Doctoral Thesis

Development of Terbium and Erbium Radiolanthanides for Radiopharmaceutical Application

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Development of Terbium and Erbium Radiolanthanides for Radiopharmaceutical Application

A thesis submitted to attain the degree of
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(Dr. Sc. ETH Zurich)

presented by
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Summary

Nowadays, the most common clinically applied radionuclide for the treatment of disseminated tumors and used in combination with peptides or antibodies is $^{177}$Lu. Phase III study of the FDA approved Lutathera® ($[^{177}]$Lu-DOTA-TATE) showed the response rates (complete and partial responses) of 18%. Even though the results are encouraging, the search for the more effective treatment of metastatic cancer with higher response rates and progression-free survival continues. $^{177}$Lu is a $\beta^–$-emitter ($E_{\beta^–}$=134 keV (100%), $T_{1/2}$=6.7 d) with an average tissue penetration range being 0.7 mm (maximum range ~2 mm). Due to the mm range in tissue, $^{177}$Lu is a promising candidate for the treatment of relatively large metastases, however, could be insufficient for killing cell clusters or single cancer cells due to the low linear energy transfer LET (~0.2 keV/µm) and cross-irradiation of the healthy tissues. In this case, $\alpha$–emitters and Auger electron emitters are believed to be better alternatives due to the delivery of higher radiation doses at a cellular and subcellular level. Alpha–emitting radionuclides are attractive candidates for the treatment of microscopic cancer clusters due to ≤100 µm range in tissue and high LET (~80 keV/µm). Preclinical research, followed by clinical trials, showed high potential for $\alpha$–particle therapy, however, with the reported toxicity and severe side effects. One of the reasons is the decay of the most medically interested $\alpha$–emitters (e.g., $^{225}$Ac, $^{227}$Th) to stable daughter via several subsequent alphas. Each $\alpha$–decay is accompanied by the alpha recoil, sufficient to break chemical bonds within the biomolecule and free the radionuclide. Therefore, the availability of the $\alpha$–emitting radionuclide, which has no $\alpha$–emitting daughters in the decay chain would be of high value. $^{149}$Tb ($E_\alpha$=3.98 MeV (16.7%), $T_{1/2}$=4.1 h) meets the requirements and demonstrated promising results on the preclinical settings. $^{149}$Tb has a range in tissue of 28 µm, making the radionuclide suitable for the treatment of micrometastases. In order to eliminate circulating in the bloodstream tumor cells, which contribute to the formation of metastases, Auger electron therapy might be an attractive option. Auger–electron emitters have high LET (~4–26 keV/µm) and a short range in tissue (~0.5 µm), meaning this type of emitters could irradiate tumors on subcellular level and eliminate single cancer cells. In literature, contradictory results about the cell toxicity of the Auger–electron emitters (with the co-emission of $\gamma$–rays) were reported, however, pure Auger–electron emitters so far have never been applied for the in vitro and in vivo experiments. Therefore, the production of pure Auger–electron emitters
(e.g., $^{165}$Er) is important for the evaluation of the possible application of such emitters for therapeutic purposes. It is believed that utilization of $\beta^–$–emitters, which decay with the co-emission of the short-range electrons (e.g., Auger electrons), might increase the therapeutic efficacy as compared to clinically approved $^{177}$Lu based therapy. In this case, a potential alternative to $^{177}$Lu is the $\beta^–$–emitting $^{161}$Tb ($E_{\beta-av}=154$ keV (100%), $T_{1/2}=7.0$ d), which was proposed by Lehenberger et al. in 2011. $^{161}$Tb has similar decay characteristics to the commercially available $^{177}$Lu but co-emits a higher percentage of short-range conversion and Auger electrons ($\sim 12$ e$^–$, $\sim 36$ keV per decay) than $^{177}$Lu. Modeling electron dose deposition from $^{161}$Tb and $^{177}$Lu in spheres with micrometer diameter and in vitro and in vivo experiments confirmed the superior antitumor effect of $^{161}$Tb over $^{177}$Lu. However, the reliable production and reproducible and efficient purification of $^{161}$Tb in order to obtain the product of the quality, comparable to the commercially available $^{177}$Lu and suitable for clinical application, until now was not possible.

In this thesis, the development of $^{161}$Tb started with the choice of the target material ($^{160}$Gd$\text{Gd}_2\text{O}_3$, 98.2% enrichment) for safe long-term irradiation at the nuclear reactors (5–21 days irradiation time). Bombardment of the target material was performed at South African Fundamental Atomic Research Installation 1 (SAFARI-1, Pelindaba, South Africa) and the high-flux reactor of Institut Laue-Langevin (HFR ILL, Grenoble, France) research nuclear reactors as well as at the Swiss Spallation Neutron Source (SINQ, PSI). The required masses of $^{160}$Gd$\text{Gd}_2\text{O}_3$ (7–33 mg) were calculated by ChainSolver code to provide the currently limited maximum 20 GBq of $^{161}$Tb at the end of bombardment (EOB), allowed for international transportations. After $^{161}$Tb production via the $^{160}$Gd(n,$\gamma$)$^{161}$Gd$\rightarrow^{161}$Tb nuclear reaction, separation of the radionuclide of interest (2–5 µg) from the target material (7–33 mg) and co-produced impurities was required. Therefore, a reproducible purification method was developed, combining the use of Sykam cation-exchange resin (styrol/divinylbenzol stationary phase, NH₄⁺ form) and LN3 extraction resin (bis(2,4,4-trimethyl-1-pentyl)phosphinic acid extractant). The method allowed to efficiently separate $^{161}$Tb from $^{160}$Gd and present impurities and to formulate $^{161}$Tb in a 500 µL 0.05 M HCl to provide specifications of the final product similar to the commercially available $^{177}$Lu. $^{161}$Tb was used for the radiolabeling of the clinically applied somatostatin analogues DOTA-NOC and DOTA-TOC in the presence of the stabilizer (ascorbic acid) at high activity concentration of 250 MBq/500 µL.
The idea of the established purification method for $^{161}$Tb (a combination of Sykam and LN3 resins) was translated to the purification of the $\alpha$–emitter $^{149}$Tb. The method was further developed for the application of Zn foils, which we use for collecting $^{149}$Tb after the production at ISOL facilities via mass separation. The use of Zn foils instead of the commonly used Zn coated gold foils will prevent loss of $^{149}$Tb within the gold (inert material) via absorption during the collection of the radionuclide. The purification method was adapted for the separation of $^{149}$Tb from ~300 mg of Zn. Processing high activities of $^{149}$Tb gave access to the radionuclidically and radiochemically pure $[^{149}\text{Tb}]\text{TbCl}_3$.

