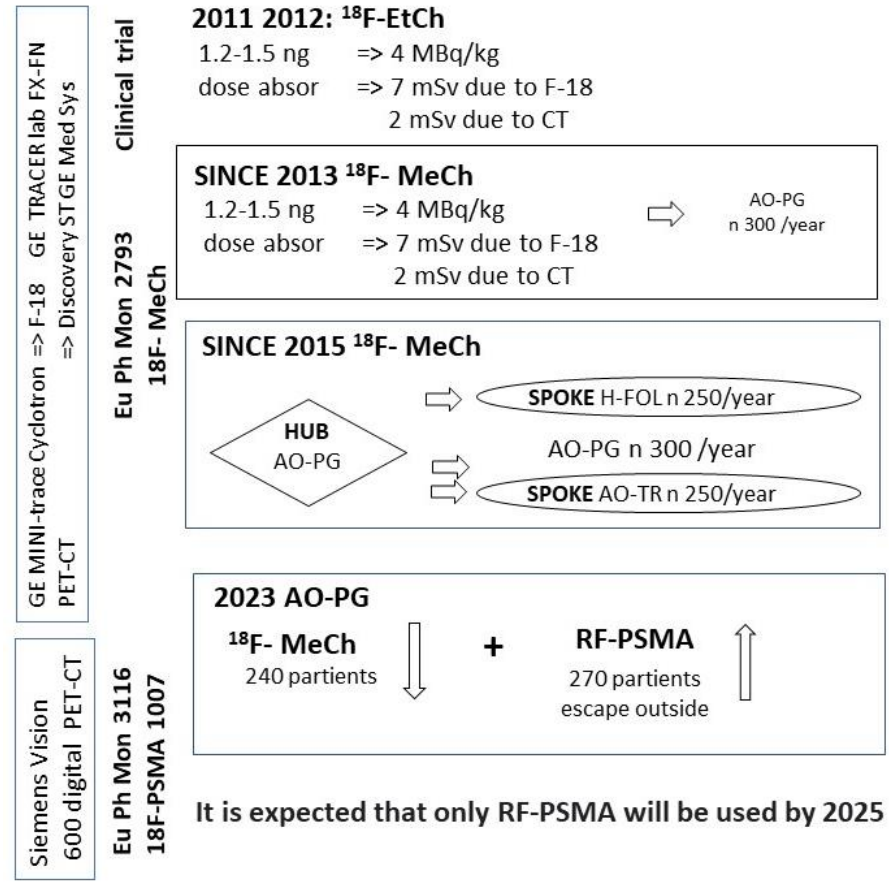
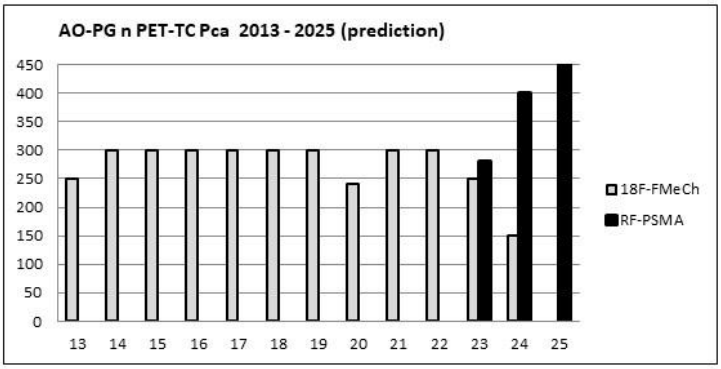
Introduction

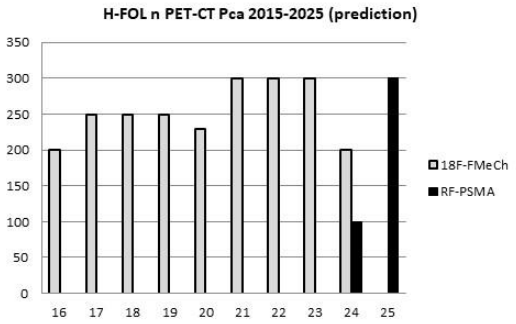
The aim of the work is to begin the use of theranostic radiopharmaceuticals in PcA. For over ten years the PET-CT Radiopharmacy of the Perugia Hospital (AO-PG) has been a HUB for the production and distribution of 18F-FDG and 18FMeCh to the PET spoke centers in the region. Recently, the gradual establishment of the use of PSMA-based radiopharmaceuticals (labelled with 68Ga or 18F) in prostate cancer currently means that our doctors refer patients towards Nuclear Medicines outside. This determines unfavorable public spending for our region together with a fragmented flow of clinical information and logistical inconvenience for the patient. The increased demand leads us to rethink a new path.

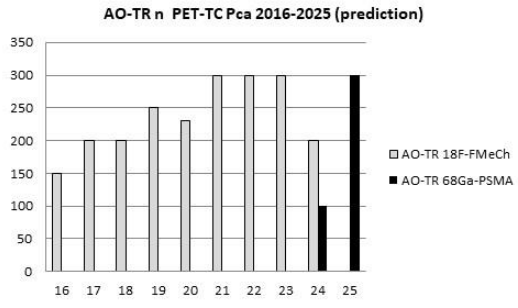
Materials and methods

Since 2012 our AO-PG PET Radiopharmacy is an important center for the Umbria region. Figure 1 shows the steps taken over the years.









We evaluate the best opportunity according to our needs:

1) Exploit the Cyclotron for the production of the F-18 and the PET radiopharmacy for the preparation of radiopharmaceuticals
18F-PSMA 1007 Monograph N.3116 European Pharmacopoeia
18F-PSMA 7 (the Monograph is awaited)
2) Cold kit Lokametz®: PSMA-11 to be labeled with Ga-68
3) Radelumin ®: F-18-PSMA-1007

Results

Umbria Region: from 2011 to 2024 January total number of PET-CT with F18-Choline is 7800:
AO-PG 3500 patients
H-FOL 2300 patients
AO-TR 2000 patients
Diagnostics
Tab 1 compares the galenic with the industrial cold kit, highlighting advantages and disadvantages.
Radelumin is not being examined at the moment, as it is not yet available in Italy
The preparation of the galenic would require the purchase of a new cassette synthesis module and the integration of instrumentation in the Quality Control laboratory.
Therapy
As regards the therapy, the choice is obligatory in the sense that our radiopharmacy is not GMP grade, therefore there is an obligation to purchase the drug.
PSMA drugs such as Lutathera® (177Lu-PSMA-617) are already in use at our Center.
Therapy with Pluvicto® hasn't started yet.

TAB 1: DIAGNOSTIC: 18F-PSMA–GALENIC versus LOKAMETZ® COLD KIT

GALENIC	**LOKAMETZ® COLD KIT**
The availability of the Cyclotron allows us to produce 18F and therefore perform a large number of diagnostic tests.	Limited number of patients due to the 68Ge-Ga68 generator for marking the kit with AIC. Multi-dose cold kit for up to 4 patients (if the generator is starting to operate)
Synthesis of other Radiopharmaceuticals: 18F-FDG, 18F-DOPA, 18F-FET, 18F-MISO 18F-FCH, 68Ga-DOTA-TOC, 18F-FAZA, 18F-UCB-H oltre a 18F-PSMA-1007, 18F-PSMA-7	With the 68Ge-68Ga generator there is the possibility of also marking the NET diagnostics
Lower risks compared to old modules: sterility, speed, improved yield. Complex QCs, however the laboratory is already equipped with almost all the instrumentation	high security Quick preparation (15 min) because the cold kit only requires marking with Ga-68. Simplified QC: TLC
Low economic impact: after an initial expense for the equipment, the costs for radiosynthesis are lower than for the drug with an MA. Amortization of the initial expense in 3 years	High cost
Possibility of continuing supply to Spoke Centers since the t1/2 of the F-18 (about 2 hours) is compatible with transport times.	Impossibility of supplying the Spoke centers since the t1/2 of the Ga-68 is approximately 1 hour, therefore incompatible with transport times.
Technical times for purchasing the equipment: from 6 months to 1 year	Technical times: short (both the 68Ge-68Ga generator and the cold kit are immediately available)

Discussion/Conclusion

The choice of the registered cold kit, undoubtedly more expensive, would guarantee a rapid start of diagnostic treatments.
The purchase of synthesis modules would require longer times but would allow us to exploit the potential of Nuclear Medicine which is still under-utilized.

Lokametz® and Radelumin ® are currently in CNN class (non-refundable) with price to be negotiated As regards therapy, PSMA drugs such as Lutathera® (177Lu-PSMA-617) and Pluvicto® are already in use at our Center and we are obliged to purchase the drug since our radiopharmacy it is not in GMP. Our goal is to increase the availability of radiopharmaceuticals and expand the use of theranostics.

Cyclotron production of ^{52}Mn-radiopharmaceuticals and on phantom PET-imaging

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Introduction

This work aims to advance the technology required for cyclotron-driven production of manganese-52 (^{52}Mn), related to the preparation of radiopharmaceuticals suitable for positron emission tomography (PET), but also for multimodal PET/MRI imaging studies when they are used in combination with analogous paramagnetic manganese-based compounds. The cyclotron-production of ^{52}Mn implies using natural or enriched in ^{52}Cr (isotopic abundance 83,789%) chromium targets and medium-low energy protons ranging from 10-20 MeV. This process predominantly relies on the nuclear reactions ^{52}Cr(p,n)^{52}Mn. The project includes: (i) designing and producing chromium metal targets; (ii) developing an automated and effective procedure for separating ^{52}Mn from the chromium bulk; (iii) labeling specific ligands with ^{52}Mn; and (iv) preliminary assessment of the imaging quality of the ^{52}Mn cyclotron produced**.**

Materials and Methods

Both natural and ^{52}Cr enriched chromium targets were produced using the Spark Plasma Sintering (SPS) technique [1]. Irradiation experiments were conducted using the ACSI TR19/300 cyclotron at the Sacro Cuore Don Calabria Hospital in Negrar di Valpolicella (Verona, Italy). The target was dissolved with HCl 6M. Then the yielded ^{52}Mn was separated and purified from the chromium bulk through a combination of anion and cation exchange chromatography with an automatic module. Preliminary labeling experiments were performed with the ligand S-2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA-SCN) at pH 5.5. Radiochemical purity has been determined by TLC chromatography. Furthermore, a preliminary evaluation of the imaging quality of ^{52}Mn, derived from ^{52}Cr enriched targets, has been carried out on a NEMA phantom, filled with 3.6 µCi of ^{52}Mn, using a microPET/CT scanner.

Results

The metal pellet of Cr was produced and joined to Nb+Au backing using the SPS technique [1]. The target was irradiated with 16 MeV proton beams at 10 µA for 15 minutes. The irradiated target was dissolved in concentrated HCl, then diluted to 3% HCl in ethanol and loaded onto a column containing AG1-X8 resin. Chromium was eluted using a solution of 3% HCl in ethanol, while manganese was eluted with 3 mL of HCl 0.1 M and directly loaded onto an AG50W-X8 resin. After

washing with HCl at various concentrations, the purified ^{52}Mn was eluted with HCl 1.5M (recovery yield was about 78%). DOTA-SCN has been labeled with the purified [^{52}Mn]$MnCl_2$ with radiochemical yield >99%. The images collected on phantom with a preclinical scanner tomograph confirm the quality of the product.

Discussions/Results

The developed technology allows obtaining cyclotron-produced ^{52}Mn in high yield and purity suitable for the labeling of DOTA-based radiopharmaceuticals. The development of new bimodal probes for manganese-based PET-MRI imaging is currently ongoing.

Acknowledgments

This work was done within the METRICS project founded by CNS5-INFN.

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Design and development of constrained DACH-derived Chelators for Radiopharmaceutical Applications

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Introduction

Nuclear medicine relies on radioactive tracers for both diagnosis and treatment of various diseases. Developing novel radiopharmaceuticals is a key aspect to advancing this field, with radiometals playing a crucial role. ^{68}Ga is a leading choice for Positron Emission Spectroscopy (PET) due to its advantageous characteristics ($t_{1/2}$ = 1.13 h, $I_{\beta+}$ = 89%, $E_{\beta+\ avg}$ = 830 keV). Effective chelation of the metal is pillar for such applications, requiring a chelating agent able to form highly stable and inert complexes. Macrocyclic chelators generally exhibit slow kinetics of metal complexation that requires harsh radiolabeling conditions incompatible with most biomolecules used as targeting vectors. For these reasons, non-macrocyclic ligands have gained particular interest as they offer faster complexation kinetics under milder conditions. However, their complexes generally exhibit lower thermodynamic stability, compared to macrocyclic counterpart. HBED[1] stands out as a promising acyclic chelator for ^{68}Ga. To address these challenges, we have developed a novel class of constrained chelators based on HBED, featuring a “rigid” *trans*-diamminocyclohexane (DACH) backbone. This design retains the aromatic portions while incorporating a pre-organized structure, offering flexibility for diverse ligating groups to form a coordinating environment suitable for the metal of interest, both on the aromatic ring and on the amine position.

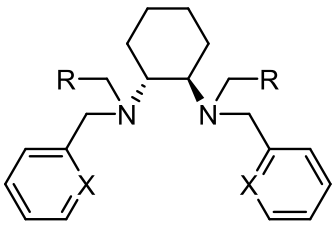

Figure 1. General Structure of DACH-derived Chelators

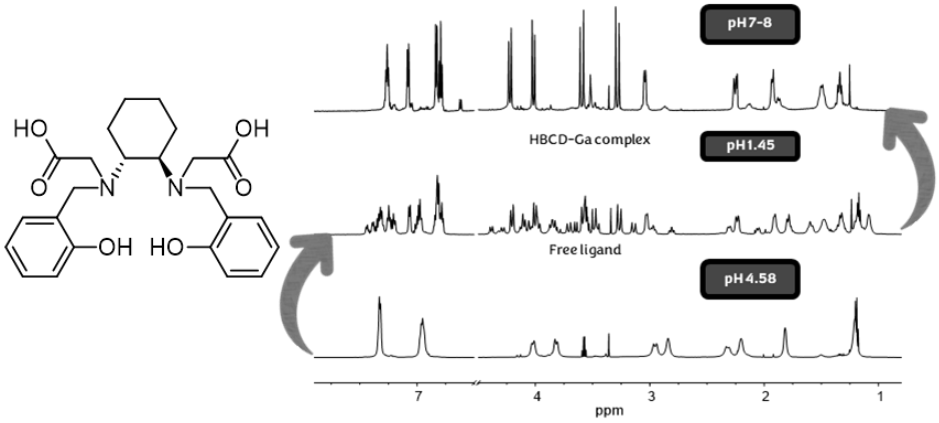

Figure 2. Structure of HBCD (bottom) and Ga-HBCD (top) complex formation with NMR spectroscopy.

Materials and Methods

NMR spectra were recorded on a Bruker Biospin FT-NMR AVANCE III HD (600 MHz) spectrometer. LC/MS was performed on an Agilent 6300 Ion Trap LC/MS system equipped with ESI interface. pH measurements were carried out using a calibrated pH-meter (Mettler-Toledo). UV–visible spectra were acquired using a JASCO V-770 UV/Vis/NIR spectrophotometer. Elemental analysis was performed on a Thermo Scientific™ FLASH 2000 CHNS Analyzer.

Results

New constrained DACH-containing chelators were synthetized and thoroughly characterized, especially for the acid-base character. As an example, we report the detailed investigation on N,N'-Di(2-HydroxyBenzyl)-(1,2-Cyclohexanediamine)-N,N'-Diacetic acid (HBCD). This ligand exhibits good affinity towards Ga(III), forming stable complexes. Ga(III) complexation was performed in D_2O and MeOD-d_4 at room temperature and followed via NMR spectroscopy. Additionally, confirmation of Ga(III) complexation was achieved through LC-MS analysis. Finally, UV-Vis titration of HBCD with $Ga(NO_3)_3$ in PBS (pH 7.4) was conducted. [Ga(III)HBCD] complex is rapidly formed at room temperature (20') and it remains intact in harsh acid conditions (HCl 2M) for several days.

Concluding remarks

These preliminary findings highlight the potential of this chelator class as a platform for developing innovative radiopharmaceuticals for ^{68}Ga. Further studies will focus on completing the investigations into these molecules for Ga, as calculating the stability constants for the complexes obtained, as well as performing the radiolabelling of the complexes obtained. Furthermore, the complexations of this molecules will undergo testing with other metals of interest.

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Advancing into the realm of innovative theranostic radionuclides: separation of silver-111 from a neutron-irradiated palladium target

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Introduction

Silver-111 ($t_{1/2}$ = 7.47 d) exhibits both medium-energy β^- ($E_{\beta-,\,max}$ = 1.04 MeV) and low-energy γ (E_γ = 245.4 keV, I_γ = 1.24 %; E_γ = 342.1 keV, I_γ = 6.7 %) emissions with promising potential for targeted radionuclide therapy and associated single photon emission computed tomography imaging. Its decay properties closely recall those of the clinically established lutetium-177, rendering it an alluring candidate for therapeutic applications. Furthermore, the clinical significance of silver-111 is heightened by the presence of a positron-emitting counterpart (silver-103; $t_{1/2}$ = 65.7 m, E_{β^+} = 2.4 MeV), thereby endowing this element with true theranostic potential. Such a well-suited pair has the potential to overcome current limitations tied to the compelled use of chemically distinct isotopes as imaging surrogates for lutetium-177. However, the utilization of radiopharmaceuticals labeled with silver isotopes has been hampered by the lack of suitable chelators capable of forming stable complexes, as well as the difficulties associated with their production and the radiochemical separation from target materials. In a noteworthy endeavor to tackle a part of these challenges, this study aims to establish a separation method for the purification of reactor-produced silver-111, affording it in a formulation suitable to the direct radiolabeling of appropriate targeting vectors.

Materials and methods

The adsorption behavior of Ag^+ and Pd^{2+} onto an extraction chromatographic LN Resin was assessed by determining the weight distribution ratios (D_w) over a wide range of HCl concentrations. Then, a separation process involving LN and TK200 resins was first developed for Ag^+ and Pd^{2+} cations in conditions mimicking a real silver-111 production. The effectiveness of the separation was assessed by ICP-OES. Silver-111 (0.6 MBq) was produced *via* the $^{110}Pd(n,\gamma)^{111}Pd$ nuclear reaction on a natural palladium target and the subsequent β^--decay of palladium-111 at TRIGA Mark II nuclear research reactor (LENA, Pavia, Italy). The separation process developed for the non-radioactive counterpart was translated to the purification of produced silver-111 from the palladium target. The effectiveness of the separations was confirmed by γ-spectrometry.

