EXECUTIVE SUMMARY PHD THESIS:

Development of terbium radioisotopes towards clinical theragnostics applications in nuclear medicine

Chiara Favaretto
DOCTOR OF SCIENCES of ETH ZÜRICH
(Dr. Sc. ETH Zürich)

This thesis investigates the pivotal role of radionuclides in radiopharmaceuticals development in the field of nuclear medicine. Radiopharmaceuticals employ a systemic approach wherein a radionuclide is coupled with a ligand designed to bind to a specific disease-related target. The primary objective is to precisely localize the drug and the radiation emitted by the radionuclide to the diseased cells, effectively minimizing accumulation in healthy tissues. By utilizing radionuclides with varying emission properties, these compounds can serve dual purposes, enabling both the diagnosis and treatment of diseases. This combination of diagnostic imaging with subsequent treatment, using the same targeting ligand but with a different isotope of the same element, is referred to as radiotheragnostics.[1] The beauty of this approach lies in the identical kinetic behavior of the two radioligands, ensuring that the treatment is precisely localized to the same diseased cells previously detected, hence the concept of "what you image is what you treat" is displayed. Therefore, it is evident that the production of diagnostic or therapeutic radionuclides with properties suitable for nuclear medicine is a crucial feature of radiopharmaceutical development for clinical use. The ease of large-scale production, as well as the production yield, radionuclidic purity (RNP), and radiolabeling yield, are pivotal considerations in the production of a specific radionuclide for applications in nuclear medicine.[2] In particular, the production yield is affected by factors such as ion beam energy, neutron flux, irradiation duration, and the number of target nuclei. Therefore, it's crucial to thoroughly explore various production methods and carefully choose the optimal reaction and energy range to achieve optimal outcomes. These factors also influence the RNP, which is defined as the ratio of the desired radionuclide's radioactivity to the total radioactivity, indicating the presence of undesired radioisotopes that might contaminate the radionuclide of interest. Ensuring a preparation suitable for human administration necessitates confirming the absence or minimal presence of other radionuclides besides the ones of interest, as these could expose the patient to unwanted radiation doses. Additionally, the radionuclide should be capable of conjugation with a ligand, allowing for the evaluation of its properties on the target

cells in preclinical and potential clinical investigations. This critical process of linking the radionuclide to the ligand is referred to as radiolabeling. Hence, it's imperative for the radionuclide to possess high radiolabeling capabilities with biologically relevant targeting agents.

Several stages can be identified in radionuclide production, each of which involves diverse scientific fields: (i) target design and irradiation parameters optimization, (ii) radiochemical separation of the desired radionuclide from undesired byproducts and target material, (iii) quality control (QC) of the radionuclide final product, (iv) radiolabeling and preclinical assessment of the radiopharmaceutical, and (v) Good Manufacturing Practice (GMP) process development for clinical evaluation.[2] Over the past decade, the Radionuclide Development group, operating within the Center for Radiopharmaceutical Sciences (CRS) at the Paul Scherrer Institute (PSI), has concentrated its efforts on the development of several radionuclides, with particular interest in terbium radioisotopes. In fact, terbium offers four radioisotopes, each possessing distinct physical decay properties ideally suited for radiotheragnostics.[3] These isotopes include terbium-152 and terbium-155, suitable for employment in diagnosis, as well as terbium-149 and terbium-161, better suited for therapeutic applications. Previous collaborations with CERN-ISOLDE involved experiments utilizing mass separation techniques, ultimately resulting in the collection and delivery of terbium isobars of 149, 152, and 155 to PSI for radiochemical separation. However, while these purified products (terbium-149, terbium-152, and terbium-155) were initially employed for preliminary preclinical research, production yields fell short of the requirements for clinical and extensive preclinical studies.

The first part of this thesis revolves around the development and optimization of production routes for terbium-149 and terbium-155. The optimization of terbium-149 production, with a half-life of 4.1 hours and suitability for α-therapy (E_α=3.98 MeV (16.7%), 28 μm range in tissue), was performed by spallation reaction Ta(p, spall)^{149}Dy→^{149}Tb, followed by online mass separation. This process involved the use of the online isotope separator facility ISOLDE at CERN (Geneva, Switzerland) and it was proved effective in generating sufficient terbium-149 for in vivo preclinical studies, achieving approximately 100 MBq per production run. In particular, a tantalum foil target (94 g/cm^2) was irradiated with 1.4 GeV protons from the CERN PS-Booster accelerator inducing a spallation reaction. The spallation products were released from the target material, ionized, accelerated, and mass-separated in a magnetic sector

field. At A = 149, the isobars were implanted, over a period of 6 to 9 hours, into zinc-coated gold foils. Afterward, an optimized radiochemical separation procedure was established, based on ion exchange and extraction chromatography, which yielded higher purity [^{149}Tb]$TbCl_3$ compared to previous methods. Notably, terbium-149 exhibited an RNP of 99.8% and allowed peptide radiolabeling at 20 MBq/nmol molar activity, whereas only 6 MBq/nmol molar activity was previously achieved.[4] Based on the RNP and Apparent Molar Activity achieved, the quality of terbium-149 was considered adequate for preclinical applications, and was used for in-vitro and in-vivo studies that will be reported in the future.