In order to obtain the pure Auger–electron emitter $^{165}$Er ($T_{1/2}=10.4$ h), we studied two production routes: the direct route via the $^{\text{nat}}$Ho(p,n)$^{165}$Er nuclear reaction and the indirect route via the $^{166}$Er(p,2n)$^{165}$Tm→$^{165}$Er nuclear reaction. The direct production of $^{165}$Er from 200 mg $^{\text{nat}}$Ho oxide or $^{\text{nat}}$Ho foil targets at 13.4 MeV proton beam energy was performed at the Injector 2 of the PSI research cyclotron or at the IBA Cyclone (Bern University Hospital (Inselspital)). The production was followed by a $^{\text{nat}}$Ho/$^{165}$Er separation with the developed purification method and provided 0.4–0.5 GBq of $^{165}$Er at the end of separation (EOS). The amount of the radioactivity enabled in vitro and in vivo experiments, however, the purity of the final $^{165}$Er product was not appropriate due to the presence of Zn (environmental impurity) and $^{\text{nat}}$Er, coming from the $^{\text{nat}}$Ho$_2$O$_3$ target material and co-produced during the bombardment. In order to remove $^{\text{nat}}$Er from the final product, Er/Ho separation should be performed before the irradiation of the $^{\text{nat}}$Ho$_2$O$_3$, provided by the supplier, and the beam energy should be decreased to <9 MeV. Unfortunately, irradiation of the 200 mg $^{\text{nat}}$Ho$_2$O$_3$ target at 8.6 MeV proton beam energy resulted in the production of only 75 MBq $^{165}$Er (EOB). Based on the obtained data, the direct production route via $^{165}$Ho(p,n)$^{165}$Er nuclear reaction was considered inappropriate for obtaining high yields of no-carrier-added $^{165}$Er. Therefore, the indirect production of $^{165}$Er via $^{166}$Er(p,2n)$^{165}$Tm→$^{165}$Er nuclear reaction was studied as an alternative. Irradiation of 98.1% enriched $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ (60 mg), followed by the developed 4-column purification method, gave access to ~500 MBq $^{165}$Er (EOS) of quality, sufficient for the preclinical research. The radionuclidic and radiochemical purity of $^{165}$Er was >99%, the possibility of performing radiolabeling of DOTA-NOC with $^{165}$Er at 30 MBq/nmol was confirmed. Provided characteristics of the radionuclide are sufficient for performing in vitro and in vivo experiments in order to determine the suitability of $^{165}$Er for the targeted radionuclide therapy.
Zusammenfassung

Im Zusammenhang mit der klinischen Behandlung von disseminierten Tumoren, ist Lutetium-177 (\(^{177}\)Lu) heutzutage das am häufigsten verwendete Radionuklid und wird in Kombination mit Peptiden oder Antikörpern eingesetzt. Die Phase-III-Studie, des von der FDA zugelassenen Lutathera® (\([^{177}\)Lu]Lu-DOTATATE), ergab eine Ausschöpfungsquote (vollständige und anteilige Ausschöpfung) von 18%. Obwohl die Ergebnisse vielversprechend sind, wird die Suche nach einer effektiveren Behandlung des metastasierten Krebses, mit höheren Ausschöpfungsquoten und einem progressionsfreien Überleben, fortgesetzt. \(^{177}\)Lu ist ein β-Emitter (\(E_{\beta_{av}}=134\) keV (100%), \(T_{1/2}=6,7\) d) mit einem durchschnittlichen Gewebedurchdringungsbereich von 0,7 mm (maximaler Bereich ~2 mm). Aufgrund des mm-Bereichs im Gewebe ist \(^{177}\)Lu ein vielversprechender Kandidat für die Behandlung von relativ großen Metastasen, könnte jedoch aufgrund des niedrigen linearen Energietransfers LET (~0,2 keV/µm) zum Abtöten von Zellclustern oder einzelnen Krebszellen unzureichend sein, da es zu einer Kreuzbestrahlung des gesunden Gewebes kommen kann. In diesem Fall werden α-Emitter und Auger-Elektronenemitter aufgrund der Abgabe höherer Strahlungsdosen aufzellulärer und subzellulärer Ebene als bessere Alternativen angesehen. Alpha-emittierende Radionuklide sind, aufgrund des Bereichs von \(\leq 100\) µm im Gewebe und des hohen LET (~80 keV/µm), attraktive Kandidaten für die Behandlung von mikroskopischen Krebsclustern. Präklinische Untersuchungen, gefolgt von klinischen Studien, zeigten ein hohes Potenzial für eine α-Partikeltherapie, allerdings wurde von Toxizität und schwerwiegenden Nebenwirkungen berichtet. Einer der Gründe ist der Zerfall, der medizinisch am interessantesten α-Emitter (z. B. \(^{225}\)Ac, \(^{227}\)Th), zu einer stabilen Tochter. Dies erfolgt durch mehrere aufeinanderfolgenden α-Zerfälle. Jeder dieser α-Zerfälle geht mit einem α-Rückstoß einher, der ausreichend ist, um chemische Bindungen innerhalb des Biomoleküls aufzubrechen und das Radionuklid freizusetzen. \(^{149}\)Tb (\(E_{\alpha}=3,98\) MeV (16,7%), \(T_{1/2}=4,1\) h) erfüllt diese Anforderungen und zeigte vielversprechende Ergebnisse bei präklinischen Tests. Im Gewebe hat \(^{149}\)Tb eine Reichweite von 28 µm, wodurch das Radionuklid zur Behandlung von Mikrometastasen geeignet ist. Um die im Blutkreislauf zirkulierenden Tumorzellen zu eliminieren, die zur Bildung von Metastasen beitragen, könnte die Auger-Elektronen-Therapie eine attraktive Option sein. Auger-Elektronen-Emitter zeichnen sich durch einen hohen LET (~4–26 keV/µm) und eine kurze Reichweite im
Gewebe (≤0,5 µm) aus, was bedeutet, dass diese Art von Emittern Tumore auf subzellulärer Ebene bestrahlen und einzelne Krebszellen eliminieren könnte. In der Literatur wurden widersprüchliche Ergebnisse zur Zelltoxizität von Auger-Elektronen-Emittern (unter Co-Emission von γ-Strahlen) berichtet. Jedoch wurden für In-vitro- und In-vivo-Experimente bisher keine reinen Auger-Elektronen-Emitter verwendet. Daher ist die Herstellung reiner Auger-Elektronen-Emitter (z. B. $^{165}\text{Er}$), für die Einschätzung der möglichen Anwendung solcher Emitter für therapeutische Zwecke, wichtig. Es wird angenommen, dass die Verwendung von β'-Emittern, die mit der gleichzeitigen Emission von Elektronen mit kurzer Reichweite (z. B. Auger-Elektronen) zerfallen, die therapeutische Wirksamkeit, im Vergleich mit einer klinisch zugelassenen $^{177}\text{Lu}$-basierten Therapie, erhöhen könnte. Eine mögliche Alternative zu $^{177}\text{Lu}$ ist in diesem Fall das von Lehenberger et al. in 2011 vorgeschlagene β'-emittierende $^{161}\text{Tb}$ (Eβ-av=154 keV (100%), T1/2=7,0 d). $^{161}\text{Tb}$ hat ähnliche Zerfallseigenschaften wie das im Handel erhältliche $^{177}\text{Lu}$, emittiert jedoch, im Gegensatz zu $^{177}\text{Lu}$, einen höheren Prozentsatz an Konversions- und Auger-Elektronen (~12 e⁻, ~36 keV pro Zerfall), die auf kurzer Reichweite im Gewebe wirksam sind. Die Modellierung der Elektronendosisdeposition von $^{161}\text{Tb}$ und $^{177}\text{Lu}$ in Kugeln mit Mikrometer Durchmesser, sowie In-vitro- und In-vivo-Experimente, bestätigten die überlegene Antitumorwirkung von $^{161}\text{Tb}$ gegenüber $^{177}\text{Lu}$. Jedoch war die zuverlässige Herstellung und reproduzierbare und effiziente Reinigung von $^{161}\text{Tb}$ zur Gewinnung, des mit dem kommerziell erhältlichen $^{177}\text{Lu}$ vergleichbaren und für die klinische Anwendung geeigneten Produkts, bisher nicht möglich.