Results

Silver-111 retrieval was afforded in 10 mL of pure water. Overall recovery was > 90% with a radionuclidic purity > 99% and a separation factor of around $4.21 \cdot 10^{-4}$ from palladium.

Conclusions

The developed separation gave the proof of concept of a method suitable to obtain silver-111 in a ready-to-use water-based formulation. A scale-up production to amounts of silver-111 suitable for pre- and clinical studies is needed to validate the process.

POSTER

X Congresso Nazionale GICR
Brescia, Centro Congressi Paolo VI
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A Template for Creating Radioimmunoconjugates

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INTRODUCTION

Immunoconjugates exploit the high affinity of monoclonal antibodies for a recognized antigen, to selectively deliver a cytotoxic payload, such as drugs or radioactive nuclides, at the site of disease. Reaction of ε-amine group of lysine residues with electrophilic reactants, such as activated esters (NHS), is the main method reported in literature as it maintains proteins in their native conformation. Traditionally, a large excess of the activated ester is reacted to the mAb working at basic pH, generating a heterogeneous mixture of conjugates which can result in decreased target affinity. Here, we report an intradomain regioselective bioconjugation between the monoclonal antibody Trastuzumab and the N-hydroxysuccinimide ester of the DOTA chelator by a kinetically controlled reaction adding substoichiometric quantities of the activated ester to the mAb working at slightly basic pH. A new proteolysis protocol named domain mapping (patent application IT202400000l524), based on a selective domain unfolding, allowed for quantification of chemical modification at a domain level (figure 1). Data analysis based on LC–MS quantification of different analytical levels (intact, reduced chains, and domains) provided a molecular formulation of the mixture of immunoconjugates.

MATERIALS AND METHODS

The immunoconjugate was synthesized by adding 0.01 eq per min. of DOTA-NHS to Trastuzumab at room temperature and pH 7.2. The total time of synthesis was 500 min. Therefore, the reaction mixture was purified through size exclusion gel filtration. The resulting immunoconjugate was digested by a trypsin enzyme developing a domain mapping mass spectrometry workflow.

RESULTS

The immunoconjugate synthesized under kinetic control showed unitary chelator to antibody ratio (CAR). Proteolysis experiments displayed that an intradomain regioselectivity was achieved, with the conjugated lysine residues not involved in the binding with the antigen. The immunoconjugate mixture was composed of 15 species, whereas up to 10^6 species are statistically possible employing traditional bioconjugations. The most abundant species in the mixture resulted in the naked Trastuzumab, with the species Trastuzumab + 1 DOTA having a relative abundance in comparison to species Trastuzumab + 2DOTA ranging from 2 to more than 20-fold (table 1).

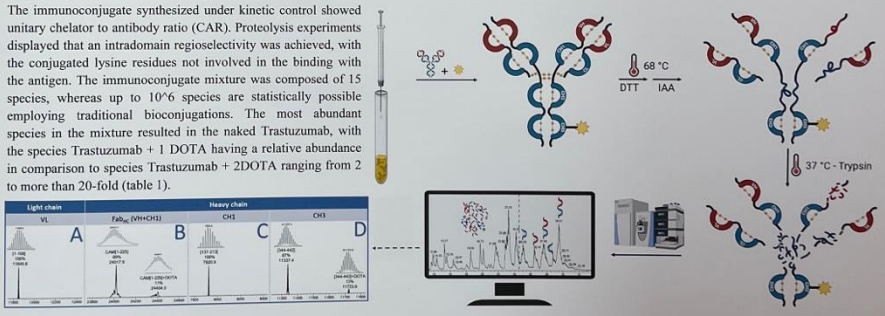

Figure 1. Schematic representation of kinetically controlled bioconjugation coupled to the domain mapping MS-workflow.

S	%
T	35
S_{CH3}	13,46
S_{CH2}	12,94
S_{VH}	11,39
S_{CL}	6,21
$S_{CH3\text{-}CH3}$	3,10
$S_{CH2\text{-}CH3}$	2,98
$S_{CH2\text{-}CH2}$	2,87
$S_{VH\text{-}CH3}$	2,62
$S_{VH\text{-}CH2}$	2,52
$S_{VH\text{-}VH}$	2,22
$S_{CH3\text{-}CL}$	1,43
$S_{CH2\text{-}CL}$	1,38
$S_{VH\text{-}CL}$	1,21
$S_{CL\text{-}CL}$	0,66

Table 1. Percentage composition of the immunoconjugate mixture. T indicate naked Trastuzumab; S indicate a generic species; subscripts indicate the conjugated domain.

DISCUSSION

During the past decade, research in radioimmunoconjugates moved toward a lower CAR. Beyond limiting the degree of modification to preserve mAb immunoreactivity, reduced CAR means more radiolabeled probes for the same quantity of radioactivity or even better reduced radioactive dose to patients to obtain a tumor to background ratio similar to that of radioimmunoconjugates with higher CAR. In the current study, we demonstrate that it is possible to synthesize immunoconjugates having unitary CAR achieving an intradomain regioselectivity, through a kinetically controlled bioconjugation. The synthesized mixture should ensure improved affinity for the antigen and lower radioactive dose to patients in comparison to traditionally synthesized radioimmunoconjugates. Preclinical in vitro and in vivo studies are currently performed to demonstrate the reduced radiotoxicity. Moreover, the choice of DOTA chelator allows for theragnostic application.

CONCLUSION

The coupling of synthesis under kinetic control with its monitoring using domain mapping could provide a model to obtain immunoconjugates which ensure a pharmaceutical quality, nowadays not achievable with traditional bioconjugation employing activated esters.

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Cytotoxic and Antiproliferative Effects of [^{64}Cu]$CuCl_2$ in Tumor Cells for Radiometabolic Therapy: a Preliminary Study

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1. Background and Goal of the present work

Peritoneal carcinomatosis (PC) represents a relatively common condition in the advanced stages of various tumors, characterized by the dissemination of malignant cells from the primary organ to the peritoneum. Its impact is global, affecting approximately 25,000 individuals in Italy and 1.4 million worldwide every year, with mostly negative outcomes [1]. The goal of this study is to delineate a therapeutic approach aimed at increasing the life expectancy of PC patients, overcoming the challenge associated with the marked genetic instability of cancer cells, which is incompatible with the receptor-targeted and antigenic therapies currently proposed [2]. In pursuit of this objective, the radionuclide copper-64, in the form of [^{64}Cu]$CuCl_2$, was employed. Recent studies have shown that copper, in its ionic form Cu^{2+}, can accumulate at significantly higher levels in cancer cells than in healthy ones, which makes its cytotoxic effect highly specific. This effect can be achieved by exploiting the nuclear decay properties of the radionuclide ^{64}Cu (^{64}Cu, $T_{1/2}$ 12.7 h; $E_{\beta+mean}$ 278 keV; $E_{\beta-mean}$ 191 keV; Auger emission) [3,4].

2. Methods

Human tumor cell lines related to the development of peritoneal metastases (MDA-MB-231, human breast adenocarcinoma cell line; NCI-N87, human gastric carcinoma cell line) and a healthy control cell line (IMR-90, normal lung fibroblast cell line) were utilized in this study. These cell lines were incubated with different activities of [^{64}Cu]$CuCl_2$ (10 µCi/mL; 100 µCi/mL; 250 µCi/mL) to evaluate their uptake and the antiproliferative and cytotoxic effects. For this purpose, an analysis was conducted to evaluate the level of ^{64}Cu incorporation in the nucleus and cytoplasm 4h after the incubation. Subsequently, in vitro studies on cell viability (XTT assay), apoptosis, and necrosis (Annexin V/SYTOX assay) were conducted following 96h and 120h of treatment in this experimental session (Figure 1). The incubation times for the XTT and Annexin V/SYTOX assays were selected after carrying out the same experiments 48h and 72h after the [^{64}Cu]$CuCl_2$ treatment.

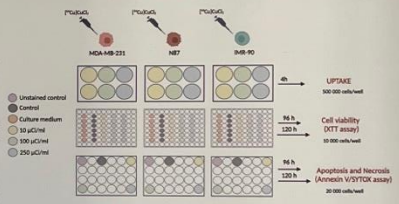


Figure 1: scheme of the experimental protocol.

3. Results

This study revealed a greater uptake of the [^{64}Cu]$CuCl_2$ in the carcinoma lines than in the healthy ones (Figure 2). As a result of the exposure to different activities of [^{64}Cu]$CuCl_2$, a greater reduction of the viability in tumor lines was observed compared to the healthy control line. Moreover, a significant increase in apoptosis in the MDA-MB-231 and NCI-N87 tumor lines was observed. The most significant results was obtained 96h after the treatment, as shown in figure 3, while up to 72 h of incubation there are no significant reductions in cell viability and induction of cell death.

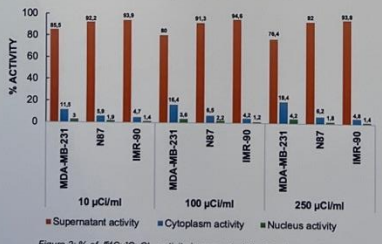
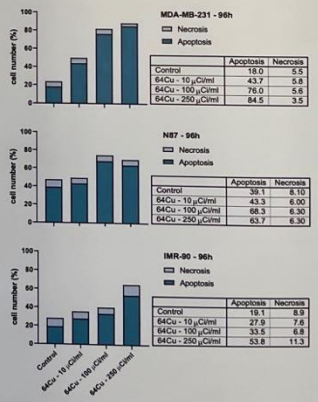


Figure 2: % of [^{64}Cu]$CuCl_2$ activity incorporated 4h after the treatment.



MDA-MB-231 - 96h	Apoptosis	Necrosis
Control	18.0	5.5
64Cu - 10 µCi/ml	43.7	5.8
64Cu - 100 µCi/ml	76.0	5.6
64Cu - 250 µCi/ml	84.5	3.5

N87 - 96h	Apoptosis	Necrosis
Control	39.1	8.10
64Cu - 10 µCi/ml	43.3	6.00
64Cu - 100 µCi/ml	68.3	6.30
64Cu - 250 µCi/ml	63.7	6.30

IMR-90 - 96h	Apoptosis	Necrosis
Control	19.1	8.9
64Cu - 10 µCi/ml	27.9	7.6
64Cu - 100 µCi/ml	33.5	6.8
64Cu - 250 µCi/ml	53.8	11.3

Figure 3: % of dead cells due to induction of apoptosis or necrosis 96h after the treatment.

4. Conclusions

Our preliminary results confirm the increased uptake of [^{64}Cu]$CuCl_2$ within the nuclear compartment of cancer cells and suggest the ability of the radiopharmaceutical to determine cell death through the induction of apoptosis. Further research to evaluate the antiproliferative and cytotoxic effects of [^{64}Cu]$CuCl_2$ using higher activities of ^{64}Cu are currently under investigation.

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GICR
Gruppo Interdisciplinare di Chimica dei Radiofarmaci

^{68}Ga-FAPi-46: radiolabelling and quality control at the European Institute of Oncology

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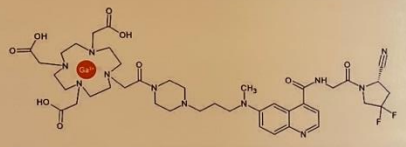


Background

^{68}Ga-FAPi is considered a pan-tumor agent capable of selectively binding to the transmembrane protein FAP overexpressed by the fibroblasts in the tumor microenvironment. In particular, ^{68}Ga-FAPi-46 has been used in tumors with low ^{18}FDG avidity, or in regions with an unfavourable ^{18}FDG tumor/background ratio.

Materials & Methods

Labelling of ^{68}Ga-FAPi-46 was carried out in a "kit-like" mode optimizing volumes and reaction conditions. A mixture of 1.5M sodium acetate and 0.07M sodium ascorbate (1.5mL, pH 8) was used as reaction buffer. Two methods of labelling were tested, as described below.

1. FAPi-46 was dissolved in the reaction buffer and subsequently mixed with 6mL of $^{68}GaCl_3$ eluate in 0.1N HCl. Reaction vial was heated in a digital thermoblock at 95°C up to a maximum of 10 minutes.

2. FAPi-46 was directly mixed with the $^{68}GaCl_3$ eluate and subsequently buffered. Reaction vial was heated in a digital thermoblock at 95°C up to a maximum of 15 minutes.

Results: RCP by Radio-TLC (ITLC-SG strips in NH_4Ac:MeOH 1:1)

1. 

^{68}Ga-FAPi-46 70,2%

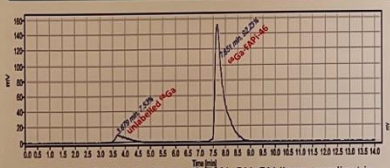
unlabelled ^{68}Ga 29,8%

2. 

^{68}Ga-FAPi-46 99,5%

unlabelled ^{68}Ga 0,5%

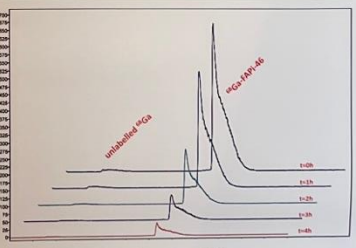
Results: RCP and Stability by Radio-HPLC



Flow: 1,0 mL/min, λ: 280nm, 0-70% CH_3CN linear gradient in 8min.

Other QC tests:

RNP	pH	Sterility	Endotoxin
<0,1 Bq/MBq	4-4,5	Sterile	<17.5 EU/mL



Stability of ^{68}Ga-FAPi-46 over 4h at RT.

Conclusion

The preparation of ^{68}Ga-FAPi-46, through the previously described labelling process, was found to be stable and reproducible with a high labelling yield and high chemical and radiochemical purity. Use in daily clinical practice confirms the results of this study.

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[^{18}F]DPA-714 synthesis, characterization and *in vivo* studies for the assessment of neuroinflammation in amyotrophic lateral sclerosis

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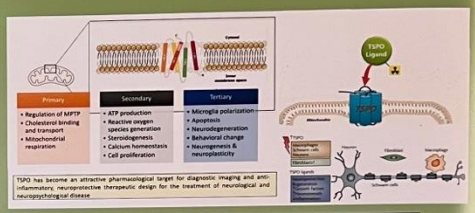
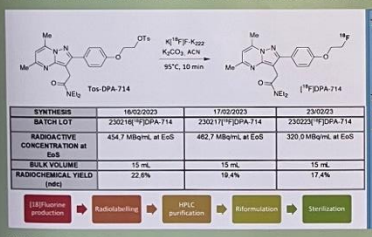
BACKGROUND AND AIM OF THE STUDY

The translocator protein 18 kDa (TSPO), a heteropolymeric mitochondrial protein, is today a validated imaging biomarker for the evaluation of neuro-inflammation and its progression, as well as a recognized target for the development of new therapeutic agents for neurological, psychiatric disorders and oncological applications. Among several radiotracers targeting TSPO, [^{18}F]DPA-714 is nowadays largely used as a PET imaging probe in many clinical trials. Here we report our preliminary experience in the production and quality controls (QCs) of the tracer as well as the collected data in experimental models of amyotrophic lateral sclerosis (ALS), a fatal type of motor neuron disease, characterized by progressive degeneration of nerve cells in the spinal cord and brain. ALS is a very difficult disease to diagnose and at the present there is no test or procedure to confirm without any doubt the final diagnoses. In this context, Nuclear Medicine with PET-TSPO may also play a role in the evaluation of patients with ALS and provide additional information to the clinicians.



[^{18}F]DPA-714 PRODUCTION AND CHARACTERIZATION (VALIDATION PROCEDURE)

The process was fully automated on a AllInOne 36 (Trasis) synthesizer equipped with semi-preparative HPLC using an in-house designed cassette and a dedicated sequence. [^{18}F]DPA-714 was synthesized by a one-step labelling process using 4.5-5.5 mg of Tos-DPA-714 as precursor, reacting with activated [^{18}F]Fluoride in ACN at 95°C for 10 min and followed by semi-preparative purification. The isolated product was trapped on C18 cartridge, then removed with ethanol 70% and reformulated with saline up to the volume of 15 mL and finally sterilized. QCs of the product were carried out according to the specifications reported in the general monographs of the European Pharmacopoeia and the literature. Following successful validation procedure, including three fully automated productions and aseptic dispensing, the described [^{18}F]DPA-714 radiosynthesis is able, with a single multidose batch, to support several patient scans.

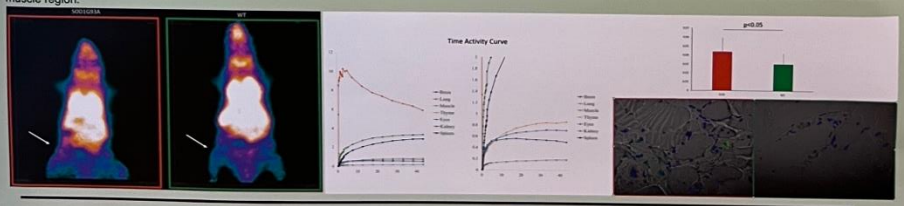


SYNTHESIS	16/02/2023	17/02/2023	23/02/23
BATCH LOT	23021B[18F]DPA-714	23021J[18F]DPA-714	23022B[18F]DPA-714
RADIOACTIVE CONCENTRATION at EoS	454,7 MBq/mL at EoS	462,7 MBq/mL at EoS	320,0 MBq/mL at EoS
BULK VOLUME	15 mL	15 mL	15 mL
RADIOCHEMICAL YIELD (ndc)	22.6%	19.4%	17.4%

[^{18}F]DPA-714 production was easily implemented with a simple and robust process with high radiochemical yields of 17,4-22,6% (ndc, n=10), molar activities of 364-613 GBq/μmol and radiochemical purities in the range 98,6-99,0% EoS. All QCs results complied with the requirements of the European Pharmacopoeia and other designed specifications, including stability studies (up to 6 hours EoS).

[^{18}F]DPA-714 *IN VIVO* PET-TSPO SMALL-ANIMAL IMAGING

The *in vivo* study included: 9 B6SJL-TgN (SOD1^{G93A})1Gur mice expressing a high number of mutant human SOD1 copies with a Gly93Ala substitution (SOD1^{G93A}) and 7 control background-matched B6SJL wild-type mice (WT, Jackson Laboratories, Bar Harbor, ME, USA). SOD1^{G93A} mice were studied both before (60/90days) and after (120 days) the onset of motor impairment. Age matched WT mice were used as controls. Mice were submitted to a dedicated micro-PET imaging (Albira, Bruker, USA) whose dual ring configuration allows the acquisition of the whole mouse body by static acquisition lasted ten minutes. A series of volumes of interest (VOIs) was drawn on the obtained images for brain, eyes, thymus, lungs, kidneys and in the skeletal muscles of both hindlimbs. The estimation of [^{18}F]DPA-714 retention was expressed as standardized uptake value (SUV). The [^{18}F]DPA-714 observed uptake in the brain was equal between SOD1^{G93A} and WT mice, while TSPO signal appeared enhanced in the skeletal muscle SOD1^{G93A} mice. The data were confirmed by the SUVs determined by the Logan slope and the specific TSPO immunofluorescence on the skeletal muscle region.