Furthermore, the development of novel production routes for terbium-155 ($T_{1/2}$=5.32 d, Eγ = 87 keV (32%) 105 keV (25%)), suitable for SPECT applications, was investigated. In particular, the ^{155}Gd(p,n)^{155}Tb and ^{156}Gd(p,2n)^{155}Tb nuclear reactions were studied utilizing the Injector 2 cyclotron at PSI. This research marks the first successful irradiation of enriched gadolinium-155 and gadolinium-156 targets with a high-energy proton beam (at 10 and 24 MeV, respectively) and subsequent radiochemical separation of terbium-155 from the target material.[5] This achievement opened doors to producing high yields of terbium-155, with rates up to 4.4 GBq and the ^{156}Gd(p,2n)^{155}Tb nuclear reaction demonstrating higher production yields. However, the latter reaction resulted in increased terbium-156 impurities (8%) compared to the ^{155}Gd(p,n)^{155}Tb route (6%). Nevertheless, consistent peptide radiolabeling at 100 MBq/nmol molar activity was achieved, enabling preclinical imaging studies confirming terbium-155's suitability for SPECT. Alternative production routes were also investigated to potentially enhance the RNP of terbium-155. This included the collaboration with CERN-ISOLDE for the production of terbium-155 by online mass separation and a comparison with the offline mass separation performed at CERN-MEDICIS. Furthermore, an indirect production route involving ^{nat}Tb(p,5n)^{155}Dy→^{155}Tb was explored, by irradiating natural terbium target with high energy proton at Injector 2 cyclotron at PSI. Online mass separation yielded higher chemical purity terbium-155 and enabled peptide radiolabeling for preclinical use at 20 MBq/nanomole, a significant improvement over previous results. However, the offline mass separation method yielded very low final activities due to inefficient mass separation (< 0.5%). The indirect production route displayed similar production yields to the direct ^{156}Gd(p,2n)^{155}Tb nuclear reaction but with superior RNP. In particular, significant proportion of terbium-156 was co-generated with terbium-155. Nevertheless, the proposed method for radiochemical separation entails an initial separation of dysprosium-155 and terbium target material, along with any co-produced terbium isotopic impurities. After the

decay of the isolated dysprosium-155, a second separation would be performed to finally isolate the terbium-155 produced by the decay, resulting in terbium-155 with particularly high RNP.

In recent years, the Radionuclide Development group achieved success in developing the production of terbium-161 ($T_{1/2}$ = 6.96 d, Eβ-av = 154 keV (100%)) by neutron irradiation of enriched gadolinium-160 targets, via the $^{160}Gd(n,\gamma)^{161}Gd \rightarrow ^{161}Tb$ nuclear reaction, followed by radiochemical separation via cation exchange chromatography.[6] This approach provided [^{161}Tb]$TbCl_3$ of sufficient quality for radiolabeling at high molar activity, suitable for extensive preclinical and potential clinical applications. Preclinical studies compared terbium-161 to lutetium-177, which is regarded as the "gold standard" of targeted radionuclide therapy, and demonstrated increased therapeutic efficacy for ^{161}Tb-labeled radiopharmaceuticals in both tumor cells in vitro and in vivo, due to the higher efficacy of terbium-161 Auger electron emission.[7,8] The second part of this thesis shifts focus to the introduction of terbium-161 into clinical practice, emphasizing the need for both the production of the radionuclide and the ^{161}Tb-radiopharmaceuticals to adhere to quality standards. To this purpose, terbium-161 was extensively characterized for therapeutic application.[9] The evaluation involved the comparison of the [^{161}Tb]$TbCl_3$ solution with the requirements of the commercially-available no-carrier added [^{177}Lu]$LuCl_3$, which is approved for the production of several radiopharmaceuticals for clinical investigations. The assessment revealed that the terbium-161 solution met clinical suitability standards, displaying radionuclidic and radiochemical purity (RCP) of > 99.9% and endotoxin levels below the permitted range (175 IU/mL) at pH 1-2. Following this evaluation, a protocol for the production of ^{161}Tb-based radiopharmaceuticals was established. Special attention was paid to preventing pharmaceutical contamination and reducing the operator radiation exposure. An automated module for radiolabeling and final radiopharmaceutical production was employed, particularly for the synthesis of the radiolabeled somatostatin agonist [^{161}Tb]Tb-DOTATOC. This synthesis was achieved for the first time using a modular automated system resulting in a product with clinically applicable specifications and activity levels, ranging from 1.0 to 7.4 GBq in 20 mL at a molar activity of 50 MBq/nmol. QC methods were also developed, confirming the product's stability (RCP $\geq$ 95%) over 24 hours.[9] In addition, recent preclinical investigations suggest that terbium-161 may be more effective than lutetium-177 when combined with somatostatin antagonists.[10] As a result, the ^{161}Tb-radiolabeled somatostatin antagonist [^{161}Tb]Tb-DOTA-LM3 emerged as a promising candidate for the treatment of neuroendocrine tumors. Proposed for a clinical study, this novel therapy is