Die vorliegende Doktorarbeit begann mit $^{161}\text{Tb}$ und der Auswahl des Targetmaterials ($[^{160}\text{Gd}]{\text{Gd}_2}\text{O}_3$, Anreicherungsgrad: 98,2%), das für eine sichere Langzeitbestrahlung der Kernreaktoren (5 bis 21 Tage Bestrahlungszeit) gedacht ist. Das Zielmaterial wurde in der südafrikanischen atomaren Grundforschungsanlage 1 (SAFARI-1, Pelindaba, Südafrika) und im Hochflussreaktor des Instituts Laue-Langevin (HFR ILL, Grenoble, Frankreich) sowie an der Schweizer Spallations-Neutronenquelle (SINQ, PSI), in Kernreaktoren beschossen. Die erforderlichen Mengen von $[^{160}\text{Gd}]{\text{Gd}_2}\text{O}_3$ (7 bis 33 mg) wurden mithilfe des ChainSolver Code berechnet, um das derzeit begrenzte Maximum von 20 GBq an $^{161}\text{Tb}$ am Ende der Bestrahlung (EOB; engl.: end of bombardment) für internationale Transporte bereitzustellen. Nach der $^{161}\text{Tb}$-Produktion über die $^{160}\text{Gd}(n,\gamma)^{161}\text{Gd}\rightarrow^{161}\text{Tb}$-Kernreaktion, mussten das wichtige Radionuklid (2 bis 5 µg) vom Zielmaterial (7 bis
33 mg) und den gleichzeitig miterzeugten Verunreinigungen abgetrennt werden. Aus diesem Grund wurde eine zuverlässige Reinigungsmethode entwickelt, bei der das Sykam-Kationenaustauscherharz (Styrol/Divinylbenzol stationäre Phase, NH₄⁺-Form) mit dem LN3-Extraktionsharz (Bis(2,4,4-trimethyl-1-penty1)phosphinsäure-Extraktionsmittel) kombiniert zum Einsatz kamen. Das Verfahren ermöglichte es, ⁶¹⁶Tb von ¹⁶⁰Gd und vorhandenen chemischen Verunreinigungen effizient abzutrennen. Anschließend konnte ¹⁶¹Tb in 500 µL 0,05 M HCl dargestellt werden, sodass das Endprodukt chemisch, mit dem bereits im Handel erhältlichen ¹⁷⁷Lu, vergleichbar ist.

Das extrahierte ¹⁶¹Tb wurde für die radioaktive Markierung der klinisch angewendeten Somatostatin-Analoga DOTA-NOC und DOTA-TOC in Verbindung mit dem Stabilisator (Ascorbinsäure) bei einer hohen Aktivitätskonzentration von 250 MBq/500 µL verwendet.


Um den reinen Auger-Elektronen-Emitter ¹⁶⁵Er (T½=10,4 h) zu erhalten, untersuchten wir zwei Produktionswege: den direkten Weg über die natHo(p, n)¹⁶⁵Er-Kernreaktion und den indirekten Weg über die ¹⁶⁶Er(p, 2n)¹⁶⁵Tm→¹⁶⁵Er Kernreaktion. Die direkte Produktion von ¹⁶⁵Er aus 200 mg natHo-Oxid- oder natHo-Folien-Targets bei 13,4 MeV Protonenstrahlenergie erfolgte am Injector 2 des PSI-Forschungszyklotrons oder am IBA Cyclone (Universitätsklinik Bern (Inselspital)). Auf dessen Produktion folgte eine natHo/¹⁶⁵Er-Trennung, basierend auf der entwickelten Reinigungsmethode, sodass am Ende der Trennung (EOS; engl.: end of separation) 0,4 bis 0,5 GBq ¹⁶⁵Er bereitgestellt wurden. Die Menge der Aktivität ermöglichte In-vitro- und In-vivo-Experimente. Allerdings war die Reinheit des ¹⁶⁵Er-Endprodukts, aufgrund der Anwesenheit von Zn (Umweltverunreinigung) und natEr, die aus dem natHo₂O₃-Targetmaterial stammten.
und während der Bestrahlung mitproduziert wurden, nicht angemessen. Um daher nat\textsuperscript{Er} aus dem Endprodukt zu entfernen, sollte vor der Bestrahlung mit dem vom Lieferanten bereitgestellten nat\textsuperscript{Ho\textsubscript{2}O\textsubscript{3}} eine Er/Ho-Trennung durchgeführt und die Strahlenergie auf <9 MeV gesenkt werden. Allerdings führte die Bestrahlung des 200 mg nat\textsuperscript{Ho\textsubscript{2}O\textsubscript{3}}-Targets mit einer Protonenstrahlenergie von 8,6 MeV zu einer Produktion von lediglich 75 MBq \textsuperscript{165}Er (EOB). Basierend auf den erhaltenen Daten wurde der direkte Produktionsweg über eine \textsuperscript{165}Ho(p, n)\textsuperscript{165}Er-Kernreaktion als ungeeignet angesehen. Um hohe Ausbeuten an \textsuperscript{165}Er ohne Trägerzusatz zu erhalten, wurde alternativ die indirekte Erzeugung von \textsuperscript{165}Er über die Kernreaktion \textsuperscript{166}Er(p, 2n)\textsuperscript{165}Tm→\textsuperscript{165}Er untersucht. Die Bestrahlung mit 98,1 % angereichertem \textsuperscript{166}Er\textsubscript{2}O\textsubscript{3} (60 mg), gefolgt von der entwickelten 4-Säulen-Reinigungs methode, ermöglichte den Zugang zu einer für die präklinische Forschung ausreichenden Qualität von ~ 500 MBq \textsuperscript{165}Er (EOS). Die radionuklidische und radiochemische Reinheit von \textsuperscript{165}Er war >99\%, sodass die Möglichkeit der Radiomarkierung von DOTA-NOC mit \textsuperscript{165}Er bei 30 MBq/nmol bestätigt wurde. Die angegebenen Charakteristika des Radionuklids reichen aus, um In-vitro- und In-vivo-Experimente durchzuführen und um die Eignung von \textsuperscript{165}Er für die gezielte Radionuklidtherapie festzulegen.
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**Abbreviations**