CONCLUSIONS

The developed [^{18}F]DPA-714 automated synthetic process is reliable and robust using disposable materials and standard reagents, being implementable for routine manufacturing and possibly for human use in clinical trials. Although usually applied for the study of neuro-inflammation, its distribution is abnormal in the studied model of amyotrophic lateral sclerosis suggesting the possible increase in TSPO expression in tissues different from the central nervous system, such as the skeletal muscle, being a valuable tool for the

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Development of an automated radiolabeling platform and preliminary results using a new potential [^{18}F]Fluorinated-PARP10 imaging agent

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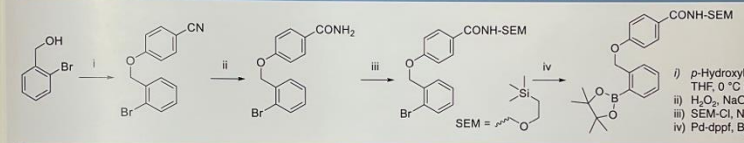
INTRODUCTION and AIM

Human poly-ADP-ribose polymerases (PARPs) are an enzyme family of 17 members that catalyze the covalent attachment of poly- or mono-ADP-ribose units post-translationally on a variety of amino acid residues of target proteins. The role of PARP10 is not completely elucidated yet, however its overexpression in various cancer cell lines led to the hypothesis that this enzyme promotes cancer proliferation and acts as an oncogene. Based on a previous work about PARP10 inhibitors (PARP10i),[1] our goal is to develop a new PET imaging agent to study the role of this enzyme in different cancer models. In this preliminary work we aimed at developing a PET tracer, namely [^{18}F]PARP10i, starting from a reported small molecule developed to inhibit PARP10 at micromolar concentrations.[2]

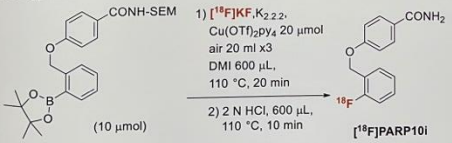
PRECURSOR SYNTHESIS at UniTO



i) *p*-Hydroxybenzonitrile, DIAD, PPH_3, dry THF, 0 °C to rt, on;
ii) H_2O_2, NaOH, MeOH, rt,72 h;
iii) SEM-Cl, NaH, dry THF, 0 °C to rt, on;
iv) Pd-dppf, B_2pin_2, AcOK, DMF, 85 °C.

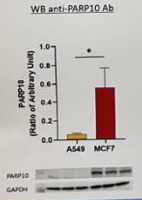
RADIOFLUORINATION and PRELIMINARY IN VITRO UPTAKE at UniGE

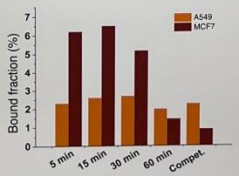
The automated radiosynthesis through an AllInOne synthesizer (Trasis) allowed a reliable production of the first promising imaging candidate [^{18}F]PARP10i *via* copper-mediated fluorination,[3] with high chemical and radiochemical purity, suitable for *in vitro* and *in vivo* experiments, in about 90 min including HPLC post-purification and final reformulation. From 1.75 to 5.42 GBq were produced with an activity yield (AY) of 10-35% (ndc) and a molar activity (Am) in the range of 72.8-222.9 GBq/μmol.

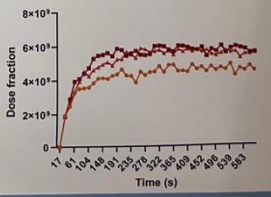


[^{18}F]PARP10i

[^{18}F]PARP10i was tested on MCF7 (breast cancer, PARP10+) and A549 (lung cancer, PARP10-) cell lines (Western-blot confirmed, below on the left). Uptake experiments (below on the center) were carried out by incubating cells with [^{18}F]PARP10i for 5, 15, 30 and 60 min (200 KBq for each well, ~1x10^5 cells) and measuring the activity with a Wizard2 2470 gamma counter (Perkin-Elmer). Competitive assay with "cold" inhibitor for 45 min, followed by further 45 min in the presence of [^{18}F]PARP10i, confirmed the specificity for the selected target. Kinetic experiments on MCF7, A549 and CT26 cells using Ligand Tracer™ confirmed a fast uptake and a plateau after few minutes (below on the right).







CONCLUSIONS

The preliminary results show that [^{18}F]PARP10i has a potential as new PET tracer for PARP10 imaging in cancer. Further experiments are in progress on tumor cell lines with and without PARP10 expression to confirm the suitability of this new PET diagnostic agent in a preclinical set using tumor-bearing mice.

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Cyclotron production of ^{52}Mn-radiopharmaceuticals and on phantom PET-imaging

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1. Background

The main goal of this work is to advance the technology required for cyclotron-driven production of manganese-52 (^{52}Mn), aiming at the preparation of radiopharmaceuticals suitable for positron emission tomography (PET), and for PET/MRI multimodal imaging studies, when they are used in combination with analogous paramagnetic manganese-based compounds. Manganese-52 may be produced by exploiting the ^{52}Cr(p,n)^{52}Mn reaction using medium-low energy protons protons within the range of 10-20 MeV.

2. Materials and methods

Manganese-52 may be produced starting from enriched ^{52}Cr or natural chromium target, which has a natural abundance of ^{52}Cr 83,789%, with medium-low energy protons (10-20 MeV) mainly by the ^{52}Cr(p,n)$^{52m/g}$Mn nuclear reaction route. Both natural and ^{52}Cr enriched chromium targets were produced using the Spark Plasma Sintering (SPS) technique [1] and then attached to a backing support composed of an Au thin inert layer bonded onto an Nb disc by SPS, as well.
Irradiation experiments were conducted using the ACSI TR19/300 cyclotron at the Sacro Cuore Don Calabria Hospital in Negrar di Valpolicella (Verona, Italy) for both target thermomecanical tests and purification process optimization. A solid target dissolution system has been manufactured by making some technical modifications to an automatic module already available on the market (Ekert&Ziegler) (Figure 1).

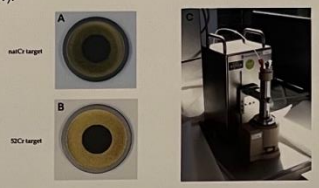

Figure 1. A, natCr target and **B**, ^{52}Cr target prepared with SPS technique, prior to irradiation; **C**, Pictures of the solid target dissolution system mounted on an E&Z module.

Several separation and purification procedures based on the combination of anionic and cationic resins have been evaluated through preliminary bench experiments with ^{nat}Cr targets. Based on the most promising results, the procedure with AG1-X8 and AG50W-X4 resin has been applied to the irradiated target purification process with the cassette-based system. The determination of Mn % recovery has been performed by γ-spectrometry analysis, whereas the amount of Cr in the final ^{52}Mn solution by ICP-OES analysis. Preliminary labeling experiments were performed with the ligand S-2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA-SCN) at pH 5.5. Radiochemical purity has been determined by TLC chromatography.
Furthermore, a preliminary evaluation of the imaging quality of ^{52}Mn, derived from ^{52}Cr enriched targets, has been carried out on a NEMA phantom, filled with 3.6 μCi of 52Mn, using a microPET/CT scanner.

3. Results

The target was irradiated with 16.8 MeV proton beams at 14.2 μA for 45 minutes. The total activity of ^{52}Mn produced on the enriched target at the End Of Bombardment (EOB) was 127 MBq. The irradiated target was dissolved in concentrated HCl, then diluted to 3% HCl in ethanol and loaded onto a column containing AG1-X8 resin. Chromium was eluted using a solution of 3% HCl in ethanol, while manganese was eluted with 3 mL of HCl 0.1 M and directly loaded onto an AG50W-X4 resin.

After washing with HCl at various concentrations, the purified ^{52}Mn was eluted with HCl 1.5M (Figure 2), resulting in a Mn recovery yield of approximately 78%. The Cr content in the final product is <10 ppm.
DOTA-SCN has been successfully labeled with the purified [^{52}Mn]$MnCl_2$,at pH 5.5 with radiochemical purity >99% reached in 15 minutes at 60°C. The images collected on phantom with a preclinical scanner tomograph confirm the quality of the product (Figure 3).

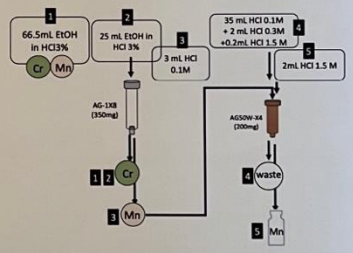

Figure 2. Scheme of the Cr/Mn separation procedure combining an AG1X-8 resin with an AG50W-X4 resin. 1.Loading a 3% HCl solution containing the mixture Cr/Mn on the anionic resin; 2. Washing with 25 mL of 3% HCl in EtOH; 3. Mn elution with 3 mL of HCl 0.1M and loading on the 270 AG50W-X4 resin; 4. Washing with 35 mL HCl 0.1 M, 2 mL HCl 0.3 M and 0.2 mL HCl 1.5 M; 5. Mn elution with 2 mL of HCl 1.5 M.

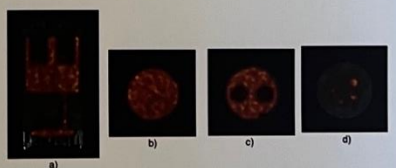

Figure 3. Reconstructed images form NEMA NU-4 image quality phantom. PET data was reconstructed with attenuation and scatter correction by using the iterative method. A single sagittal slice is shown highlighting the different sections in the phantom (a); a single transverse slice through the uniform section of phantom (b); a single transverse slice through the section of the phantom containing air (c); and a single transverse slice through the 5 rods region (d).

4. Conclusions

In this work we reported the development of a technology that allows obtaining cyclotron-produced ^{52}Mn in high yield and purity. Labeling tests were conducted using DOTA-SCN to produce radioactive complexes (^{52}Mn-DOTA-SCN) that underwent preliminary studies on phantom imaging using a microPET imaging system. Results indicated the effectiveness of the radiopharmaceutical preparation. The development of new bimodal probes for manganese-based PET-MRI imaging is currently ongoing.

References
1. Pupillo G et al. Cyclotron-based production of innovative medical radionuclides at the INFN-LNL: state of the art and perspective. Eur. Phys. J. Plus. 2023; 138:1095.

^{177}Lu and ^{161}Tb: comparison of cell damage produced by somatostatin analog radiopharmaceuticals

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12-14 aprile 2024
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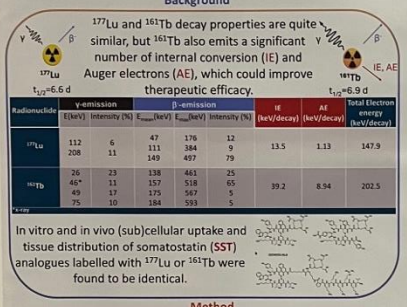
Background



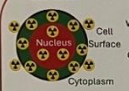
^{177}Lu and ^{161}Tb decay properties are quite similar, but ^{161}Tb also emits a significant number of internal conversion (IE) and Auger electrons (AE), which could improve therapeutic efficacy.

Radionuclide	γ-emission		β⁻-emission			IE (keV/decay)	AE (keV/decay)	Total Electron energy (keV/decay)
	E(keV)	Intensity (%)	E_{max}(keV)	E_{max}(keV)	Intensity (%)			
^{177}Lu	112	6	47	176	12	13.5	1.13	147.9
	208	11	111	384	9			
			149	497	79			
^{161}Tb	26	23	138	461	25	39.2	8.94	202.5
	46*	11	157	518	65			
	49	17	175	567	5			
	75	10	184	593	5			

*X-rays

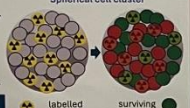
In vitro and in vivo (sub)cellular uptake and tissue distribution of somatostatin (SST) analogues labelled with ^{177}Lu or ^{161}Tb were found to be identical.

Method



Absorbed dose (AD) to the nuclei of AR42J cells and survival fraction (Sf) were obtained with the MIRDcell code (v. 4.14) [Katugampola S *et al* J Nucl Med 2022, 63] using the linear quadratic model equation and experimentally determined α, β parameters and cell size.

Subcellular distribution of ^{161}Tb or ^{177}Lu SST radiopharmaceuticals (RPs) according to the experimental data [Borgna F *et al* EJNMMI 2022, 49].



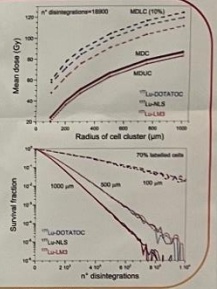
Cell uptake and localization (%)	DOTATOC	NLS	LM3
Cell Surface	19	16	91
Cytoplasm	80	78	7
Nucleus	1	6	2
Uptake	10	15	70

Results: AD and Sf of ^{177}Lu-RPs

ADs to unlabeled (MDUC), labeled (MDLC) and all cells (MDC) increase with the dimension of the cell cluster.

MDC and MDUC do not depend on the kind of RP; differences are found for MDLC, but decreasing with increasing % of labeled cells.

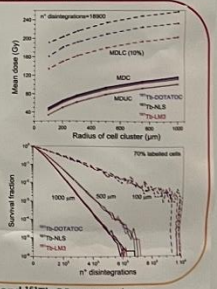
For a given cluster size and % of labelled cells, Sf are very similar for different RPs.



Results: AD and Sf of ^{161}Tb-RPs

MDC shows a slight dependence on the type of RP, but, for a given cluster size and % of labelled cells, Sf are again very similar for different RPs.

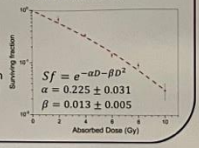
For the same number of disintegrations and cell cluster size, MDC, MDUC and MDLC are significantly larger than for ^{177}Lu-RPs, resulting in a lower Sf.



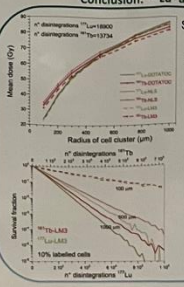
Results: AR42J cells size and survival parameters

The mean radius of the cell and nucleus obtained from cell imaging of a 3D cell culture in alginate beads after microscopic measurements were 8 ± 1 μm and 6 ± 1 μm, respectively.

The α and β radiobiological parameters of the linear quadratic model were estimated by fitting cell proliferation data obtained 24 h after irradiation with a Gammacell 220 (^{60}Co-source, dose rate 7.5 Gy/h).



Conclusion: ^{177}Lu- and ^{161}Tb-RPs comparison



Scaling the disintegration number by a factor 1.37, taking into account the different amount of energy emitted in the form of electrons, ^{161}Tb results more efficient than ^{177}Lu for small clusters, but less or similarly efficient for larger clusters.

[18F]F-JKPSMA7 from HPLC to SPE purification

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INTRODUCTION

^{18}F-JK-PSMA-7 is a new promising PSMA specific tracer under clinical evaluation to evaluate the prostatic malignancy recurracy in oncologic patients. [1] The radiopharmaceutical is produced in a Trasis synthesis module through nucleophilic aromatic substitutions followed by acid deprotection and purified by HPLC. HPLC purification is a bottleneck step on Rph production due to the limited numbers of synthesis modules equipmed with HPLC and due to the multiples possible failure caused by this technique.
To overcome these limitations, we had to study a possible purification pathway to reduce the synthesis steps and time and make synthesis stability and easiness.

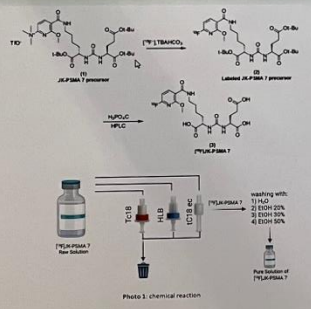

Photo 1: chemical reaction

RESULTS

Evaluating the profile of the reactor solution we identify some impurity like free fluorine-18, partially deprotected moieties and the product. C18 trapping was evaluated in different sorbent materials and amounts to better understand the best trapping conditions. Water wash allows to remove all the inorganic impurities and leat the product on cartridge. fractionated elution with different ethanol concentrations allowed to find the best ratio to use, final Aluminum cartridge was used to completely remove the free fluorine-18.

MATERIALS AND METHODS

The [18F]F-JKPMSA7 was produced on miniAIO TRASIS synthesis module, the reaction was copied from the AIO procedure up to crude reactor step. On this step the reaction was quenched by an appropriate solution and then transferred out to the module.
THe crude reaction solution was diluted with different solvent like pure distilled water or physiological solution. The final dilutes solutions were divided on multiple samples to assess different SPE purification methods.
SPE cartridges tested were supplied by:

- C18, C18 Long, OASIS, HLB, Al, QMA were supplied by Waters GmbH.
- C18 ec was supplied by Mackerey-Nagle.

Starting from the different solutions with a radioactive HPLC profile shown in figure 1, we would like to obtain a similar qualitative HPLC profile recorded from [18F]F-JKPMSA7 purified through HPLC.

Photo 2: on the left: Trasis MiniAIO module; on the right: TLC Cyclone instrument

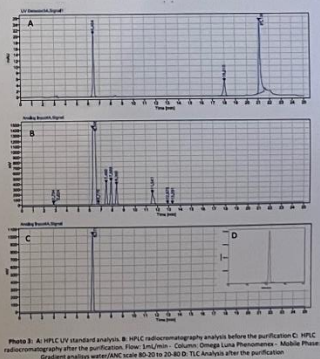

Photo 3: A: HPLC UV standard analysis. B: HPLC radiocromatography analysis before the purification C: HPLC radiocromatography after the purification. Flow: 1mL/min - Column: Omega Luna Phenomenex - Mobile Phase: Gradient analisys water/ANC scale 80-20 to 20-80 D: TLC Analysis after the purification

CONCLUSION

The SPE purification applied to radiopharmaceutical preparation simplifies the operation and increases the yield reducing synthesis time. The quality control profile is to comply with the [18F]F-JKPMSA7 monograph draft published in Pharmeuropa.