contingent on the establishment of Good Manufacturing Practice (GMP)-compliant production methods to ensure clinical viability. This thesis encompasses the development of a GMP-compliant purification method for terbium-161 and the subsequent synthesis of [^{161}Tb]Tb-DOTA-LM3 using an automated module system. The terbium-161 module-based purification procedure yielded activities of up to 7.4 GBq with a final yield ranging from 62% to 93%. This approach was validated for the range of activity (0.5 to 1 GBq/dose) intended for the initial phase of a clinical trial, designed for dosimetry evaluation prior to therapeutic dose escalation. The production of the final medicinal product [^{161}Tb]Tb-DOTA-LM3 was then established and validated using the same automated module, following protocols developed for the similarly ^{161}Tb-labeled radiopeptide [^{161}Tb]Tb-DOTATOC. Synthesis and QC for [^{161}Tb]Tb-DOTA-LM3 were performed for activities up to 4.5 GBq, but also validated for the 0.5-1 GBq activity range, affirming the product's suitability for initial clinical use. QC assessments on the synthesis batches demonstrated good product quality, with RCP ≥ 95% up to 24 hours. Ethanol levels (≤ 7%) and bacterial endotoxin levels (≤ 175 UI/20 mL) remained within acceptable limits. However, further efforts, such as sterility evaluation and process controls, were required to establish a GMP-compliant process, which were not required for the DOTATOC pilot project.

In conclusion, in this thesis, considerable developments were made at various stages in the production of three terbium radioisotopes that are of great relevance for potential future radiotheragnostic clinical applications in nuclear medicine. Terbium-149 production was successfully optimized, resulting in terbium-149 of greatly improved quality for preclinical therapy studies. On the other hand, several terbium-155 production alternatives were exhaustively investigated, and the best alternatives produced terbium-155 in quantities and quality adequate for potential clinical uses. Finally, the purity of terbium-161 was proved to be sufficient for the development of a radiopharmaceutical production protocol for [^{161}Tb]Tb-DOTATOC and [^{161}Tb]Tb-DOTALM3, and, thanks to this study, terbium-161 was used for the first time in clinical practice for the investigation of [^{161}Tb]Tb-DOTALM3 in the treatment of neuroendocrine neoplasms. Furthermore, the automated radiolabeling procedures devised for this research have the potential to be applied to other DOTA-derivatized peptides, broadening the application of various new radiopharmaceuticals in clinical settings.

References

1. Rösch, F., Herzog, H. & Qaim, S. M. The beginning and development of the theranostic approach in nuclear medicine, as exemplified by the radionuclide pair ^{86}Y and ^{90}Y. *Pharmaceuticals* **10**, 1–28 (2017).

2. Talip, Z., Favaretto, C., Geistlich, S. & Van Der Meulen, N. P. A step-by-step guide for the novel radiometal production for medical applications: Case studies with ^{68}Ga, ^{44}Sc, ^{177}Lu and ^{161}Tb. *Molecules* **25**, 1–28 (2020).

3. Müller, C. *et al.* A Unique Matched Quadruplet of Terbium Radioisotopes for PET and SPECT and for α- and β--Radionuclide Therapy: An In Vivo Proof-of-Concept Study with a New Receptor-Targeted Folate Derivative. *J. Nucl. Med.* **53**, 1951–1959 (2012).

4. Umbricht, C. A. *et al.* Alpha-PET for Prostate Cancer: Preclinical investigation using ^{149}Tb-PSMA-617. *Sci. Rep.* **9**, 1–10 (2019).

5. Favaretto, C. *et al.* Cyclotron production and radiochemical purification of terbium-155 for SPECT imaging. *EJNMMI Radiopharm. Chem.* **6**, (2021).

6. Gracheva, N. *et al.* Production and characterization of no-carrier-added ^{161}Tb as an alternative to the clinically-applied ^{177}Lu for radionuclide therapy. *EJNMMI Radiopharm. Chem.* **4**, 1–16 (2019).

7. Müller, C. *et al.* Direct in vitro and in vivo comparison of ^{161}Tb and ^{177}Lu using a tumour-targeting folate conjugate. *Eur. J. Nucl. Med. Mol. Imaging* **41**, 476–485 (2014).

8. Müller, C. *et al.* Terbium-161 for PSMA-targeted radionuclide therapy of prostate cancer. *Eur. J. Nucl. Med. Mol. Imaging* **46**, 1919–1930 (2019).

9. Favaretto, C. *et al.* ^{161}Tb-DOTATOC Production Using a Fully Automated Disposable Cassette System : A First Step Toward the Introduction of ^{161}Tb into the Clinic. *J. Nucl. Med.* 1–8 (2023). doi:10.2967/jnumed.122.265268

10. Borgna, F. *et al.* Combination of terbium-161 with somatostatin receptor antagonists - a potential paradigm shift for the treatment of neuroendocrine neoplasms. *Eur J Nucl Med Mol Imaging* (2021).