- **DGA resin**: N,N,N',N'-tetra-octyldiglycolamide
- **DNA**: Deoxyribonucleic acid
- **DOTA**: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
- **DOTA-NOC**: [DOTA,1-Na³]-octreotide
- **DOTA-TATE**: (DOTA⁰,-Tyr³)-octreotate
- **DOTA-TOC**: (DOTA⁰,-Phe¹-Tyr³)-octreotide
- **DSB**: Double-strand break
- **DTPA**: Diethylenetriaminepentaacetic acid
- **E_{β-av}**: Average beta minus energy
- **E_{β+av}**: Average positron energy
- **E_γ**: Gamma energy
- **EC**: Electron capture
- **EOB**: End of bombardment
- **EOS**: End of separation
- **FDA**: Food and Drug Administration
- **HFR**: High-flux reactor
- **HPGe**: High purity Germanium
- **HPLC**: High performance liquid chromatography
- **IC**: Internal conversion
- **ICP-OES**: Inductively coupled plasma-atomic emission spectrometry
- **ICP-MS**: Inductively coupled plasma-mass spectrometry
- **ILL**: Institut Laue-Langevin, Grenoble, France
- **ITG**: Isotope Technologies Garching
- **ISOLDE**: The Isotope mass Separator On-Line facility, CERN, Switzerland
- **LET**: Linear energy transfer
- **Ln**: Lanthanide
- **LN3 resin**: bis(2,4,4-trimethyl-1-pentyl)phosphinic acid extraction resin
- **NET**: Neuroendocrine tumor
- **PET**: Positron emission tomography
- **PSI**: Paul Scherrer Institut, Villigen PSI, Switzerland
- **PSMA**: Prostate specific membrane antigen
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE</td>
<td>Rare-earth elements</td>
</tr>
<tr>
<td>SAFARI-1</td>
<td>South African Fundamental Atomic Research Installation 1</td>
</tr>
<tr>
<td>SINQ</td>
<td>Swiss Spallation Neutron Source</td>
</tr>
<tr>
<td>SMC</td>
<td>Small medical cyclotrons</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>Half-life</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TRNT</td>
<td>Targeted radionuclide therapy</td>
</tr>
<tr>
<td>$\alpha$-HIBA</td>
<td>$\alpha$-hydroxyisobutyric acid</td>
</tr>
</tbody>
</table>
Chapter 1

General Introduction
1.1 Radionuclides for Targeted Radionuclide Therapy – from the origins to the new trends

1.1.1 Genesis and development of Targeted Radionuclide Therapy

The history of radionuclide therapy stemmed from the discovery of radioactivity by Henri Becquerel (1896) and Marie Curie (1898) [1]. In 1901, Becquerel recorded an important observation (severe inflammation of the skin) after holding a Radium (Ra) tube inside his waistcoat pocket for several hours. This discovery was a starting point for the application of radioactivity for therapeutic purposes: Henri Alexandre Danlos and Eugene Bloch placed Ra onto tuberculous skin lesions and Alexander Graham Bell suggested the same procedure for the treatment of tumors (1903) [2]. In 1913 a Hungarian radiochemist, George de Hevesy, performed first trace experiments to observe the distribution of native elements in plants (210Pb) or animals (210Pb, 210Bi), which led to the recognition of the application of trace approach for therapy [3, 4]. However, the number of the suitable and available tracers was limited and the production of artificial radionuclides was mandatory in order to develop the new field of cancer treatment – radionuclide therapy. The production of artificial radionuclides started in 1930, when Ernest Orlando Lawrence invented and built the first cyclotron. His brother, John Lawrence (a medical doctor), studied total body irradiation after intravenous administration of radioactive Phosphorus (32P) for cancer therapy using an animal model of leukemic mice. In 1937, a patient with chronic myeloid leukemia (CML) successfully received the first-ever human injection of a radionuclide – 32P [5, 6]. The described experiments and the recognition of artificial radioactivity gave rise to the fast development of an encouraging field in cancer research – Targeted Radionuclide Therapy (TRNT).

TRNT is a form of non-surgical treatment, which is based on the delivery (either by vehicle molecule or by specific metabolic pathways within the body) of therapeutic doses of radiation to malignant tumors [7]. Nowadays, TRNT is going through an exciting phase of growth and development, with the approval of a number of new radiopharmaceuticals such as Xofigo® ([223Ra]RaCl2) for the treatment of bone metastases and bone pain, arising from castration-resistant prostate cancer (CRPC), or Lutathera® ([177Lu]Lu-DOTA-TATE), applied for metastatic neuroendocrine tumors (NETs). TRNT could be appropriate as the main treatment or combined with other treatments (immunotherapy, chemotherapy) for patients with multiple types of cancer.
The specificity, efficacy and great potential of TRNT is demonstrated in Figure 1.1, an example of a body scan before, during and after the successful treatment of a patient having Merkel Cell Cancer with Lutathera®, in combination with immunotherapy (avelumab) [10].

Figure 1.1. (a) The diagnostic image ([68Ga]Ga-DOTA-TATE, pre-treatment) of a patient with metastasized NET. (b) Post-injection scan (4 hours after the infusion of Lutathera®), showing the distribution of [177Lu]Lu-DOTA-TATE in all the locations of somatostatin-receptor positivity. (c) The diagnostic image ([68Ga]Ga-DOTA-TATE, post-treatment) showing near complete remission of all the metastases. The figure adapted from Kasi et al., 2019 [10].

1.1.2 How to choose a suitable radionuclide for cancer treatment?

TRNT has the advantage of selective delivery of a radiopharmaceutical to the targeted tumor, limiting the exposure of the surrounding normal tissue and leading to minimal non-severe immediate or late side effects [11]. The biological effect is achieved by absorption of the energy emitted by the radionuclide. A radiopharmaceutical is a radiolabeled molecule designed for in vivo application (Figure 1.2). The design can be described based on two parts: (1) a molecular structure (vehicle molecule), responsible for the selectivity and action of the radiopharmaceutical within the organism (pharmacokinetics and pharmacodynamics) and (2) a radioactive part (radionuclide, complexed with the bifunctional chelator), which dictates the efficacy of the treatment [12]. A linker connects a radioactive part with the vehicle molecule. A small number of radionuclides can act as radiopharmaceuticals without being linked to the vehicle molecule, but possessing specific biological pathways. For example,
abnormal bone metabolism of $[^{89}\text{Sr}]\text{RaCl}_3$ or $[^{223}\text{Ra}]\text{RaCl}_2$ leading to the accumulation of calcium mimetic $^{89}\text{Sr}$ or $^{223}\text{Ra}$ within the targeted area from the bloodstream [13].