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Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"
Istituto di Ricovero e Cura a Carattere Scientifico

ISTITUTO ROMAGNOLO PER LO STUDIO DEI TUMORI DINO AMADORI

Production and Quality Control of [68Ga]GaFAPI46: Development of an Investigational Medicinal Product Dossier for Clinical Trials for a multicentric clinical trial

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INTRODUCTION

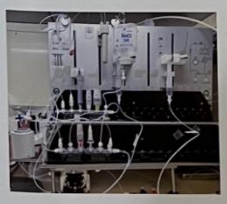
FAPI-46 is a quinoline-structured inhibitor of Fibroblast Activating Protein (FAP) a type II membrane serine protease overexpressed by tumour stroma-associated fibroblasts (CAFs). FAP is also associated with a poor prognosis in cancer patients and it's involved in biological mechanisms of tumour development, for this reason actually FAP is an ideal target for diagnostic and therapeutic radiopharmaceuticals. [68Ga]GaFAPI46 is an experimental drug useful for PET imaging of tumour tissues that overexpresses FAP.

The aim of this abstract is to describe the structure of an investigational medicinal product dossier (IMPD) for a multicenter clinical trial.

MATERIALS AND METHODS

Our clinical trial involves two clinical centers. The drug substances described in the IMPD are precursor FAPI46 (Sofie) and Gallium-68 obtained by Ge-68/Ga-68 generator GalliaPharm (Eckert Ziegler) with marketing authorization. The radiolabelling of [68Ga]GaFAPI46 is carried out by two different synthesis modules palced in the two clinical centers, in particular Eazy (Eckert Ziegler) and MiniAIO (Trasis).

Consumables and the reagent kit are different in the two centers based on the different manufacturing process. The quality control equipments are different, but the release specifications are the same.



RESULTS

The IMP produced in the two sites consists in a multidose solution of [68Ga]GaFAPI46 with a radioactive concentration between 50-70 MBq/ml at the End of Synthesis (EOS) that is considered ART. Acceptance criteria, specifications, and release timing are the same for both centers and were chosen in compliance with the general texts and monographs of the current European Pharmacopoeia. All the tests, except sterility are carried out before the release. The sterility test are performed by the same external Laboratory. The validation of the analytical procedures, the acceptance limits, and the parameters considered (specificity, linearity, range, accuracy, precision, quantification, and detection limit) were carried out by the two centers according to the ICH guideline Q2(R1). Both sites performed process validation by three different batches of [68Ga]GaFAPI46. Each batch was fully characterized from the analytical point of view, to confirm the compliance with the established acceptance criteria. The acceptance criteria were verified also to verify the two-hour stability at room temperature for all three validation batches.

DISCUSSION/CONCLUSIONS

This work demonstrates that [68Ga]Ga-FAPI-46 can be prepared as an IMP by different centers involved in the same clinical trials.In this case Regulatory Agency requires a single integrated IMPD detailing both manufacturing processes. The center applicant need to demonstrate the consistency of radiopharmaceuticals produced at the different sites, justifying and detailing any differences in manufacturing processes, controls, and/or specifications. The dossier should report the process validation obtained for each site, while information common to both sites should be reported only once.

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12-14 aprile 2024 X CONGRESSO NAZIONALE GICR

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Azienda Unità Sanitaria Locale di Reggio Emilia

A Roadmap to the Rational Development of Radium-223 and Barium-131/135m Chelators for Targeted Theranostics

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Aim. ^{223}Ra ($t_{1/2}$ = 11.43 d) is an **α-emitter**, currently approved as [^{223}Ra]RaCl$_2$ (Xofigo®) for the palliative treatment of bone metastases in castration-resistant prostate cancer. ^{131}Ba ($t_{1/2}$ = 11.50 d) and ^{135m}Ba ($t_{1/2}$ = 28.7 h) are **γ-emitters** that could serve as ^{223}Ra surrogates for SPECT imaging, allowing a ^{223}Ra/^{131,135m}Ba-based **theranostic** approach.
To widen the possible treatable tumors, the spontaneous accumulation of Ra^{2+}/Ba^{2+} in the bones must be overcome by stably complexing Ra^{2+}/Ba^{2+} through a chelator, in turn conjugated to a targeting vector to direct radiation to the tumor site. However, the so-far **poorly explored coordination chemistry** of Ba^{2+} and Ra^{2+} has complicated the rational design of chelating agents capable to firmly trap these radionuclides *in vivo*, hindering their utilization.
Herein we delve into the **coordination preferences of Ra^{2+} and Ba^{2+}**, assessing their affinity to different donor groups. This exploration lays the groundwork for the subsequent **design of tailored chelators**, crucial for the development of **^{223}Ra/$^{131/135m}$Ba-based radiopharmaceuticals**.

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References
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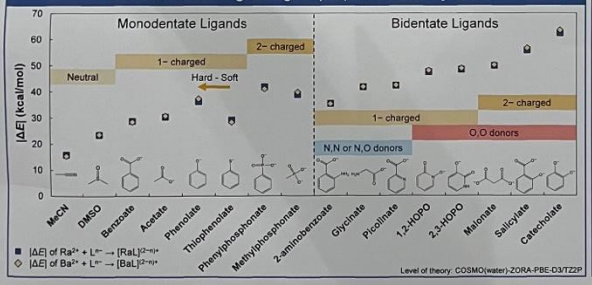
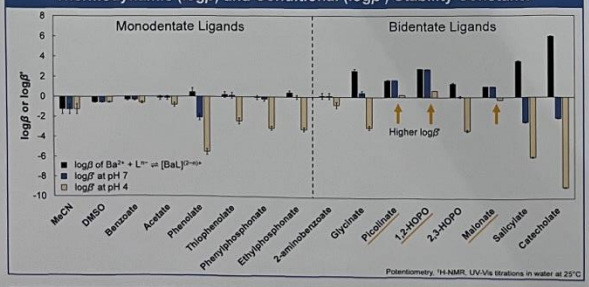
Reissig *et al., Nucl. Med. Biol.* **2021**, 98: 59-68.
Ivanov *et al., Chem. Commun.* **2022**, 58 (71): 9938-9941.
Brown *et al., Radiochim. Acta* **2022**, 110 (6-9): 505-513.
Franchi *et al., J. Inorg. Biochem.* **2024**, *Submitted*.

Electronic Binding Energies (Δ*E*) calculated by DFT

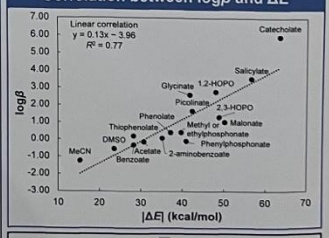


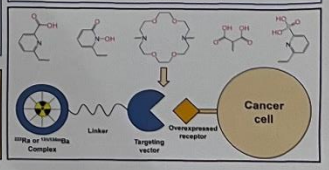
Results. DFT calculations unveiled **striking similarities** in the behavior of **Ba^{2+} and Ra^{2+}**, as evidenced by their nearly identical Δ*E* values across all the investigated ligands. The **correlation** between computed Δ*E* and experimental log*β* of Ba^{2+} complexes is quite good. Both methods converge in revealing the preference of Ra^{2+} and Ba^{2+} for more **negatively charged** ligands and for **oxygen** rather than nitrogen or sulfur donors.
To design chelators for radiopharmaceutical applications, log*β'* values should be considered because they represent the **effective stability** of the complexes at a given pH. At **pH 4 and 7.4** (both common in radiolabeling experiments and 7.4 also being the physiological pH) **picolinate**, 2-hydroxypyridine 1-oxide (**1,2-HOPO**), and **malonate** provide the most stable $[BaL]^{(2-n)+}$ complexes among those investigated.

Thermodynamic (log*β*) and Conditional (log*β'*) Stability Constants



Correlation between log*β* and Δ*E*





Conclusions. We are currently developing **multidentate chelators for Ra^{2+} and Ba^{2+}** utilizing the most promising **building blocks** identified through this research. This endeavor aims to pave the way for the creation of cutting-edge **^{223}Ra/^{131,135m}Ba**-based radiopharmaceuticals for **targeted theranostics** of cancer.

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UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

GICR Gruppo Interdisciplinare di Chimica dei Radiofarmaci

Novel PET Radiotracers for measuring P-glycoprotein function in Neural Disorders

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Introduction

P-glycoprotein (P-gp, Mdr1) is an ATPase protein, belonging to the ATP-Binding Cassette (ABC) transporter and is involved in the absorption and elimination of xenobiotics. P-gp is localized at the apical side of cell membranes of the same organs such as the liver, kidney, and gut, and in some barriers such as the blood-brain barrier.[1] Changes in the P-gp expression and function are involved in several neurological disorders such as Alzheimer's and Parkinson's disease. For this reason, the development of PET radioligands to detect P-gp activity can be useful in the clinical setting of neurodegenerative disorders.[2] This study relates to a novel PET-radiotracer [^{18}F]MC225, patented by our group,[3] with high affinity and selectivity towards P-gp and its potential for the in *in vivo* evaluation of P-gp role in neural disorders.

Background

Currently, The most widely used radiopharmaceutical for P-gp imaging is [^{11}C]-verapamil (fig.1). Verapamil was developed to be an inhibitor for Pgp in tumor cells but it shows a low affinity for the target. Was observed that at tracer level it behave as Pgp substrate to this was labelled to be used as radiotracer for functional studies of Pgp in neural diseases. Other two P-gp inhibitors tariquidar and elacridar (fig.1) proved to behave as substrates at tracer level and were labeled with Carbon-11 and used for PET imaging. Most of the labeled compounds that are currently in use are labeled with carbon-11, while labeling with fluorine-18 would lead to a radiopharmaceutical that can be transported to other imaging centers without on-site cyclotron due to the longer half-life of fluorine-18 of 110 min vs 20 min for carbon-11. In addition, the longer half-life would enable prolonged imaging times and more subjects could be dosed from one radiopharmaceutical production. Clearly, there was an unmet need to develop 18F-radiolabeled compounds to study P-gp function with PET at the BBB. [^{18}F]MC225 (fig.1) is a promising novel PET tracer developed within a library of compounds which was patented by our group.

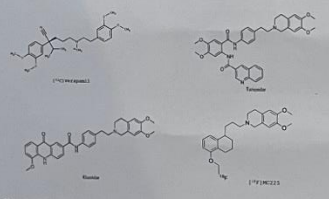
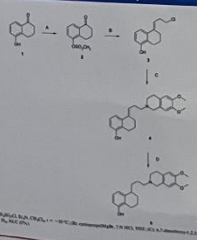
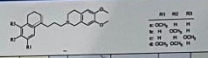


Figure 1. PET radiotracers for in *vivo imaging* of P-gp.

Synthesis of the pharmacophore

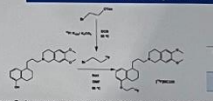


Structure - activity relationship



CPD		Pgp inhibition EC_{50} ± SEM (µM)	Pgp (BA/AB)	CPD		Pgp inhibition EC_{50} ± SEM (µM)	Pgp (BA/AB)	CPD		Pgp inhibition EC_{50} ± SEM (µM)	Pgp (BA/AB)
PB28	–	0.55 ± 0.02	1.7	7a		10.2 ± 0.80	3.7	8a		35.5 ± 2.5	5.4
Verapamil	–	20 ± 1.0	1.2								
6a		4.86 ± 1.10	3.9	7b		8.15 ± 0.30	4.2	8c		15 ± 2.5	7.1
6c		1.64 ± 0.20	1.6	7c		6.35 ± 0.25	18	8d		31.6 ± 2.7	1.5

Radiosynthesis



Production time	Radiochemical yield	Radiochemical purity	Specific activity
74 min	12%	> 99%	> 100 GBq/µmol

Figure 2. Automated synthesis of [^{18}F]MC225 approved for human use by the Institutional PET Drug Committee and MHLW Certified Clinical Research Review Board, Tokyo Metropolitan Geriatric Medical Center for first-in human clinical research (jRCTs031190136).

Preclinical and Clinical Evaluation of [^{18}F]MC225

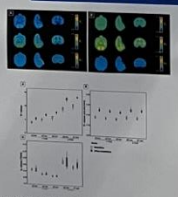


Figure 3. Evaluation of [18F]MC225 in Non-Human Primates. Parametric images calculated using 91-min scan duration and 2-TCM at baseline (A) and after-inhibition (B). Boxplot showing the third quartile and first quartile range of K1 (A), VT (B), and k2 (C) values of the whole-brain region at baseline and after-inhibition scans in different scan durations. The black line within the box marks the median and the whisker above and below the box indicates the maximum and the minimum value excluding the outliers.

Figure 4. standardized uptake value (SUV) images. first [18F]MC225 PET brain scans in a healthy human subject in both unblocked (A) and blocked (B) P-gp state. This image was awarded by the Eur J of Nuclear Medicine and Molecular Imaging as the image of the month.

Conclusions

The first [^{18}F]MC225 PET brain scans in a healthy human subject in both unblocked (A) and blocked (B) P-gp state (Fig.4) quantitatively show higher uptake at baseline levels (VT = 4.38) and after P-gp inhibition (VT = 5.48) with respect [^{11}C]Verapamil (VT = 1.28 at baseline, VT = 2.00 after P-gp inhibition) facilitating the measurement of P-gp function in the brain.

References

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2. Knox E.G., Aburto M.R., Clarke G. et al. [2022], Mol Psych. 27: 2659–2673
3. Ghaffr El Idrissi I., Colabufo N.A. et al. [2016], WO2016174496

Acknowledgments

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12-14 aprile 2024

Centro Congressi Paolo VI
Brescia

X CONGRESSO NAZIONALE GICR

SOMAKIT-TOC, PERSONALIZED DOSES AND RISK ASSESSMENT

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Introduction

The availability of authorized kits for the labelling of Gallium-68 radiopharmaceuticals also allows Hospital Radiopharmacies that do not prepare Officinal Formulae to carry out the labelling in a simple, rapid, and safe way. It is the case of SomaKit-TOC, which, after radiolabelling with a Gallium-68 chloride solution, is authorized for Positron Emission Tomography (PET) imaging of somatostatin receptor overexpression in adult patients with confirmed or suspected well-differentiated gastroenteropancreatic neuroendocrine tumors (GEP-NET) for localizing primary tumors and their metastases.

The preparation of the SomaKit-TOC requires the use of a full $^{68}Ge/^{68}Ga$ generator eluate volume (5 ml), so the available Gallium-68 activity is until 1.2-1.3 GBq for a 1.85 GBq $^{68}Ge/^{68}Ga$ generator at the calibration date. Nevertheless, the license of the kit is for a single dose, which is between 100 and 200 MBq [1, 2]. The single dose use and the time required (4h) to achieve full yield after the last elution of the $^{68}Ge/^{68}Ga$ generator make it impossible to perform more than a single PET scan on the same day, even though each kit has a high activity (MBq) of finished product potentially suitable for 3-4 patients.

The aim of this work was to identify a pharmaceutical approach, regarding technical and regulatory, for overcoming this limitation.

Materials and methods

Joint meetings between the Radiopharmacies of Modena, Ferrara, and Cesena/Meldola occurred to analyze the current pharmaceutical legislation regarding the dispensing in personalized doses, intended for the individual patient, of a drug approved for single-use according to a medical prescription and to evaluate its applicability to the SomaKit-TOC [3-5].

A risk assessment was performed by applying FMEA-FMECA analysis to the full process, including the production and dispensing of the drug. To validate the method of multiple personalized doses-dispensing of the drug, a sterility test on the residual of the SomaKit-TOC preparation was performed in triplicate (on three preparations).

Results

The only critical issue that occurred during the risk assessment of the process of production and dispensing in multiple doses of the SomaKit-TOC was the maintenance of the sterility of the preparation, performing multiple manual doses (Index Priority Risk = 11).

Tabella FMECA relativa alle attività critiche:

Punti critici	Possibili modi di guasto	Effetti/conseguenze	G	P	R	IPR	Analisi delle cause	Azioni correttive
Mantenimento dell'asepsi durante il frazionamento manuale	Contaminazione microbiologica del prodotto in fase di frazionamento in dosi personalizzate.	Immissione in circolo ematico di materiale infetto/pirogeno	5	3	3	11	Mancato rispetto delle tecniche di lavorazione in asepsi	Nuovo addestramento e sensibilizzazione dell'operatore con ripetizione della convalida iniziale.

Tabella di graduazione del rischio

Gravità \ Probabilità	Frequente 5	Probabile 4	Occasionale 3	Remota 2	Improbabile 1
Catastrofica 9-10	N 45 - 50	N 36 - 40	N 27 - 30	[illegible]	L 9 - 10
Critica 7-8	N 35 - 40	N 28 - 32	[illegible]	M 14 - 16	[illegible]
Seria 5-6	[illegible]	[illegible]	M 15 - 18	L 10 - 12	[illegible]
Minore 3-4	M 15 - 20	M 12 - 16	L 9 - 12	[illegible]	[illegible]
Trascurabile 1-2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

T = Rischio tollerabile: non sono necessari controlli aggiuntivi
L = rischio Basso: sarebbe meglio aggiungere qualche ulteriore intervento
M = rischio Medio: è necessario identificare le cause di errore e aggiungere controlli ulteriori
H = rischio alto: occorre definire stretti controlli per gestire il rischio ed, eventualmente, cercare metodi alternativi
N = rischio Intollerabile: Necessario bloccare la produzione o l'attività o il cambiamento proposto.

The result of the sterility tests performed during the validation process highlighted the maintenance of the sterility .

Discussion/conclusion

We analyzed the current pharmaceutical legislation and performed the risk analysis to guarantee access to the PET imaging of somatostatin receptors for more cancer patients by dispensing in multiple personalized doses the radiopharmaceutical [^{68}Ga]Ga-DOTATOC, obtained from the SomaKit-TOC prepared according to RCP. According to the regulatory system for drugs, the partition of medicine in personalized doses is comparable to a Magistral Formula [3-5]. This classification allows the Pharmacist to dispense the Somakit-Toc final preparation in individual doses according to a nuclear medicine physician single prescription **in order to satisfy a clinical need** [6, 7]. The physician's prescription ensures the safety and efficacy of dosage, but the pharmaceutical quality depends on the operator, who must apply validated procedures.

References:

[^{89}Zr]ZrDOTA reactivity evaluation to optimize [^{89}Zr]ZrDOTA-c(RGDfK) preparation starting from zirconium-89 produced through solid target.

M.Malachini [1*],Moussou Diakhate[1], Armando D'Angelo[1], Jonathan Amico[1], Valentino Andrea Bragaja[1], Daniele Peruzzi[1], Nicolò Bergamaschi[1], Paola Bovone[1], Sara Cisternino2, Juan Esposito2, Giancarlo Gorgoni[1], Emiliano Cazzola[1].