![Schematic illustration of a radiopharmaceutical for TRNT and its interaction with the target site.](image)

**Figure 1.2.** Schematic illustration of a radiopharmaceutical for TRNT and its interaction with the target site. The figure adapted from Wadsak et al., 2010 [12].

The efficiency of the tumor-internalized radiopharmaceutical is highly dependent on the decay characteristics of the radionuclide employed for the purpose – physical and biochemical [14]. Physical characteristics include the radionuclide half-life ($T_{1/2}$), type and energy of the emitted radiation, daughter product(s) and purity [15]. The biochemical characteristics determine targeting of the tissue, radioactivity retention in the tumor, in vivo stability, and toxicity [16].

Radionuclides with a half-life between 4 h and 7 d, decaying ideally to the stable daughter by the following three modes – $\beta^–$–particle emission, $\alpha$–particle emission and emission of short-range electron (Auger electrons) – are considered suitable for the application in TRNT [17]. These radiations possess different ranges of tissue penetration and different linear-energy transfer values (LET, energy deposition of an ionizing particle along the track) (Table 1.1). Based on these characteristics, $\beta^–$–particles, $\alpha$–particles and Auger electrons, could irradiate tumor volumes of multicellular, cellular and subcellular dimensions (Figure 1.3), respectively, with different ionization densities.

**Table 1.1.** General characteristics of therapeutic radionuclides. The table is adapted from Kassis, 2008 [16].

<table>
<thead>
<tr>
<th>Emitted radiation</th>
<th>Range</th>
<th>LET</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$–particle</td>
<td>40–100 µm</td>
<td>~80 keV/µm</td>
</tr>
<tr>
<td>$\beta^–$–particle</td>
<td>0.1–10 mm</td>
<td>~0.2 keV/µm</td>
</tr>
<tr>
<td>Auger electron</td>
<td>2–500 nm</td>
<td>~4–26 keV/µm</td>
</tr>
</tbody>
</table>
Figure 1.3. Schematic representation of α–particles, β–particles and Auger electrons tracks in tissue (blue circle corresponds to a single cell) as well as ionization densities produced along the tracks (DNA scale). The figure adapted from Lee et al., 2012 [18].

Most of the clinically-applied radionuclides used for therapeutic purposes are β–emitters, due to the availability of the radionuclides (e.g., $^{90}$Y, $^{131}$I, $^{177}$Lu) with suitable decay characteristics [19]. Based on the low LET (compared to LET of α–particles and Auger electrons), β–emitters produce slightly ionizing tracks with relatively low killing efficacy. However, due to the long penetration paths (100 to 500 cell diameters), β–emitting radionuclides create crossfire effect on nearby tumor cells [20]. This means cells which are not directly targeted with a radionuclide may still receive a dose from the crossfire irradiation from a neighboring targeted cell. Therefore, not every cell has to be targeted within the tumor, as long as the cells are within the range of the decaying atoms. This characteristic is favorable, when the therapy is applied for treatment of large metastases or tumor masses [21]. However, TRNT is usually applied when the patient had already failed several rounds of the standard primary treatments (surgery, chemotherapy, brachytherapy etc.) or the tumor relapsed after the initial therapy and the disease had progressed to the highly metastasized stage [22, 23]. In this case, targeting metastases, including micrometastases or circulating cancer cells, is of primary importance. Maximizing the absorbed radiation dose within
the metastatic volume may achieve an increased therapeutic efficacy of the applied TRNT. Therefore, \(\alpha\)-particle or Auger–electron therapy may be preferable due to the high LET of \(\alpha\)-particles and short-range electrons (Table 1.1), which result in highly localized energy deposition within the cell clusters (\(\alpha\)-emitters) or single cancer cells (Auger–electron emitters), while sparing surrounding normal tissues [24].

### 1.1.3 Characteristics of radiation possessing high LET

Two mechanisms of cell damage (direct or indirect) induced by particle radiation (both low LET and high LET) are believed to play the main role in provoking cell destruction or death (Figure 1.4). In the direct action, the radiation directly strikes deoxyribonucleic acid (DNA) and the damage is initiated with the breaking O-H, N-H and C-H bonds within a timeframe of \(10^{-14}\)–\(10^{-12}\) s [25]. The mechanism of the indirect cell damage is following: radiation hits the water (the major constituent of the cell), resulting in the production of reactive oxygen species (ROS) (hydroxyl radical (\(\cdot\)OH), ionized water (\(\text{H}_2\text{O}^+\)), superoxide (\(\text{O}_2\cdot^-\)) and peroxide (\(\text{H}_2\text{O}_2\)), as well as reactive nitrogen species (RNS) (nitric oxide (\(\cdot\)NO), nitrogen dioxide (\(\text{NO}_2\cdot\)), peroxynitrite anion (\(\text{ONOO}^-\)), which induce modifications of biomolecule and DNA damage.

![Diagram of direct and indirect damage](image)

**Figure 1.4.** Illustration of the direct and indirect (formation of the potent intracellular oxidants) DNA damage by ionizing radiation. Radiation can directly interact with cellular DNA and cause damage. The figure adapted from Hur et al., 2017 [26].
The direct action of ionizing radiation is the main mechanism of cell destruction for high LET radionuclides (α–particle and Auger–electron emitters), while in the case of low LET emitters (β–emitters) formation of the reactive species via indirect action is predominantly dictating the success of the treatment. This is an advantage of high LET radionuclides over β–emitters, as the presence of hypoxic regions (regions with poor oxygenation) within the tumor is believed to be a major cause of failure (low efficacy, local recurrences) of β–radionuclide therapy [16, 27, 28].

**α–particle emitters**

Over the years, the therapeutic potential of radionuclides emitting α–particles has been intensively studied. Alpha decay is a disintegration of the parent nuclei to the daughter, occurring via emission of an α–particle (Figure 1.5). The alpha particle is an energetic nuclei of Helium (\(^4\)He), which consists of two protons and two neutrons, bound together and possessing the double charge (+2). Therefore, these particles primarily interact with matter through Coulomb forces between the positive charge of the particle and the negative charge of the atomic electrons within the absorber. A single α–particle is barely deflected while passing through matter (straight travelling lines) due to its heavy mass (6.642\cdot10^{-4} \text{ g}) and can produce several thousand ion pairs per µm of air [29].

\[
\frac{AX}{Z} = \frac{A-4}{Z-2}Y + \frac{4}{2}\text{He}
\]

**Figure 1.5.** Illustration of the decay mode via emission of α–particles; the figure adapted from https://www.nuclear-power.net/nuclear-power/reactor-physics/atomic-nuclear-physics/radioactive-decay/alpha-decay-alpha-radioactivity/.