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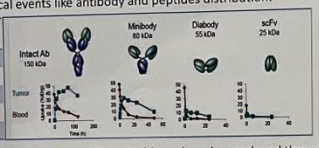
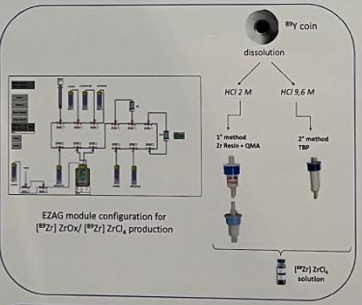
Introduction

[^{89}Zr] Zirconium is one of the emergent isotopes in the last years due to the favorable PET imaging characteristics. Because of its nuclear properties, like half life of 78.8 h (β^+_{max} 0.9 MeV, 22.7%) allows to acquire late imaging of the radiopharmaceuticals, in order to, to study long biological events like antibody and peptides distribution.

Isotope	T ½ (h)
^{68}Ga	1.1
^{18}F	1.8
^{64}Cu	12.7
^{89}Zr	78.4
^{124}I	100.3



DOTA complex of zirconium-89 are stable and can be produced through [^{89}Zr]$ZrCl_4$ precursor, that can be produced by two different pathways, the first one request a previews production of oxalate formulation and then a conversion on chloridric form using a QMA-SPE. The second procedure requests a different, more acid, dissolution a purification by TBP SPE.



EZAG module configuration for [^{89}Zr] ZrOx/ [^{89}Zr] $ZrCl_4$ production

Methods

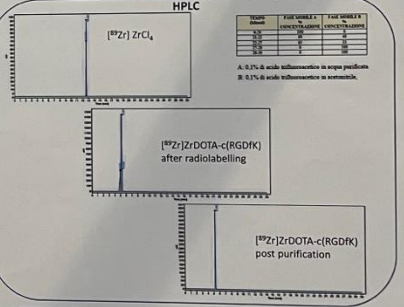
[^{89}Zr] $ZrCl_4$ solution was prepared starting from yttrium coin shaped. ZR and TBP for recovery was from Triskem and QMA and C18 from Waters. DOTA was provided by Macrocyclics, DOTA-cyclo(RGDfK) fromABX.

[^{89}Zr] $ZrCl_4$ solution was coupled with DOTA and a reactivity study was conducted, in order to, optimize it. For radiolabeling 200-500 µl of [^{89}Zr]$ZrCl_4$ (26-188 MBq) were added with different volumes HEPES 0,5M pH 6,5 and buffered with 2M sodium carbonate solution. The final reactions were carried out for 1h at 95°C in agitation (550 rpm).
Radiolabeled and references, were injected on HPLC (Primesep B2 C18) with a gradient method (CH_3CN 0,1%TFA and H_2O 0,1%TFA) and TLC SiO2 eluted with different mobile phases (DTPA 50mM, EDTA 50mM and MeOH/H_2O(1:1)4%TFA).

Results

Once we tested two different pathways for [^{89}Zr] $ZrCl_4$ and with the first method (Zr Resin + QMA) the final solution was better in terms of yields, residual contaminants and stability. For optimization of DOTA reactivity trials, we conducted by increasing ligand amount, activity and the quantity of stabiliser (HEPES) to find the best ratio.
Reaction kinetics was evaluated at different timepoints 15, 30, 60 minutes,
The reaction yield was evaluated under iTLC-SA. The [^{89}Zr]ZrDOTA-cyclo (RGDfK) was obtained in 88% of yield, under the 95 % set up a goal.
With the aim to increasing the yield a SPE based purification procedure was developed and allow to isolate the pure product as shown by HPLC and iTLC-SA.

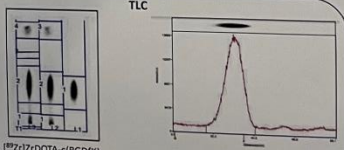
HPLC



TEMPO (Minuti)	FASE MOBILE A % CONCENTRAZIONE	FASE MOBILE B % CONCENTRAZIONE
0,00	95	5
20,00	0	100
25,00	0	100
26,00	95	5
30,00	95	5

A: 0,1% di acido trifluoroacetico in acqua purificata
B: 0,1% di acido trifluoroacetico in acetonitrile.

TLC



L1: [^{89}Zr]ZrDOTA-c(RGDfK)
L2: [^{89}Zr]ZrDOTA-c(RGDfK) post 1st cartridge
L3: [^{89}Zr]ZrDOTA-c(RGDfK) post 2nd cartridge

L3: [^{89}Zr]ZrDOTA-c(RGDfK)
FINAL PRODUCT

Conclusion

Under developing a reaction protocol between zirconium-89 in different formulations and DOTA we identify the optimal reaction condition to conjugate DOTA ligands to zirconium-89. The application of the identity protocol on ligand DOTA-cyclo(RGDfK) gave high coordination yield over 88%. To obtain a pure radiopharmaceuticals, purification and identity study was performed to isolate a pure compound shown on HPLC and iTLC-SA.

DEVELOPMENT OF COUMARIN-no3py DERIVATIVES FOR THERANOSTIC APPLICATIONS IN NUCLEAR MEDICINE

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[b]Univ de Brest, UMR-CNRS 6521 CEMCA, 6 avenue Victor le Gorgeu, F-29200 Brest, France.
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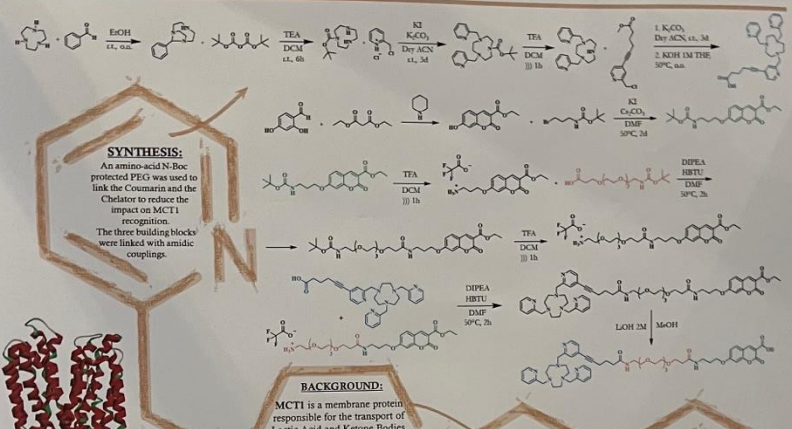
e-mail: matteo.mari@unimore.it

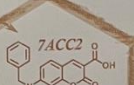


SYNTHESIS:
An amino-acid N-Boc protected PEG was used to link the Coumarin and the Chelator to reduce the impact on MCT1 recognition.
The three building blocks were linked with amidic couplings.

MCT1 Crystal Structure

N. Wang et al. Cell, vol. 184, iss. 2, pp. 370-383.e13. Jan. 2021.

BACKGROUND:
MCT1 is a membrane protein responsible for the transport of Lactic Acid and Ketone Bodies. A few inhibitors for MCT1 are known, like the Coumarin derivative 7ACC2.

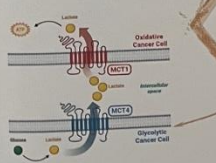


7ACC2

Cu^{2+}

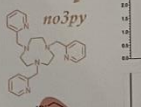
MCT1 structure hasn't been resolved, MCT1 is a potential target for theranostic applications because of the Warburg effect.

^{64}Cu (II), ^{67}Cu (II) and ^{68}Cu (II) are used in nuclear medicine for theranostic applications. no3py is a macrocycle that forms inert complex with Cu (II).



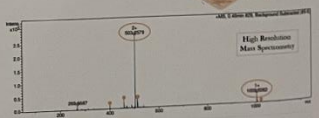
Oxidative Cancer Cell
Glycolytic Cancer Cell
MCT1
MCT4
Lactate
Glucose
ATP

Warburg Effect, Created with BioRender.com

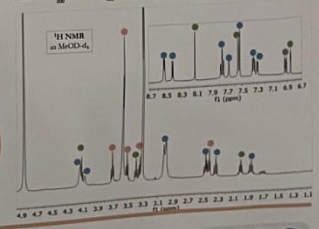


no3py

CONCLUSIONS:
The final compound was characterized with ^{1}H and ^{13}C NMR spectroscopy and High-Resolution Mass Spectrometry.

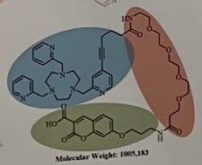


High Resolution Mass Spectrometry



^{1}H NMR in MeOD-d_4

NEXT STEPS:
- Physic-chemical characterization
- Radio-labelling
- *In vitro* and *in vivo* studies



Molecular Weight: 1005,183

CUMA-PEG-no3py

REFERENCES:

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2. N. Wang, *et al. Cell* **2021**, *184*, 370.

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^{99m}Tc-scFvD2B a potential theragnostic pair for ^{177}Lu-scFvD2B

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XCONGRESSO NAZIONALE GICR

BACKGROUND

Preclinical studies have shown that the ^{177}Lu-labeled single-chain variable fragment of the anti-prostate specific membrane antigen D2B antibody (^{77}**Lu-scFvD2B) exhibits greater uptake and internalization in prostate cancer cells compared to ^{177}Lu-PSMA inhibitory peptides** [1].This results in an increased tumor/kidney ratio and higher tumor radiation dose [2]. However, currently **there is no diagnostic radiopharmaceutical available that can be used as a theragnostic pair**.

OBJECTIVE

The aim of this study was to synthesize and characterize a new radiotracer, ^{99m}Tc-scFvD2B, and assess its potential as a theragnostic pair for ^{177}Lu-scFvD2B.

MATERIALS AND METHODS

The scFvD2B-Tag and scFvD2B antibody fragments were produced by ExcellGene in a prokaryotic and in a eukaryotic system, respectively, and purified by affinity chromatography, as reported by Frigerio et al [3]. Two HYNIC derivative chelators, HYNIC-Gly-Gly-Cys-NH2 (HYNIC-GGC) and succinimidyl-HYNIC (S-HYNIC), were used to conjugate the scFvD2B-Tag and scFvD2B isoforms, respectively. ELISA assays were performed to evaluate the affinity and specificity of each conjugate for the PSMA receptors.

The conjugates were radiolabeled to obtain ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2, and then the stability of the radiolabeled complexes was tested. Finally, cell uptake and internalization of ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2 were evaluated in PC3-PIP(++), LNCaP(+) and PC3(-) cell lines.

RESULTS

Conjugations of HYNIC with scFvD2B-tag or scFvD2B were performed as shown in ***Figure 1***.

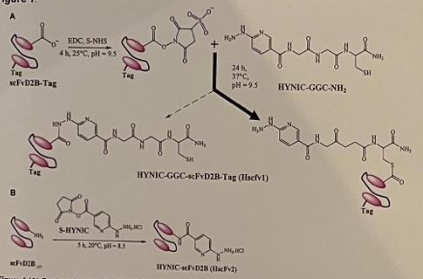

Figure 1 *(A) Conjugation of scFvD2B-Tag with HYNIC-GGC (B) Conjugation of scFvD2B with S-HYNIC*

The SE-HPLC chromatogram, mass spectroscopy and spectrometry **confirmed the conjugation of HYNIC to the scFv** and showed no antibody degradation or aggregation (***Figure 2***).

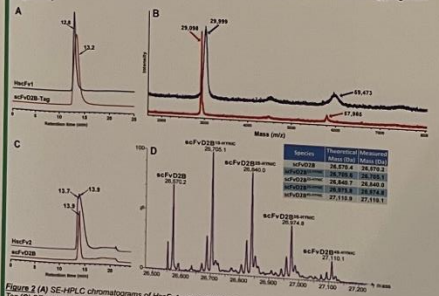

Figure 2 *(A) SE-HPLC chromatograms of HscFv1 and scFvD2B-Tag (B) MALDI-MS spectra of HscFv1 and scFvD2B-Tag (C) SE-HPLC chromatograms of HscFv2 and scFvD2B (D) LC-MS spectra of scFvD2B and HscFv2.*

ELISA test and flow cytometric displacement experiments demonstrated that the **conjugation procedure did not significantly reduce the affinity or specificity of the scFv for the receptor** ***(Fig. 3)***.

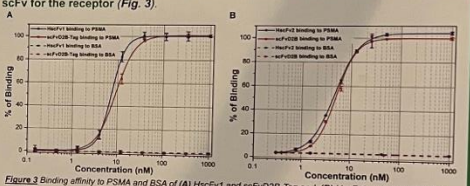

Figure 3 *Binding affinity to PSMA and BSA of* ***(A)*** *HscFv1 and scFvD2B-Tag and* ***(B)*** *HscFv2 and scFvD2B-Tag*

The radiolabeled yields of ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2 analyzed by C_4-RP-HPLC were 5% and 45%, respectively ***(Fig. 4)*** Upon purification the radiochemical purity of both the ^{99m}Tc-HscFv was > 95%. **Both purified radiocomplexes showed high stability in saline, PBS and human serum**.

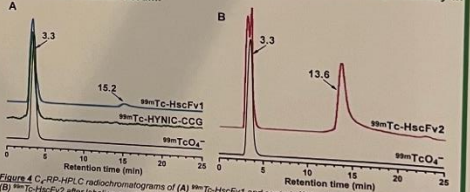

Figure 4 *C_4-RP-HPLC radiochromatograms of* ***(A)*** *^{99m}Tc-HscFv1 and controls ^{99m}Tc-HYNIC-CCG and ^{99m}TcO4$^-$. (B) ^{99m}Tc-HscFv2 after labeling reaction and ^{99m}TcO4$^-$ as control.*

In vitro studies demonstrated that **^{99m}Tc-HscFv agents were highly uptaken and internalized by PSMA(+) cells**. There were no statistical differences between ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2. In both cases, the blocking assay confirmed the site-specific interaction (***Fig. 5***).

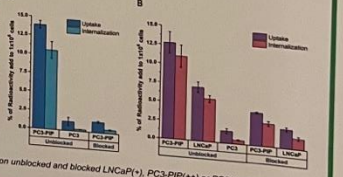

Figure 5 *Uptake and internalization on unblocked and blocked LNCaP(+), PC3-PIP(++) or PC3(-) of* ***(A)*** *^{99m}Tc-HscFv1 and* ***(B)*** *^{99m}Tc-HscFv2.*

CONCLUSIONS

The HYNIC-scFvD2B conjugates exhibited high in vitro stability. Both ^{99m}Tc-HscFv1 and ^{99m}Tc-HscFv2 showed high site-specific interaction with PSMA receptors, high uptake and internalization in PSMA(+) cells. However, the reduced labeling yield makes the use of ^{99m}Tc-HscFv1 unfeasible, whereas the labeling yields obtained for ^{99m}Tc-HscFv2 allow for further studies to optimize its production.

Preclinical studies are needed to determine whether the in vivo pharmacokinetics and tumor uptake of ^{99m}Tc-HscFv still offer sufficient advantages over HYNIC-peptide conjugates.