The typical energies of α–particles are in the range of 4 to 9 MeV, possessing tissue ranges of approximately five mammalian-cell diameters. Therefore, internalization of α–emitters within the cell nucleus is not required and highly ionizing
α-particles may cause irreversible DNA damage, independent on cell cycle positions and oxygen levels [16]. α-emitting radionuclides suitable for application in TRNT include $^{225}\text{Ac}$, $^{223}\text{Ra}$, $^{213}\text{Bi}$, $^{211}\text{At}$ and $^{149}\text{Tb}$ [30, 31].

**Auger-electron emitters**

The decay of the radionuclide via electron capture (EC) or internal conversion (IC) creates an electron vacancy (hole) in the atomic shell. During EC, an electron of the inner shell is drawn into the nucleus where it combines with a proton, forming a neutron and a neutrino (ejected from the atomic nucleus). The vacancy in the electron energy level is rapidly filled with an electron from a higher energy state, followed by the emission of the remaining energy as an X-ray or by the ejection of low-energy orbital electrons, called Auger electrons (Figure 1.6). The de-excitation process is repeated, so that the vacancies are moved towards the outermost shell, resulting in the generation of an Auger electron cascade (on average 5 – 30 electrons with energies of few eV to dozens of keV) [32]. The energy of the Auger electron will correspond to the differences in the energy levels of the electrons in the outer and inner shells less the binding energy of the electron [33].

During IC an unstable nucleus transmits its decay energy to an atomic electron (conversion electron), which is emitted from the atom with an energy corresponding to the nuclear decay energy minus the binding energy of the atomic electron. The formed vacancy is filled via the previously-described de-excitation process, resulting in the emission of X-rays or Auger electrons (Figure 1.6). Both IC electrons and Auger electrons have well-defined energy values, resulting in the discrete energy spectrum.

![Figure 1.6](image)

**Figure 1.6.** Atomic de-excitation after the decay of the nuclide by EC or IC. The figure adapted from IARC Monographs [34].
As mentioned above, Auger electrons possess short path lengths in the tissue and a very high LET, which limits cytotoxic effects to a sphere of a few nanometers in the immediate vicinity of the decay site [35]. Therefore, Auger-electron emitters are highly selective for killing single cancer cells once they are internalized in the cell nucleus. The cell death predominantly occurs due to the primary double-strand break (DSB) of DNA, triggering an apoptosis [36]. Recently, another mechanism of cell death, caused by high LET emitters, was discovered – a cytotoxic bystander effect produced by radiolabeled cells. Bystander effect is a biological response in the nonirradiated (non-targeted) cells, induced by signaling from targeted cells (e.g., local charge effects, cytokines communications), which may contribute to the additional therapeutic effect of the radionuclide [16]. A number of short-range-electron emitters are of high interest for application in TRNT; namely, $^{165}$Er, $^{161}$Tb, $^{125}$I, $^{119}$Sb and $^{58m}$Co [37-40].

1.2 Production of medical radionuclides for therapeutic purposes

1.2.1 Radionuclides produced by neutron irradiation

The primary source for the production of $\beta^-$-emitting radionuclides (neutron rich radionuclides) is a research nuclear reactor. A target nucleus absorbs a neutron and emits a discrete quantity of electromagnetic energy. The occurring (n, $\gamma$) nuclear reaction is called neutron capture or neutron radiative capture. Unlike charged particles, no Coulomb barrier hinders neutrons from reaching the target nucleus, leading to higher reaction probabilities (cross-sections) for neutrons, particularly at very low energies. For the production of artificial radionuclides, thermal neutrons are of primary importance (0.025 eV kinetic energy), as these neutrons easily penetrate nuclei, resulting in the production of high quantities of radioactivity [41].

The production of the radionuclide of interest by neutron capture may result in both carrier-added and no-carrier-added (free from any stable isotopes) product. The production of no-carrier-added radionuclide is preferable as it allows to achieve radiolabeling of biomolecules at high molar activities (measured radioactivity per mole of compound) and to avoid competition for binding sites on cancer cells between cold and radioactive isotopes [11]. In order to obtain no-carrier-added radionuclide, the neutron-induced reaction is obliged to follow fast $\beta^-$-decay, producing neutron-rich
radionuclides with atomic numbers different from that of the target element (Figure 1.7, a) [42]. Production of no-carrier-added $^{177}$Lu via the “indirect” route $^{176}$Yb(n,$\gamma$)$^{177}$Yb$\rightarrow$$^{177}$Lu could serve as an example: bombardment of the highly enriched $^{176}$Yb targets (>99 % enrichment) result in the production of $^{177}$Yb ($T_{1/2}$=1.9 h) as an intermediate product, which undergoes $\beta^-$-decay to $^{177}$Lu [43]. The example of carrier-added radionuclide, used for treatment of unrespectable liver tumors, is $^{166}$Ho in the form of poly-L-lactic acid (PLLA) microspheres QuiremSpheres®. $^{nat}$Ho-PLLA spheres are synthesized prior to irradiation and $[^{166}$Ho]$^{166}$Ho-PLLA microspheres, obtained after neutron activation, are injected into the liver via a microcatheter in the hepatic artery and serves as a liver implant [44]. $^{166}$Ho is produced via neutron activation of natural Ho via “direct” route, i.e. the $^{165}$Ho(n, $\gamma$)$^{166}$Ho nuclear reaction (Figure 1.7, b).

Figure 1.7. Schematic illustration of the: (a) indirect production route to obtain no-carrier-added radionuclide by neutron activation of the target material (e.g., production of no-carrier-added $^{177}$Lu via $^{176}$Yb(n,$\gamma$)$^{177}$Yb$\rightarrow$$^{177}$Lu nuclear reaction); (b) direct production route to obtain carrier-added radionuclide (e.g., $^{166}$Ho via $^{165}$Ho(n, $\gamma$)$^{166}$Ho nuclear reaction). The figure adapted from the Karlsruhe Chart of Nuclides [45].

Neutron-rich radionuclides for medical application are produced at research nuclear reactors – civil and commercial reactors, not used for power generation but providing a neutron source for research purposes. There are 226 operational nuclear reactors worldwide (December 2018) of which 72 are involved in radionuclide production [46]. In the present work, two research nuclear reactors have been used for the production of the therapeutic radionuclide $^{161}$Tb: South African Fundamental Atomic Research Installation 1 (SAFARI-1, Pelindaba, South Africa) and the high-flux
reactor of Institut Laue-Langevin (HFR ILL, Grenoble, France). These reactors provide thermal neutron fluxes of $2 \times 10^{14}$ n.cm$^{-2}$.s$^{-1}$ and $1 \times 10^{15}$ n.cm$^{-2}$.s$^{-1}$, respectively.