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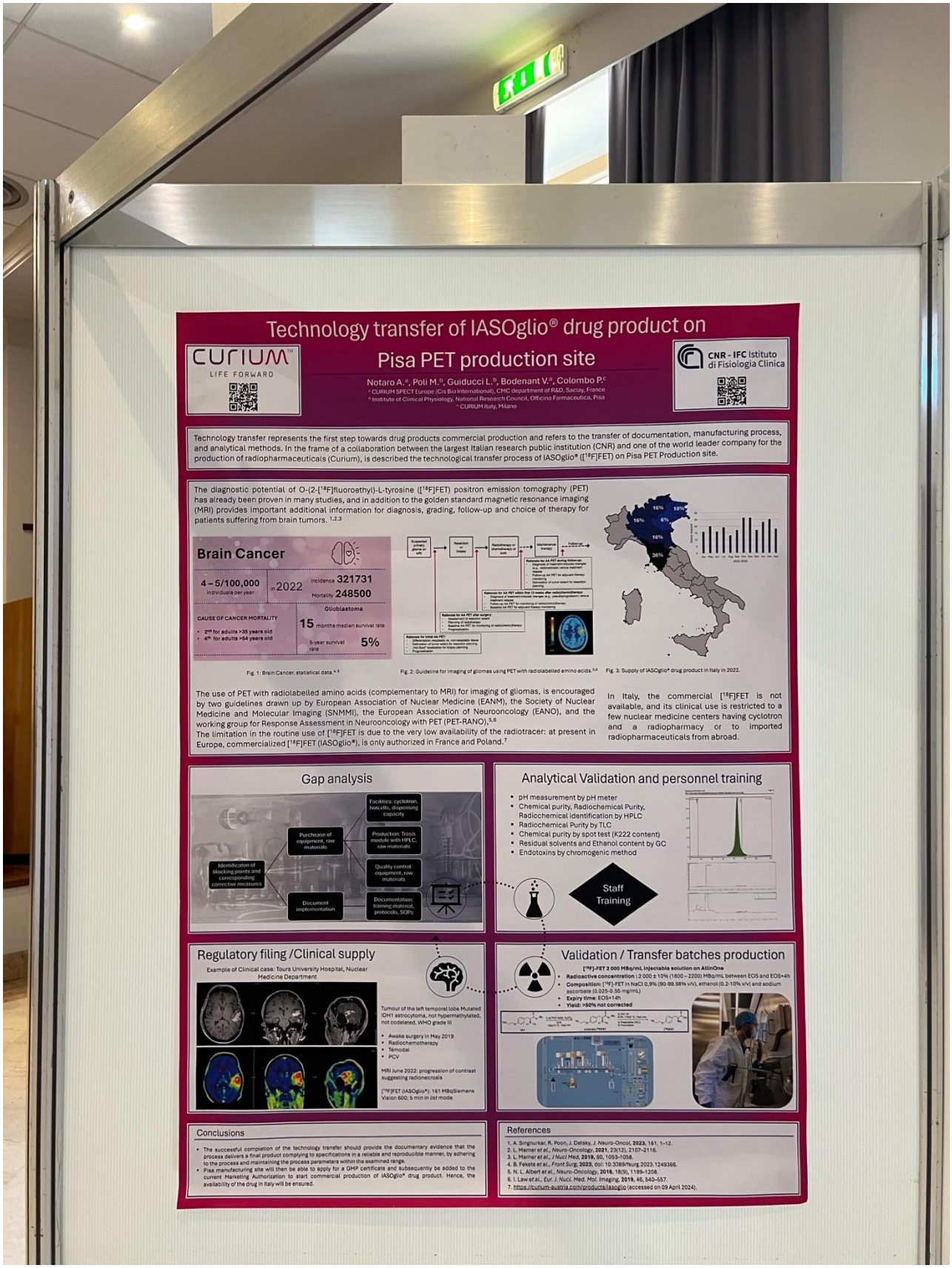
Technology transfer of IASOglio® drug product on Pisa PET production site
CURIUM LIFE FORWARD
CNR - IFC Istituto di Fisiologia Clinica
Notaro A.[a], Poli M.[b], Guiducci L.[b], Bodenant V.[a], Colombo P.[c]
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Technology transfer represents the first step towards drug products commercial production and refers to the transfer of documentation, manufacturing process, and analytical methods. In the frame of a collaboration between the largest Italian research public institution (CNR) and one of the world leader company for the production of radiopharmaceuticals (Curium), is described the technological transfer process of IASOglio® ([18F]FET) on Pisa PET Production site.
The diagnostic potential of O-(2-[18F]fluoroethyl)-L-tyrosine ([18F]FET) positron emission tomography (PET) has already been proven in many studies, and in addition to the golden standard magnetic resonance imaging (MRI) provides important additional information for diagnosis, grading, follow-up and choice of therapy for patients suffering from brain tumors. 1,2,3
Brain Cancer
4 – 5/100,000 individuals per year
in 2022
Incidence 321731
Mortality 248500
Glioblastoma
CAUSE OF CANCER MORTALITY
2nd for adults >35 years old
4th for adults >54 years old
15 months median survival rate
5-year survival rate 5%
Fig. 1: Brain Cancer, statistical data. 4,5
Fig. 2: Guideline for imaging of gliomas using PET with radiolabelled amino acids. 5,6
16%
10%
16%
6%
16%
36%
Fig. 3: Supply of IASOglio® drug product in Italy in 2022.
The use of PET with radiolabelled amino acids (complementary to MRI) for imaging of gliomas, is encouraged by two guidelines drawn up by European Association of Nuclear Medicine (EANM), the Society of Nuclear Medicine and Molecular Imaging (SNMMI), the European Association of Neurooncology (EANO), and the working group for Response Assessment in Neurooncology with PET (PET-RANO).5,6
The limitation in the routine use of [18F]FET is due to the very low availability of the radiotracer: at present in Europe, commercialized [18F]FET (IASOglio®), is only authorized in France and Poland.7
In Italy, the commercial [18F]FET is not available, and its clinical use is restricted to a few nuclear medicine centers having cyclotron and a radiopharmacy or to imported radiopharmaceuticals from abroad.
Gap analysis
Facilities: cyclotron, hotcells, dispensing capacity
Purchase of equipment, raw materials
Production: Trasis module with HPLC, raw materials
Identification of blocking points and corresponding corrective measures
Quality control: equipment, raw materials
Document implementation
Documentation: training material, protocols, SOPs
Analytical Validation and personnel training
pH measurement by pH meter
Chemical purity, Radiochemical Purity, Radiochemical identification by HPLC
Radiochemical Purity by TLC
Chemical purity by spot test (K222 content)
Residual solvents and Ethanol content by GC
Endotoxins by chromogenic method
Staff Training
Regulatory filing /Clinical supply
Example of Clinical case: Tours University Hospital, Nuclear Medicine Department
Tumour of the left temporal lobe Mutated IDH1 astrocytoma, not hypermethylated, not codeleted, WHO grade III
Awake surgery in May 2019
Radiochemotherapy
Temodal
PCV
MRI June 2022: progression of contrast suggesting radionecrosis
[18F]FET (IASOglio®): 161 MBqSiemens Vision 600; 5 min in list mode
Validation / Transfer batches production
[18F]-FET 2 000 MBq/mL injectable solution on AllinOne
Radioactive concentration : 2 000 ± 10% (1800 – 2200) MBq/mL between EOS and EOS+4h
Composition: [18F]-FET in NaCl 0,9% (90-99,98% v/v), ethanol (0,2-10% v/v) and sodium ascorbate (0.025-0.55 mg/mL)
Expiry time: EOS+14h
Yield: >50% not corrected
Conclusions
The successful completion of the technology transfer should provide the documentary evidence that the process delivers a final product complying to specifications in a reliable and reproducible manner, by adhering to the process and maintaining the process parameters within the examined range.
Pisa manufacturing site will then be able to apply for a GMP certificate and subsequently be added to the current Marketing Authorization to start commercial production of IASOglio® drug product. Hence, the availability of the drug in Italy will be ensured.
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^{68}Ga-Labelling of the potential theragnostic agent NOTA-R54 on an E&Z ModularLab PharmTracer

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Introduction

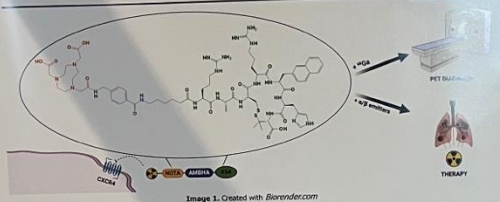


Image 1. Created with *Biorender.com*

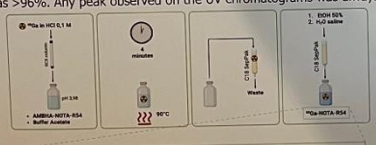
CXCR-4 is a receptor abundantly expressed in different human cancers and involved in tumor growth, invasion, angiogenesis and metastasis. Its crucial role in tumor progression has made it an interesting target for the development of new theragnostic radiopharmaceuticals. Recently a new family of low-molecular weight cyclic peptides has been designed by engineering the N-terminal region of CXCL12, the endogenous ligand, with a chelating agent (NOTA) using an amide linkage (AMBHA).[1] By so doing the resulting molecule, named NOTA-R54, can be labelled with either ^{68}Ga for PET diagnosis and with appropriate alpha/beta emitters for therapy.

Material and Methods

The precursor NOTA-R54 was aliquoted in H2O TraceSELECT™ and kept for months at -20°C. Radiolabelling with ^{68}Ga was carried out on an E&Z ModularLab PharmTracer fully automated cassette module, using an E&Z GalliaPharm ^{68}Ga generator. A pre-purification of the generator eluate on a SCX cartridge and a final purification on a C18 SepPak were applied. Different temperatures, pHs, precursor amount and batches were tested in order to optimize both radiochemical yield and radiochemical purity of the radiolabelled peptide. The latter was investigated by means of two differents radioTLC methods (*Image 3 and 4*) and two gradient HPLC analyses (*Image 5 and 6*). A UV detector set at 220 nm and a Raytest detector Gabi Star were placed at the columns exit. In a preliminary study, the Kinetex-EVO column was associated to a Diode array to determine the correct absorption wavelength of the peptide (220 nm).

Results

Radiochemical yields between 63-82% (d.c. at SOS) were obtained in ca. 16 min by using 50 µg precursor of two different batches dissolved in acetate buffer at different pHs and a 90°C x 240 s reaction. The iTLCs showed the absence of free or colloidal ^{68}Ga, while on radioHPLC the radiochemical purity was >96%. Any peak observed on the UV chromatograms was always of much lower intensity than the precursor one.



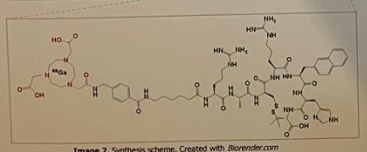
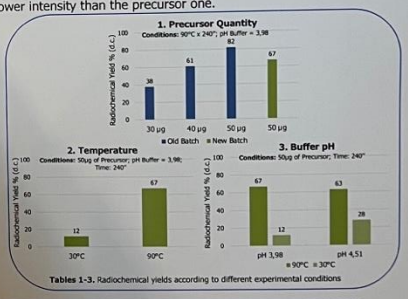


Image 2. Synthesis scheme. Created with *Biorender.com*



Tables 1-3. Radiochemical yields according to different experimental conditions

iTLC Analyses

Image 3. iTLC – SG with MeOH/$AcONH_4$ (0,77g/l) 1:1

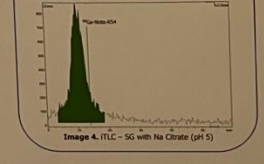


Image 4. iTLC – SG with Na Citrate (pH 5)

HPLC Analyses

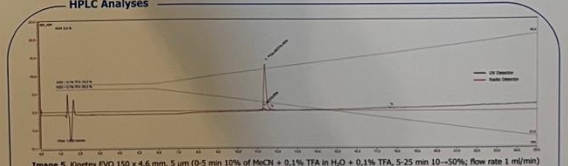


Image 5. Kinetex EVO 150 x 4.6 mm, 5 µm (0-5 min 10% of MeCN + 0,1% TFA in H_2O + 0,1% TFA, 5-25 min 10→50%; flow rate 1 ml/min)

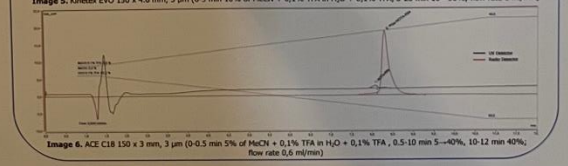


Image 6. ACE C18 150 x 3 mm, 3 µm (0-0.5 min 5% of MeCN + 0,1% TFA in H_2O + 0,1% TFA , 0.5-10 min 5→40%, 10-12 min 40%; flow rate 0,6 ml/min)

Conclusions

The observed radiochemical purity 96,4-99% can probably be further improved in view of the fact that the two batches of precursor (belonging to the same production lot) showed only an 75-77% purity on the HPLC. However, these impurities were almost entirely removed by the final C18 purification. Thus, apart from applying new reaction conditions, additional tests will be carried out starting from a new, fresh batch.
Finally, tests of the radiotracer on NSCLC-PDX models are expected to start soon.

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Fondazione IRCCS Policlinico San Matteo

Sistema Socio Sanitario Regione Lombardia

Feasibility of LASER-assisted radiolabelling: the case of [^{68}Ga]Ga-MAA.

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12-14 aprile 2024
Centro Congressi Pavia VI
X CONGRESSO NAZIONALE GICR

Introduction: ^{68}Ga radiolabelling of commercially available Macro-Aggregate of Albumin (MAA) kits has recently gained interest, due to the availability of ^{68}Ge/^{68}Ga generators and the enhanced spatial resolution offered by PET imaging, with superior imaging characteristics and quantification capabilities. There are numerous clinical investigations focusing on the evaluation of [^{68}Ga]Ga-MAA, but setting up a standardized and effective production procedure remains crucial. Conventionally, a heating procedure up to 90°C is employed. Nevertheless, this method is associated with temperature-induced partial disintegration of MAA particles, leading to the need for an additional step of purification.
In this study, we present the outcomes of a novel radiolabelling LASER-assisted method for [^{68}Ga]Ga-MAA preparation.

Materials and Methods: Three vials of MAA (Pulmocis ®) were reconstituted with 4 ml of saline each. To each vial, 200 MBq of buffered ^{68}Ga solution in 1.25 mL were added. Vial-1 was kept at room temperature (RT) for 15 min, Vial-2 was heated at 75 °C for 15 minutes, and Vial-3 underwent irradiation with a blue LASER (Techhodd PWM/TTL Blue laser OEM Module, China) (wavelength 450 nm, power 7 W, frequency 30 Hz) for 15 minutes in a custom-built facility designed to shield irradiation (Figure 1). The temperature of Vial-3 was monitored during irradiation using a thermocouple thermometer.
The Labelling Yield was assessed after the labelling procedure using thin-layer chromatography, with ITLC-SG and as mobile phase 0.1 M tribasic citrate solution. Stability tests in serum were also conducted. Particle dimensions were measured using a series of two polycarbonate membrane filters (3 µm and 400 nm pores) and observed in Burker chamber. The particle sizes were then compared to those obtained after standard ^{99m}Tc labelling of MAA.

Figure 1: Extremely simple equipment used for LASER-assisted radiolabelling: LASER, standard lead shielded containers for vials with a drill hole and the MAA kit vial

Results: Labelling Yield (LY) of [^{68}Ga]Ga-MAA obtained in Vial-1 was very low (38%), while reached 88% in Vial-2 (heat method) and to > 95% in Vial-3 (LASER method) (Figure 2). In vial-3 only a minimum increase of temperature, up to 38°C, was reported. LY was stable after serum incubation. Moreover, the percentage of particles with size <400 nm was negligible in Vial-1 and Vial-3 (about 1%), similar to the results of routine ^{99m}Tc labelling procedure, while it was dramatically higher (up to 68%) in Vial-2 (heat method), then observed in Burker chamber (Figure 3 and 4).

Figure 3: Comparison [68Ga]Ga-MAA 25° C (a), LASER (b) and 75° C (c) radiolabelling in Burker chamber

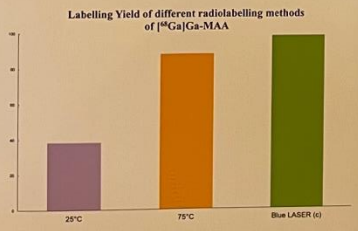

Figure 2: Radiochemical yield of [68Ga]Ga-MAA with three different radiolabelling techniques at room temperature (25°C), 75°C and LASER irradiation

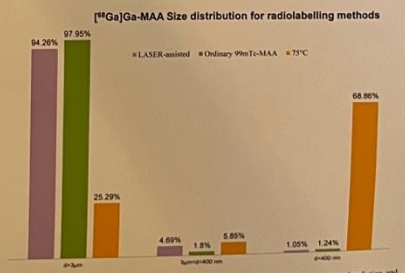

Figure 4: Differences in the distribution in Size Classes of MAA particles between Laser Irradiation, and traditional [99mTc]Tc-MAA and Heating to 75°C of [68Ga]Ga-MAA .

Conclusion: LASER assisted radiolabelling of [^{68}Ga]Ga-MAA assures a labelling yield higher than the usual heating method and does not affect the MAA size, thus avoiding the need of further purification steps necessary for the methods proposed so far. LASER Induced chemical reaction is an interesting approach under development, with no previous applications in the field of radiopharmaceuticals production.

07

AUTOMATED PRODUCTION OF [68GA]FAPI-46 FOR CLINICAL USE: EVALUATION OF SYNTHESIS PROCESS PERFORMANCE OVER A 3 YEARS PERIOD

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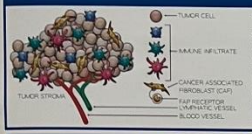
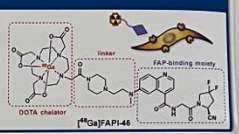
BACKGROUND



The fibroblast activation protein (FAP) is a type 2 transmembrane serine protease that is selectively overexpressed on the so-called cancer associated fibroblasts (CAFs) in the stroma of many malignant neoplasms.[1-3] Thanks to this innate tumor-specific distribution, FAP has become a target of great interest for tumor diagnosis. **[68Ga]FAPI-46 is one of the most clinically investigated FAP-targeting radiotracers**, with a widespread application in the diagnosis by PET/CT of a large spectrum of tumor types.[4-6]



AIM OF THE WORK

Our group recently developed and validated an **automated synthesis method able to provide multiple-doses of [68Ga]FAPI-46 with high radiochemical purity (RCP) and good yield (RCY) for PET/CT imaging**. Process optimization and scale up were performed by evaluating the influence of **different key parameters**:

- labeling reaction molarity
- different anti-radiolysis agents
- reaction time
- amount of precursor
- stability over a wide range of eluted $^{68}GaCl_3$

Therefore, the aim of this work was to **assess the long-term performance of [68Ga]FAPI-46 production in our hospital setting** within two prospective investigational trials.

RADIOSYNTHESIS OPTIMIZATION AND SCALE UP

Different reaction conditions were evaluated by performing the related radio-labeling process in triplicate on a Modular Lab PharmTracer module equipped with disposable single-use cassettes. The process was based on the concentration and pre-purification of the eluted gallium-68 (GalliaPharm Eckert & Ziegler) on a SCX cartridge, and on the final purification of the tracer trough a C18 SPE cartridge. Overall, the labeling conditions leading to the best RCY and highest RCP using the eluates provided by two generators were identified as **40 µg of precursor, 3 mg/ml ascorbic acid as anti-radiolysis agent, 0.4M sodium acetate buffer pH 4.5 and 15 min heating at 95°C** (Figure A – C). Furthermore, **vitamin C (Bayer) was added to the final formulation as stabilizer** to ensure a RCP >95% in a time frame of 2h after the end of synthesis (EOS) (Figure D).

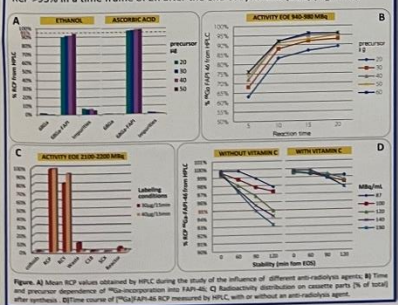


Figure. **A)** Mean RCP values obtained by HPLC during the study of the influence of different anti-radiolysis agents; **B)** Time and precursor dependence of ^{68}Ga-incorporation into FAPI-46; **C)** Radioactivity distribution on cassette parts (% of total) after synthesis . **D)**Time course of [68Ga]FAPI-46 RCP measured by HPLC, with or without an anti-radiolysis agent.

LONG-TERM PROCESS PERFORMANCE

To assess process efficacy along time we measured RCYs, RCPs, and number of injected patients per batch over 43 synthesis for clinical purpose. The fully automated production of [68Ga]FAPI-46 was **completed in 33 min**. The **mean decay-corrected yield was 91%±3%** (range 85,8-97,6%), with minimum fluctuations even if batch activity decreased proportionally to generators shelf-life (Figure).

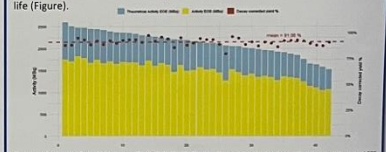


Figure. Performance of synthesis process for [68Ga]FAPI-46. The batches were sorted in descending order of theoretical EOE activity, calculated according to decay laws, generators shelf-lives and elution yields. Labeling yields were corrected for the decay in 25 minutes, excluding generators elution. EOE = end of elution; EOS = end of synthesis.

QUALITY CONTROL

Test	Acceptance criteria	[68Gd FAPI 46
RCP from HPLC/TLC	≥ 95%	99.3%±0.3%
Colloidal 68Ga from TLC	≤ 3%	0.33%±0.1%
Radiochemical identity from HPLC (t_R)	t_R compared with reference Std	conforms
Radionuclidic identity (half life)	61 – 75 min	conforms
Final radioactivity concentration		125±18 MBq/ml
Radionuclide identity	511 keV, 1077 keV	conforms
68Ge breakthrough	≤ 0.001%	≤ 0.0002%
Ethanol content	≤ 10% (v/v)	conforms
pH	4 - 8	5.5 ± 0.3
Endotoxins	≤ 175/V IU	conforms
Sterility	sterile	conforms

Mean RCP value as determined by radio-HPLC and radio-iTLC was 99.3%±0.3% and the quantity of **colloids from radio-iTLC was 0.33%±0.1% (n=43).** All the other tested quality controls were in accordance with the European Pharmacopoeia (see Table).