Nowadays, the interest is growing towards alternative non-reactor methods of neutron-rich radionuclide production, as the number of the reactors in the world is decreasing. Production using spallation-neutron sources could be applied as an alternative and in this work production of $^{161}$Tb was also performed at the Swiss Spallation Neutron Source (SINQ). Spallation is a nuclear reaction where high-energy particles (protons) fragment a heavy nucleus, removing one or more nucleons from it and producing lighter nuclei [47] (Figure 1.8). As a result of the collision of high-energy particles with the excited nuclei, high-energy neutrons and protons are emitted and the excited nuclei shed the energy excess by evaporating particles, predominantly neutrons. Particularly in SINQ, a beam of fast protons strikes the target (an array of lead rods, enclosed in zircalloy tubes), heating up the nucleus, followed by the ejection of 10 to 20 neutrons [48].

![Figure 1.8](https://www.psi.ch/en/media/the-sinq-neutron-source)

1.2.2 Radionuclides production at particle accelerators

Proton-rich radionuclides are essentially produced via charged particle reactions in accelerators (cyclotrons, linacs), utilizing protons ($^1$H$^+$ or p), deuterons ($^2$H$^+$ or d) and α-particles ($^4$He$^{2+}$ or α) as projectiles. In order to facilitate a nuclear reaction of interest in case of charged particles, the incoming particle must have sufficient energy to overcome two potential barriers: Coulomb barrier (electrostatic repulsion between the positively charged particles and the positively charged nucleus) and the mass...
difference energy [49]. The total probability that a compound nucleus will be formed and then decomposed along a particular channel is characterized by the nuclear reaction cross-section, which is often called the “excitation function” and is measured in barns (b), where $1 \text{ b} = 1 \cdot 10^{-24} \text{ cm}^2$. The excitation function determines the amount of a radionuclide, which can be obtained, along with the levels of contamination with other radioisotopes that can be present in the target material. An example for the production of no-carrier-added $^{165}$Er, using $^{165}$Ho(p,n)$^{165}$Er nuclear reaction is shown in Figure 1.9: in order to obtain maximum production yield of $^{165}$Er without the formation of $^{164}$Er as a side product, the proton energy on the target should be kept below 9 MeV, based on the theoretically calculated cross-sections [39].

![Figure 1.9. Excitation function of the $^{165}$Ho(p,n)$^{165}$Er nuclear reaction. The proton energy region for the production of carrier-free $^{165}$Er is outlined in red. The figure adapted from Sadeghi et al., 2010 [39].](image)

Different energies of the projectile will provide different excitation levels of the target nucleus. At low excitation energies (e.g., protons within 2–8 MeV energy range), the electromagnetic radiation ($\gamma$–rays) or a single nucleon (e.g., neutron) is emitted. At higher excitation energies, a number of individual nucleons may be released, e.g., several neutrons. Some proton-induced processes are illustrated in Figure 1.10 as (p,$\gamma$), (p,n) and (p,2n) nuclear reactions. The product, formed after the proton irradiation, represents another chemical element, allowing to obtain no-carrier-added radionuclide of interest after the chemical separation from the target material.
Figure 1.10. Schematic illustration of the proton-induced nuclear reactions (products outlined in red). The figure adapted from the Karlsruhe Chart of Nuclides [45].

In this work, the production of the Auger-electron emitter $^{165}$Er was performed at the PSI facility, which consists of three connected accelerators, generating and hastening protons to achieve different beam energies (Figure 1.11). The first linear accelerator (a Cockroft-Walton accelerator) generates the beam, pre-accelerates protons to 0.87 MeV and directs the beam to Injector 2 – a separated sector cyclotron. Injector 2 accelerates the received beam to 38% of the speed of light, providing 72 MeV protons, ~2% of which is used for the production of medical radionuclides. The rest of the beam is transported for further acceleration to the large 590 MeV “ring” cyclotron, where protons are accelerated to 80% of the speed of light. Part of this proton beam is forwarded to physics muon experimental vaults, while the remainder is collected in a Pb-zircalloy beam dump used as a target for SINQ – continuous spallation source, which was discussed in the previous section.
Exotic (far from stability) nuclei could be also produced at ISOL (isotope separation on-line) facilities, e.g., ISOLDE facility (CERN, Switzerland), which was used in the current work to obtain $^{149}$Tb. In this production route, the radioactive ion beams are delivered based on the high-energy proton-induced spallation of Tantalum (Ta) targets. The simple illustration of the production process is shown in Figure 1.12. The primary proton beam (1.4 GeV) heats the Ta target, causing the spallation of Ta foils inside the target container. The only orifice of the container is connected via a line transfer to the ion source, from which ions are injected into the acceleration stage of the electromagnetic isotope separator (EMIS). Charged particles of different masses (same charge and kinetic energy) will have different trajectories when passing through a uniform magnetic field, with the heavier ions possessing larger diameter. In the end of the on-line isotope mass separation the isotopically pure beams (isobars are normally present) are collected in the collector stations [51, 52].
Figure 1.12. Schematic illustration of the on-line isotope separator at ISOLDE facility. The figure adapted from https://op-webtools.web.cern.ch/beamdoc/PSB/NORMGPS-HRS/psb_NORMGPS-HRS.php

1.3 Radiolanthanides for Targeted Radionuclide Therapy

The application of the radiolanthanides for TRNT was developed over the last decade and proved to be reasonable and successful by entering the market as part of a number of radiopharmaceuticals. Lanthanides have similar chemical properties and coordination chemistry, which implies identical conditions for radiolabeling. Therefore, clinically-applied radiolanthanides for TRNT (e.g., $^{153}$Sm, $^{166}$Ho, $^{177}$Lu etc.) could be easily replaced with novel radiolanthanides without changing the bifunctional chelator and the vehicle molecule. The radiolanthanides, which are currently in use in clinics or could be potentially used for TRNT are presented in Table 1.2.
Table 1.2. Radiolanthanides relevant for application in TRNT. The table is adapted from Uusijärvi et al., 2006 [53] and Rösch, 2007 [54].