DIAGNOSTIC PERFORMANCE

65 patients with different **lung, ovarian, head, neck, breast cancers and sarcomas** (EudraCT:2020-005549-17) and 63 patients with **lung cancers** (EudraCT:2021-006570-23) were subjected to [68Ga]FAPI-46 PET/CT (Figure), with **up to 7 injected patients per batch** imaged by 4 PET/CT scanners acquiring simultaneously.

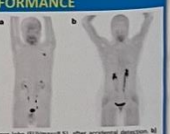


Figure. **a)** Characterization of parenchymal thickening in the left upper lobe (SUVmean=8.5), after accidental detection. **b)** Staging of left upper lobe lung adenocarcinoma (SUVmax=2.5) (uptake not seen from the MIP).

CONCLUSIONS AND PERSPECTIVES

In this study, the performance of synthesis process for multiple doses of [68Ga]FAPI-46 was evaluated, demonstrating **quantitative RCY, high reproducibility over a wide range of theoretical activities of eluted $^{68}GaCl_3$** (range 1522-2598 MBq) and **highly stable RCP values**, thus assessing **great process robustness.** In conclusion, our results suggest that the developed method can provide **high throughput clinical activity** along with the possibility of extending the fields of application of FAPI-PET modality to new clinical protocols.

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06

Development of [^{99m}Tc][Tc(N)(PNP)]-based PSMA targeting agents: the PNP3OH experience

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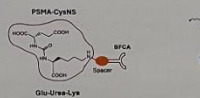
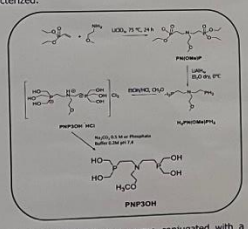
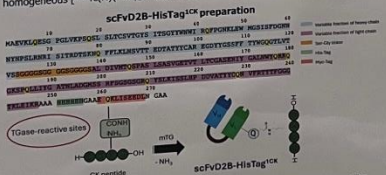
Background. [^{99m}Tc][Tc(N)(PNP)]-approach has long been used to label biomolecules. The most important advantage of this technology is its high chemical flexibility that allows for a fine modulation of the chemical-physical properties of the corresponding tagged targeting vector; nevertheless, the usage of traditional alkoxy-alkyl PNPs leads to the need for heating overextended times to attain high radiochemical yields. These conditions are not suitable for labeling temperature-sensitive biomolecules. Water-soluble phosphines are an attractive class of oxidatively stable ligands that generate stable hydrophilic chelates with good pharmacokinetics. Modification of the substituents on the P atoms induces the variation of electronic and sterical properties of the ligand, which affect its reactivity for the metal ions, influencing the reaction rate, and the stereochemistry of the final complex. Hence, the water-soluble (*ws*) PNP3OH, ($[(OHCH_2)_3PCH_2CH_2]_2NCH_2CH_2OCH_3$), was designed and the effect of the substituents on the corresponding *ws*-[Tc(N)(PNP3OH)]$^{2+}$framework was investigated for the preparation of target specific compounds at room temperature and mild reaction conditions.[1]
AIM Within, we reported our experience in the usage of [^{99m}Tc][Tc(N)(PNP3OH)]-framework to label PSMA targeting molecules, including a small molecular weight PSMA inhibitor and the fragment scFvD2B for PSMA imaging.



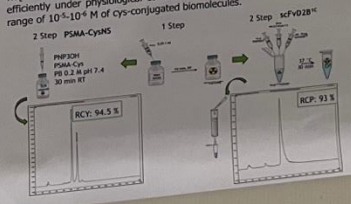
Results. PNP3OH was synthesized according to the following reaction scheme and characterized.



The biomolecules mentioned above were conjugated with a terminal cysteine residue, cys~, to allow the coordination of the *ws*[^{99m}Tc][Tc(N)(PNP3OH)]-synthon. scFvD2B does not contain reactive cys residues; hence, it was derivatized via a site-specific enzymatic reaction catalyzed by transglutaminase (TGase), with the H-Cys-GlyLys-Gly-OH tetrapeptide (H_2Cys) to produce site-specific and homogeneous [^{99m}Tc(N)(PNP)]-tagged protein conjugates.



Radiosyntheses were conveniently carried out using a two-step reaction. The insertion of water-soluble groups on PNP improves the reactivity of *ws*[^{99m}Tc][Tc(N)(PNP3OH)]-framework towards cys~. Reactions were performed efficiently under physiological conditions at RT in 30 min, using a concentration range of 10^{-5}-10^{-6} M of cys-conjugated biomolecules.

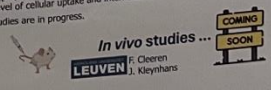


The stability of the pure radiolabeled PSMA targeting molecules was evaluated in different media and after cells exposure. Their receptor specificity was assessed in-vitro in pertinent cell lines.

Summary

- [^{99m}Tc(N)(PNP3OH)(PSMA)] compounds are efficiently produced under mild reaction conditions; RCP are > 93%.
- They are stable in injectable solutions, against an excess of small molecule challengers, and after incubation in sera, murine kidney and liver homogenates, and cell exposure.
- Data clearly show that PSMA targeting molecules labeled with [^{99m}Tc][Tc(N)(PNP3OH)]-synthon preserve their receptor targeting ability with high level of cellular uptake and internalization.

In vivo studies are in progress.



Conclusions
Data support the effective application of [^{99m}Tc(N)(PNP3OH)]-technology to labeling molecular effectors including temperature-sensitive protein derivatives.

Acknowledgment to Bracco Imaging and AIRC (IG-2020 ID 24528)

References

1.Bolzati C. *et al.* [2022], Mol Pharm, 19: 876-894

05

Consiglio Nazionale delle Ricerche

Istituto di Chimica e della Materia Condensata e di Tecnologie per l'Energia — ICMATE

Synthesis and characterization of Rhenium and Technetium-99m nitrido complexes with tridentate thiosemicarbazones ligands

Nicola Salvarese,[1*] Davide Lucchini,[2] Carolina Gobbi,[1] Marco Baron,[2] Alessandro Dolmella,[3] Alessio Zavaroni,[4] Dominga Rogolino,[4] Mauro Carcelli,[4] Cristina Bolzati[1]

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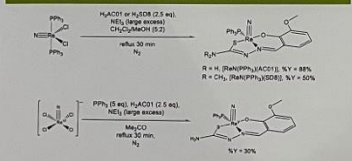
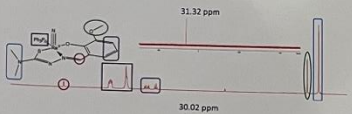
INTRODUCTION and AIM

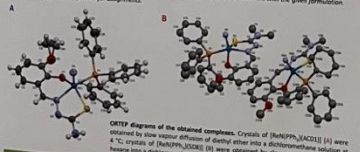
Because of the position in the periodic table of elements, the coordination chemistry of technetium and rhenium is intrinsically connected and remains under current interest. Regardless of the increasing general relevance of PET technology, SPECT remains the first-line imaging modality in nuclear medicine. This is because SPECT imaging relies mostly on technetium-99m, which, thanks to its ideal physical-chemical properties and convenient availability, is the most used radionuclide in clinical practice. To emphasize this radionuclide, the existence of radioactive rhenium congeners ($^{186/188}$Re) that have been shown to be very attractive candidates for endoradiotherapy.[1] This makes ^{99m}Tc and $^{186/188}$Re ideal for the development of a theranostic pair that combines diagnosis and therapy. Thiosemicarbazones (TSC) represent an interesting class of ligands that form stable complexes with rhenium and technetium. Most of our research activity is based on the study of the M≡N core (M=Tc, Re).[2,3] Believing in the potential to develop novel radiopharmaceuticals based on the M≡N core with TSC, here we describe the reactivity of two tridentate TSC (3-methoxysalicylaldehyde thiosemicarbazone, H_2ACO1; 3-methoxysalicylaldehyde N,N-dimethyl-thiosemicarbazone, H_2SD8) with the M≡N core, where M=Re and ^{99m}Tc.

"COLD" NITRIDO-RHENIUM COMPLEXES



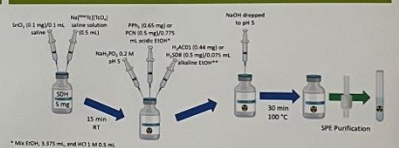


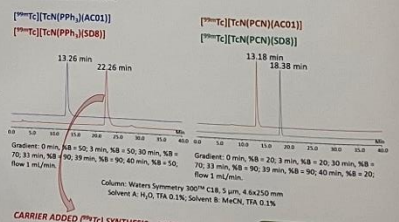
^{1}H and ^{31}P NMR characterization in CD_2Cl_2. ^{13}C NMR was also performed and found coherent with the given formulation. 2D experiments were performed for assignments.



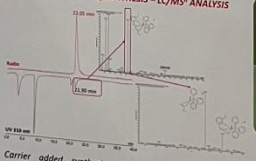
ORTEP diagrams of the obtained complexes. Crystals of [ReN(PPh$_3$)(ACO1)] (A) were obtained by slow vapour diffusion of diethyl ether into a dichloromethane solution at 4 °C; crystals of [ReN(PPh$_3$)(SD8)] (B) were obtained by slow vapour diffusion of n-hexane into a dichloromethane solution at 4 °C.

NITRIDO-TECHNETIUM-99m COMPLEXES





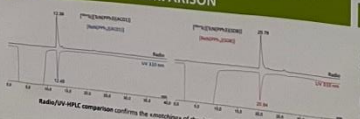
CARRIER ADDED (^{99m}Tc) SYNTHESIS – LC/MS^n ANALYSIS



Carrier added synthesis was performed for all the complexes, and LC-MS^n analysis confirmed the formulation in all the cases.

- ✓ *All the complexes are adequately STABLE;*
- ✓ *Binding to serum proteins: 50-80%*

RHENIUM/TECHNETIUM-99m COMPLEXES HPLC COMPARISON



Radio/UV-HPLC comparison confirms the «matching» of rhenium and technetium-99m analogues.

CONCLUSION

Within the aim of synthesizing complexes with a view to medical applications, the basic coordination chemistry of the M≡N core (M = Re, ^{99m}Tc) toward tridentate TSCs was evaluated. Stable compounds were efficiently prepared as prototypes with potential to be used in radiopharmaceuticals development.

References

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04

High-purity ^{155}Tb production by hospital-cyclotrons: enriched ^{155}Gd targets at comparison

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Introduction

^{155}**Tb** ($T_{1/2}$ = 5.32 d, E_γ = 87 keV (32%) and 105 keV (25%)) **is a good candidate for SPECT imaging**, its long half-life allows the biodistribution of radiopharmaceuticals to be studied over several days and it can be paired with other Tb radionuclides for theranostic purposes [1]. However, to find feasible production routes for medical applications is still an open issue. This work focuses on the ^{155}Gd(p,n)^{155}Tb reaction. **The challenge is to minimize the co-production of ^{156}Tb** ($T_{1/2}$ = 5.35 d) due to its high-energy γ emission, which compromises image quality and increases the absorbed dose to the patient [2].

Methods

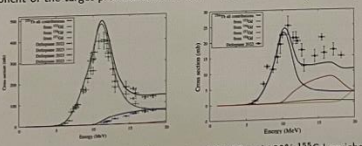
A) Nuclear reaction models [3] were used to describe the relevant production cross sections, to be compared with experimental measures [4,5]. From the theoretical cross sections thick-target yields and radionuclidic purity (RNP) have been derived.

B) Dosimetric evaluations were accomplished with the OLINDA software [6], using biodistribution data from ^{161}Tb-cm09 [1]. The dose increase (DI) was determined considering the yield of all Tb radioisotopes produced.

C) The **imaging quality** of ^{155}Tb is assessed by calculating the Compton-to-peak ratio that expresses the noise contribution of high-energy γ-rays emitted by Tb-contaminants.

Cross sections and yields

The contribution from the main Gd components of the ^{155}Gd-enriched target (^{155}Gd 91.9%, ^{156}Gd 5.87%; ^{157}Gd 0.81%; ^{158}Gd 0.65%) [4] to the ^{155}Tb and ^{156}Tb cross sections are highlighted. It is evident that the ^{156}Gd component of the target provides most of the ^{156}Tb cross-section (blue curve).



Yields of Tb radioisotopes for this target and 98, 99 and 100% ^{155}Gd-enriched target (with only ^{156}Gd as impurity) are compared in Table 1. **The higher the ^{156}Gd component in the target, the higher the ^{156}Tb contamination.**

	Yields (MBq/μA-h)				
Target enrichment	^{154g}Tb	^{155}Tb	^{156g}Tb	^{156m1}Tb	^{156m2}Tb
^{155}Gd-100%	0.0022	2.60	0.002	8.63E-05	4.27E-08
^{155}Gd-99%	0.0022	2.58	0.026	0.0014	1.93E-07
^{155}Gd-98%	0.0022	2.55	0.051	0.0028	3.43E-07
^{155}Gd-91.90%	0.0053	2.40	0.149	0.0081	9.46E-07

Table 1:Tb radioisotopes yields

References
[1] Müller C et al., [2014], Nucl Med Biol, 41:e58–65
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[3] Koning A J, Hilaire S, Goriely S, [2023] EPJ A, 59
[4] Delle Piane G et al.,[2022], Appl Radiat Isot., 184 110175
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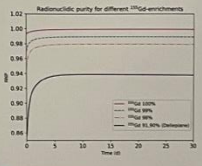


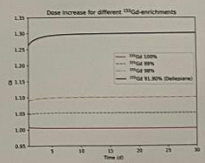
Accordingly, the ^{155}Tb RNP shows significantly high values for 98, 99, and 100% enriched-targets.

Dosimetry

The effective doses (ED) listed in Table 2 correspond to an unitary-activity administration of each Tb-cm09. **Labelling the radiopharmaceutical with ^{156g}Tb or ^{154g}Tb implies an ED 5.9 and 2.4 times bigger than ^{155}Tb-cm09.**

	^{154g}Tb	^{155}Tb	^{156g}Tb	^{156m1}Tb	^{156m2}Tb
Effective dose (mSv/MBq)	4.44E-02	1.86E-02	1.09E-01	2.47E-03	2.06E-03

Table 2:Effective doses



The assessment of the DI illustrates that a 2% content of ^{156}Gd in the target is the maximum limit that still guarantees a safe clinical application.

Imaging

The noise in the SPECT image introduced by the higher-energy γ-rays from ^{156g}Tb and ^{154g}Tb is illustrated in Table 3. It has been shown that **the image quality is comparable to ^{111}In (currently used in clinics)** [2].

	Compton-to-peak ratio 96 h after EoB		
Peak energy (keV)	100% ^{155}Gd	99% ^{155}Gd	98% ^{155}Gd
88.5 keV	18.89%	21.52%	22.61%
167 keV	8.91%	15.95%	19.32%
262 keV	1.71%	19.8%	26.55%

Table 3:Compton-to-peak ratio for the three principal peaks of ^{155}Tb

Conclusions

The presence of ^{156}Gd as impurity of the enriched ^{155}Gd target may increase the production of the contaminant ^{156}Tb. **This study demonstrates that a 2% content of ^{156}Gd in the target could be still suitable for clinical applications since it guarantees a 98% RNP value combined with a DI lower than 10%.**

03

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Sistema Socio Sanitario Regione Lombardia

GICR

An assessment of the effects of autoclave sterilization on the stability of O-(2-[^{18}F]fluoroethyl)-L-tyrosine

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INTRODUCTION

The study aimed to investigate the negative effects of heat treatments on an amino acid like [^{18}F]FET, as well as executing an assessment of the stability of the diagnostic radiopharmaceutical over seven hours.

MATERIALS AND METHODS

The synthesis of [^{18}F]FET was accomplished in about 50 minutes with a radiochemical yield of 23,3%. Autoclave sterilization was carried out at temperatures between 36°C and 137°C over about 8 minutes.
The samples were evaluated at t0, t4, and t7 according to the European Pharmacopoeia (EP) monograph.

RESULTS

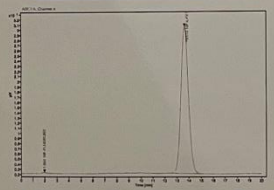
Both sterilized (AC) and non-sterilized (NA) samples were compliant with EP specifications.
A slight difference in the quantification of L-FET was observed in the tests for chemical and enantiomeric purity, which also showed rising levels of D-FET for both sets of samples from t0 to t7 (Table below).
A modest variation in the content of ethanol and acetonitrile between AC and NA samples was noted.

Time	L-FET Area (AC)	L-FET Area (NA)	Variation (%)	D-FET Area (AC)	D- FET Area (NA)
t0	15,216	16,350	7,45	0,496	0,559
t4	13,997	15,235	8,85	1,264	0,904
t7	13,470	15,016	11,28	1,374	0,733

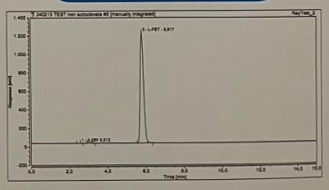
Table 2: Areas of L-FET and D-FET observed via HPLC-UV, and variation between AC and NA samples.

Time	EtOH Area (AC)	EtOH Area (NA)	Variation (%)	AcN Area (AC)	AcN Area (NA)	Variation (%)
t0	370,94	419,87	13,19	0,69	0,81	16,98
t4	372,71	419,15	12,46	0,70	0,79	13,18
t7	368,72	417,94	13,35	0,66	0,77	17,30

Table 3: Areas of solvents evaluated via GC-FID, and variation between AC and NA samples.



Chromatogram 5: AC t7, test for impurities C and D performed via HPLC connected to a radioactivity detector. Column: size: l = 0.15 m, Ø = 4.0 mm; stationary phase: crown-ether silica gel for chiral separation R (5 µm). Mobile phase: methanol R, 2.9 g/L solution of perchloric acid R (10:90 V/V). Flow rate: 1 mL/min.



Chromatogram 12: NA t7, test for radiochemical purity performed via HPLC connected to a radioactivity detector. Column: size: l = 0.25 m, Ø = 4.6 mm; stationary phase: end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R (5 µm). Mobile phase A (water for chromatography R, protected from the atmosphere during chromatography) and mobile phase B (acetonitrile for chromatography R) were used in gradient elution mode. Flow rate: 1 mL/min.

DISCUSSION

The radiochemical purity is not affected by heat treatments: values higher than 99% were maintained throughout the study. Autoclave sterilization has proven to be easily applied in the routine preparation of a promising ^{18}F-labeled amino acid.

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Polo di ricerca, cura e formazione universitaria

UNIVERSITÀ DEGLI STUDI DI MILANO

02

Production of Copper-61 from natZn(p,α)61Cu reaction route by solid target irradiation: preliminary results.

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2 National Institute for Nuclear Physics - Legnaro National Laboratories (INFN-LNL), Legnaro (Pd), Italy
3 Dipartimento di Medicina Traslazionale e per la Romagna Università di Ferrara
4 Dipartimento di Scienze chimiche e Farmaceutiche Università di Ferrara
5 Dipartimento di Scienze dell'ambiente e della prevenzione Università di Ferrara

Introduction

Copper-61 (Cu-61) is emerging as a promising alternative PTE radioisotope for the development of copper-6X based radiopharmaceuticals, driven by its cost-effectiveness and favorable nuclear properties, such as a lower production cost and proper nuclear characteristics (Half-live of 3.34h, β⁺).[1,2]
Different production pathways are now under investigation, with one particularly promising application involving the $^{nat}Zn(p,\alpha)^{61}Cu$ route by using medical cyclotrons. Alves [2] conducted a study utilizing a liquid target for this nuclear reaction, yielding positive results. The process involved initiating the reaction from a nitric zinc solution, showcasing the viability of such a method.
That being stated, we have explored the feasibility of applying the same pathway to a solid target instead, by using two different zinc-based materials: ^{nat}Zn and ^{nat}ZnO. The study aims at assessing and overcoming the material's limits posed by the low melting temperature of the zinc element, which is approximately 420°C. This temperature could present a potential issue with the increasing beam current, and the study seeks to address and mitigate any challenges associated with that.

^{nat}ZnO pellet

^{nat}ZnO pellet with support

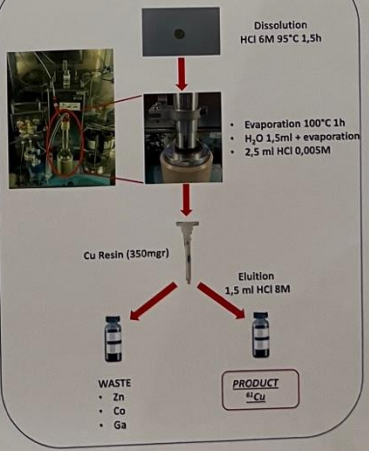
^{nat}ZnO pellet with support after beam

Target	Beam E [MeV]	Beam on target [MeV]	Current [μA]	Time [min]
^{nat}Zn foil 1	18.6 MeV	14 MeV	5 μA	5 min
^{nat}Zn foil 2	18.6 MeV	14 MeV	15 μA	5min
^{nat}ZnO pellet 1	18.6 MeV	14 MeV	15 μA	5min
^{nat}ZnO pellet 2	18.6 MeV	14 MeV	30 μA	5min
^{nat}ZnO pellet 3	18.6 MeV	14 MeV	25 μA	30min
^{nat}ZnO pellet 4	18.6 MeV	14 MeV	17 μA	20min
^{nat}ZnO pellet 5	18.6 MeV	14 Me	4 μA	20min
^{nat}ZnO pellet 6	18.6 MeV	14 MeV	4 μA	20min

Methods

The ACSI TR19/300 proton cyclotron, located at the Radiopharmacutical department of IRCCS Sacro Cuore Hospital (Verona, Italy) with variable beam energy and equipped with a vertical solid target station was used to irradiate two different coin-shaped targets configurations: foil disc of ^{nat}Zn metal and sintered ^{nat}ZnO pellet
Foil disc of 99.99% ^{nat}Zn 0.5 mm thickness.
Pellet of 99.9% ^{nat}ZnO around 0.6 mm thickness and density around 99%.
A sandwich target configuration was explored about the disc foil configuration, incorporating a Nb backing target and an Al frontal foil, while for the pellet target one, we have innovated by developing a magnetic closing target capsule made of aluminum alloy.

Dissolution and purification



Results

This study involved target irradiations with increasing beam currents, starting from 5 μA, to assess the mechanical and thermal resistance of the ^{nat}Zn foil as well as ^{nat}ZnO pellet. The pellet successfully withstood the challenging conditions even at 30 μA without breaking (Figure 1 and Table 1). Various dissolution strategies, coupled with purification procedures, are currently being tested. Commercial resins, including Tk201, CU, and TBP, are being utilized in these tests.
Table 1. Irradiation parameters used for ^{nat}Zn foils and ^{nat}ZnO pellets.

Conclusion

The zinc oxide targets have withstood a 30 μA beam at an energy of 14 MeV, demonstrating their suitability for the production of copper-61.

References
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[2] S. do Carmo, V.H.P. Alves, F. Alves and A. Abrunhosa. Fast and cost-effective cyclotron production of ^{61}Cu using a ^{nat}Zn liquid target: an opportunity for radiopharmaceutical production and R&D. Dalton Transactions. 2017; 46.14556-14560

Advancing Into the Realm of Innovative Theranostic Radionuclides: Separation of Silver-111 from a Neutron-Irradiated Palladium Target

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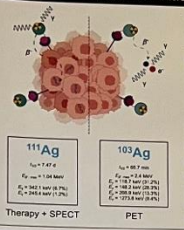
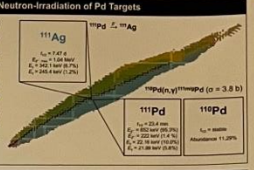
1. Introduction: Theranostic Properties of Ag Radioisotopes



^{111}Ag exhibits both medium-energy β^- and γ emissions with promising potential for **targeted radiopharmaceutical therapy** and associated **SPECT** imaging [1,2,3]. Its decay properties closely recall those of the clinically established ^{177}Lu, rendering it an alluring candidate for therapeutic applications. Furthermore, the clinical significance of ^{111}Ag is heightened by the presence of a β^+-emitting counterpart **^{103}Ag**, thereby endowing this element with true **theranostic** potential. Such pair could overcome current limitations tied to the compelled use of chemically distinct isotopes as imaging surrogates for ^{177}Lu.[3] Notwithstanding these attractive properties, the use of Ag radioisotopes has been hindered so far by the **challenges** related to their **production** and **separation from** the **target** material. The absence of chelators able to stably incorporate Ag radioisotopes in tumor-targeting vectors *in vivo* is a significant drawback as well.

2. ^{111}Ag Production: Neutron-Irradiation of Pd Targets

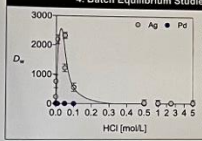
^{111}Ag was produced *via* **neutron irradiation** of **^{nat}Pd targets** at TRIGA Mark II nuclear research reactor at the Laboratory of Applied Nuclear Energy (LENA) of the University of Pavia, Italy.[4] ^{109}Pd was co-produced (^{108}**Pd(n,γ)^{109m,g}Pd**, σ = 148.9 b). ^{nat}Pd was used to allow the direct evaluation of the Ag/Pd separation by γ-spectrometry. To avoid side reactions on ^{nat}Pd isotopes and obtain ^{111}Ag in high molar activity, highly enriched ^{110}Pd will be employed.



3. Current Challenges in ^{111}Ag/Pd Separation and Study Aim

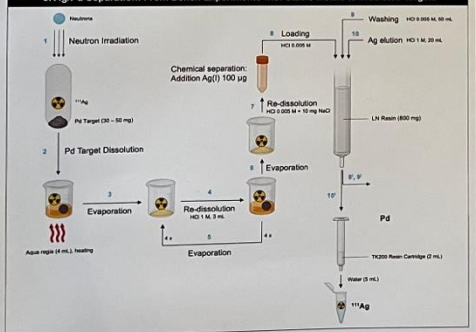
Various methods for extracting ^{111}Ag from neutron-irradiated Pd targets exist.[1] However, they afforded ^{111}Ag at (*i*) unacceptably **low molar activity** and **chemical purity** (due to the addition of ^{nat}Ag carrier or other chemicals) or (*ii*) in a **large volume highly concentrated acidic solutions**, unsuitable for direct labelling of biological vectors (this formulation forces evaporations and reconstruction in weakly acidic solutions, reducing yields and introducing contaminants). To surmount these issues, this work introduces an innovative two-steps chromatographic separation process able to extract **^{111}Ag** selectively and efficiently from bulk Pd matrix and obtain it in a **formulation suitable for direct labelling**.

4. Batch Equilibrium Studies: Ag and Pd Weight Distribution Ratios (D_w)



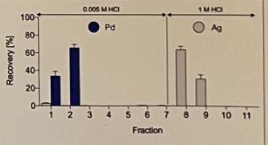
Ag and Pd behaviour on LN resin was explored, aiming to optimize their separation. LN resin displayed high Ag(I) selectivity over Pd(II), with strong adsorption of Ag(I) at low HCl concentrations (0.01 - 0.05 M) and negligible retention at higher concentrations (> 0.2 M). Pd(II) always exhibited minimal affinity for LN resin. This behaviour has been ascribed to the different stability of the complexes formed with the stationary phase and the varying species formed by the two metals upon the whole range of HCl concentrations.

5. Ag/Pd Separation: From Bench Experiments with Stable Metals to Irradiated Targets

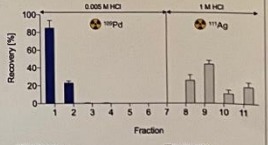


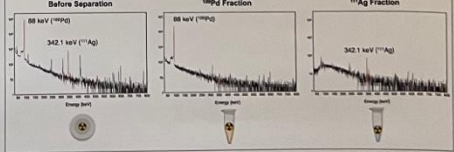
6. (Radio)Chemical Ag/Pd Separation – Step 1: LN Resin

Chemical Separation (ICP-OES):
Pd(II) was removed in the loading and the first washing steps (25 mL, 0.005 M HCl) (**Pd(II) recovery > 99.96%**). Ag(I) was recovered adding 1 M HCl (40 mL, **Ag(I) recovery > 95%, Ag purity > 99%**, separation factor ($S_{Pd,Ag}$) ~ 4.21·10^{-4}). The separation progress can be qualitatively assessed by observing colour changes: Pd(II)-Cl complexes are reddish-brown, while Ag(I) ones are colourless.

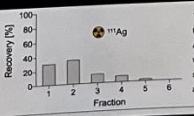


Radiochemical Separation (γ-spec):
^{109}Pd was eluted in the first two fractions (**^{109}Pd recovery > 90%**) while ^{111}Ag was stripped with 1 M HCl (40 mL, **^{111}Ag recovery > 92%, radionuclidic purity > 99%**).





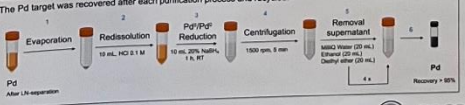
7. Radiochemical Ag/Pd Separation – Step 2: TK200 Resin



^{111}Ag was loaded onto a second column (TK200 resin) to **decrease the volume** and provide it in a **ready-to-use formulation**. Although the complete elution of ^{111}Ag requested around 10 mL of water, the harvesting of the first 6 mL provided a formulation suitable for direct radiolabeling of bioconjugates still ensuring an average yield of 80 ± 5%.

8. Pd Target Recovery

The Pd target was recovered after each purification process and recycled.[5]



9. Conclusions

A new process was developed to separate ^{111}Ag from neutron-irradiated Pd with high yield and purity. Our method represents a significant improvement over the previously reported procedures as the **recovery of ^{111}Ag in a small volume of water** enables its immediate use in the labelling of sensitive vectors without the need for time-consuming evaporation and reformulation steps. These findings open new avenues for advancing the utilization of ^{111}Ag labelled radiotracers in therapeutic applications.

References: [1] Tosato M., Pharmaceuticals, 2023, 16(7), 929; [2] Maastren T., Anal. Chim. Acta, 2016, 908, 75; [3] Tosato, M., EJNMMI Radiopharm. Chem., 2023, 8, 43; [4] Morselli L., Appl Radiat Isot, 2023, 197, 110798; [5] Chrya T. Radiochim. Acta, 2020, 108(8), 641

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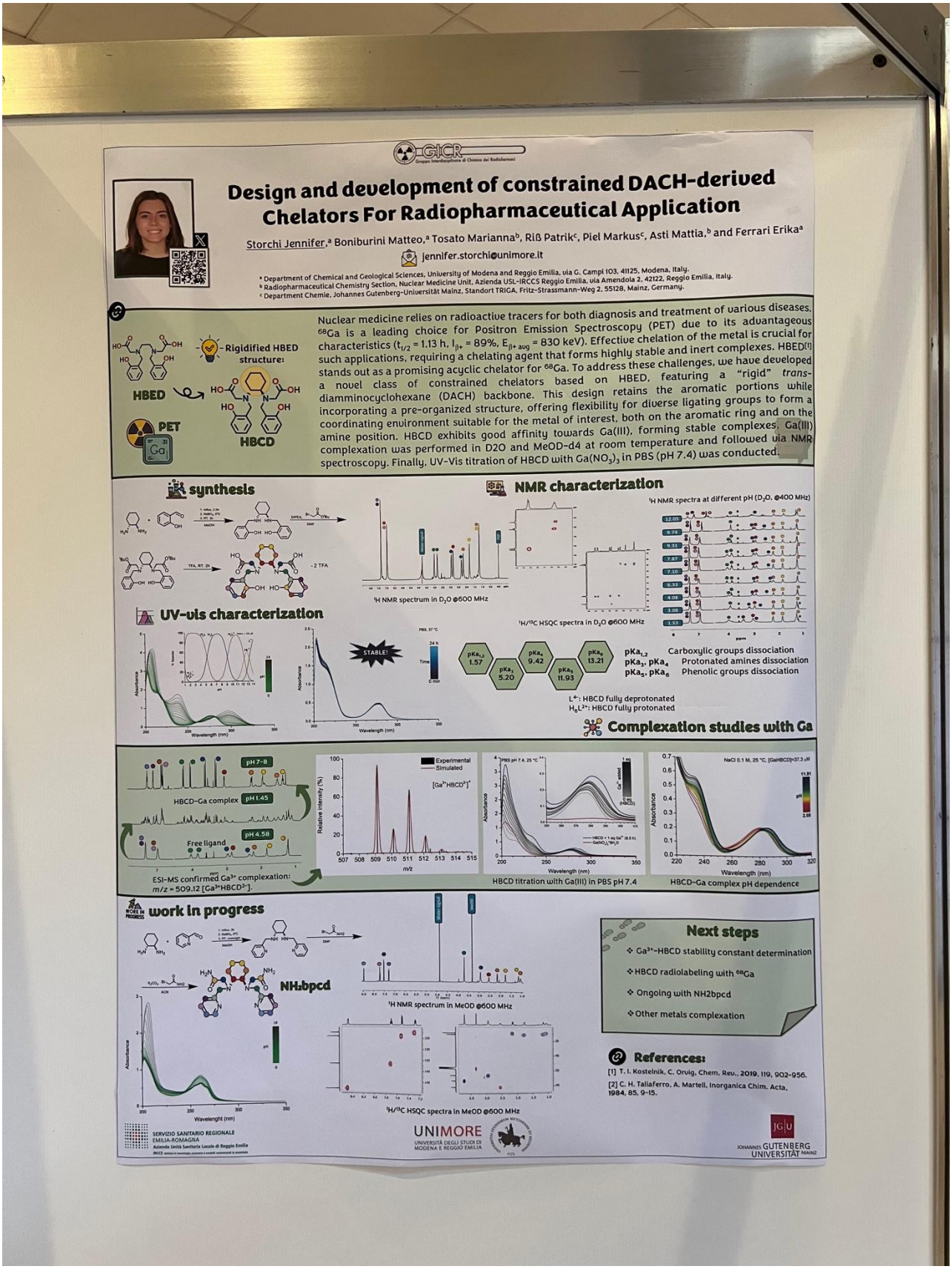
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Design and development of constrained DACH-derived Chelators For Radiopharmaceutical Application
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HBED
Rigidified HBED structure:
HBCD
PET
Nuclear medicine relies on radioactive tracers for both diagnosis and treatment of various diseases. 68Ga is a leading choice for Positron Emission Spectroscopy (PET) due to its advantageous characteristics ($t_{1/2}$ = 1.13 h, $I_{\beta+}$ = 89%, $E_{\beta+\,avg}$ = 830 keV). Effective chelation of the metal is crucial for such applications, requiring a chelating agent that forms highly stable and inert complexes. HBED[1] stands out as a promising acyclic chelator for 68Ga. To address these challenges, we have developed a novel class of constrained chelators based on HBED, featuring a "rigid" trans-diamminocyclohexane (DACH) backbone. This design retains the aromatic portions while incorporating a pre-organized structure, offering flexibility for diverse ligating groups to form a coordinating environment suitable for the metal of interest, both on the aromatic ring and on the amine position. HBCD exhibits good affinity towards Ga(III), forming stable complexes. Ga(III) complexation was performed in D2O and MeOD-d4 at room temperature and followed via NMR spectroscopy. Finally, UV-Vis titration of HBCD with $Ga(NO_3)_3$ in PBS (pH 7.4) was conducted.
synthesis
- 2 TFA
NMR characterization
1H NMR spectra at different pH (D2O, @400 MHz)
1H NMR spectrum in D2O @600 MHz
1H/13C HSQC spectra in D2O @600 MHz
UV-vis characterization
STABLE!
pKa1,2 1.57
pKa3 5.20
pKa4 9.42
pKa5 11.93
pKa6 13.21
pKa1,2 Carboxylic groups dissociation
pKa3, pKa4 Protonated amines dissociation
pKa5, pKa6 Phenolic groups dissociation
L4-: HBCD fully deprotonated
H6L2+: HBCD fully protonated
Complexation studies with Ga
pH 7-8
HBCD-Ga complex
pH 1.45
Free ligand
pH 4.58
ESI-MS confirmed Ga3+ complexation: m/z = 509.12 [Ga3+HBCD2-].
Experimental
Simulated
[Ga3+HBCD2-]+
HBCD titration with Ga(III) in PBS pH 7.4
HBCD-Ga complex pH dependence
work in progress
NH2bpcd
1H NMR spectrum in MeOD @600 MHz
1H/13C HSQC spectra in MeOD @600 MHz
Next steps
Ga3+-HBCD stability constant determination
HBCD radiolabeling with 68Ga
Ongoing with NH2bpcd
Other metals complexation
References:
[1] T. I. Kostelnik, C. Orvig, Chem. Rev., 2019, 119, 902-956.
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