<table>
<thead>
<tr>
<th>Radiolanthanide</th>
<th>Decay mode (mean E of the emitted particle)*</th>
<th>Half-life</th>
<th>Commercial name of the product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{140}$La</td>
<td>$\beta^-$ (525 keV)</td>
<td>1.7 d</td>
<td>–</td>
</tr>
<tr>
<td>$^{143}$Pr</td>
<td>$\beta^-$ (315 keV)</td>
<td>13.6 d</td>
<td>–</td>
</tr>
<tr>
<td>$^{146}$Tb</td>
<td>$\alpha$ (3.98 MeV)</td>
<td>4.1 h</td>
<td>–</td>
</tr>
<tr>
<td>$^{153}$Sm</td>
<td>$\beta^-$ (225 keV)</td>
<td>1.9 d</td>
<td>[${^{153}}$Sm]EDTMP (Quadramet®)</td>
</tr>
<tr>
<td>$^{161}$Tb</td>
<td>$\beta^-$ (154 keV)</td>
<td>6.9 d</td>
<td>–</td>
</tr>
<tr>
<td>$^{165}$Er</td>
<td>EC (6.6 keV)</td>
<td>10.4 h</td>
<td>–</td>
</tr>
<tr>
<td>$^{166}$Ho</td>
<td>$\beta^-$ (711 keV)</td>
<td>1.1 d</td>
<td>[${^{166}}$Ho]Ho-PLLA spheres (QuiremSpheres®)</td>
</tr>
<tr>
<td>$^{169}$Er</td>
<td>$\beta^-$ (100 keV)</td>
<td>9.4 d</td>
<td>–</td>
</tr>
<tr>
<td>$^{177}$Lu</td>
<td>$\beta^-$ (134 keV)</td>
<td>6.7 d</td>
<td>[${^{177}}$Lu]Lu-DOTA-TATE (Lutathera®)</td>
</tr>
</tbody>
</table>

* Only emissions, valuable for therapeutic application, are mentioned.

The most common bifunctional chelator (“gold standard”), used for the complexation of radiolanthanides for pharmaceutical purposes, is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (Figure 1.13) [55]. Lanthanides showed two coordination numbers (9 and 8) in the solution, depending on the presence of a water molecule within the coordination sphere of the Ln-DOTA complex. The coordination number decreases from 9 to 8 when the water molecule is expelled from the inner sphere, in a process written as: [Ln-DOTA(H$_2$O)]$^-$ $\rightleftharpoons$ [Ln-DOTA)]$^- + $H$_2$O [56].

![Figure 1.13. Structure of Ln-DOTA complexes (Ln corresponds to lanthanide). The figure adapted from Fusaro et al., 2014 [57].](image-url)
Thermodynamic stability constants of DOTA with rare earth elements (REE) reach high values, leading to the formation of stable (e.g., log $K$ (Tb-DOTA) = 24.2 [58]) and remarkably rigid complexes under usual biological conditions. For example, Y dissociation from a DOTA-type ligand was <0.5% over 18 days in serum (pH 7.4, 37°C) [59]. The thermodynamic stability of the Ln-DOTA complexes decreases under acidic conditions (pH<3.5), resulting in the formation of the protonated [Ln-H₂DOTA]^+, [Ln-HDOTA]^# as well as deprotonated forms [Ln-DOTA]^- (ML]^# represents intermediate species in which Ln³⁺ is incompletely coordinated). Moreover, at pH>6 lanthanides form insoluble hydroxides, that applies the use of the pH range in between 4 and 6 for the quantitative complexation of Ln-DOTA as [Ln-DOTA]⁻ [57].

1.4 Chromatographic methods for the purification of radiolanthanides

In order to facilitate preclinical studies, the radiolanthanide should possess high purity (chemical, radionuclidic, radiochemical) and be formulated in a small volume of diluted acid. Therefore, after the production, the complete separation of the radionuclide of interest (nano or micro amounts) from the target material (macro amounts) and possible co-produced impurities is required. The purification (chemical separation) is a vital part of radionuclide processing, which dictates the further possible implementation of the radionuclide to preclinical and clinical research.

The discovery of chromatography belongs to the Russian botanist M. S. Tswett, who illustrated in 1903 the separation of various leave pigments on different adsorbents (soils, silicates and inorganic substances (ion-exchangers)) [60, 61]. The success of Tswett immediately found a reflection in the synthesis and use of the novel ion-exchange sorbents, giving a rise to a new field of chromatography, later called ion-exchange chromatography [62]. The use of ion-exchange chromatography for lanthanide separation attracted researchers who were working on the Manhattan Project (1939-1947) during and immediately following World War II. The goal of the Manhattan Project was to develop and to build the first atomic bomb, based on $^{235}$U or $^{239}$Pu fuel. These type of bombs were detonated over the Japanese cities of Hiroshima and Nagasaki in August 1945. The fission of $^{235}$U or $^{239}$Pu results in the production of over 100 radioisotopes, including many “unknown” elements of that time – lanthanides (e.g., $^{147}$Nd, $^{141}$Ce, $^{155}$Eu etc.) [63]. In order to understand the physics and chemistry of
each lanthanide, as well as the possible effect of the fission products on humans, the chemical separation of elements was necessary. The efficient separation of neighboring lanthanides is a difficult task, due to their similar physical and chemical properties (e.g., ionic radii, equal ionic charge, and coordination chemistry) and required massive research by testing different experimental methods and conditions. It was determined that cation-exchange chromatography served as the most effective way to separate elements in microquantities, being straightforward, reliable and simple in operation. As a result, different resins, eluents and experimental conditions were tested in order to find the most suitable method for lanthanide separation (Table 1.4).

Table 1.4. Experimental conditions for the first successful separations of lanthanides, using cation exchange chromatography.

<table>
<thead>
<tr>
<th>Element</th>
<th>Eluent</th>
<th>Cation-exchange resin</th>
<th>Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nd, Pr</td>
<td>Citric Acid</td>
<td>Amberlite IR-1</td>
<td>[64]</td>
</tr>
<tr>
<td>La, Ce, Pr, Nd</td>
<td>Citric Acid</td>
<td>Dowex 30</td>
<td>[65]</td>
</tr>
<tr>
<td>Nd, Tm, Er, Ho</td>
<td>Citric Acid</td>
<td>Dowex 50, Amberlite IR-1</td>
<td>[66]</td>
</tr>
<tr>
<td>Tb, Eu, Sm</td>
<td>Citric Acid</td>
<td>Dowex 50</td>
<td>[67]</td>
</tr>
<tr>
<td>Tb, Dy</td>
<td>Lactic Acid, 87°C</td>
<td>Dowex 50</td>
<td>[68]</td>
</tr>
<tr>
<td>Lu, Tm, Er</td>
<td>Glycolic Acid</td>
<td>Dowex 50</td>
<td>[69]</td>
</tr>
<tr>
<td>Sm, Eu, Tb</td>
<td>α-HIBA</td>
<td>Dowex 50</td>
<td>[70]</td>
</tr>
</tbody>
</table>

Cation-exchange chromatography is an analytical technique used to exchange ions of the stationary phase (insoluble substances containing loosely held ions, e.g., resins with fixed functional groups) with the dissolved ions from a solution (ionic solutes). The ion-exchange process consists of three main steps – sorption, elution and regeneration [71]. During the first step (sorption), the targeted ions are passed through the column and bound onto the resin; the primary ions of the resin are released (exchanged) (Figure 1.14). During the second step (elution), the eluent replaces and releases the target ions from the resin into the solution phase (e.g., by interaction of the target ion with the complexing agent). The goal of the last step (regeneration) is to remove the residual ions after the sorption and elution in order to regenerate the exchanger to the initial state (normally by applying strong acids).
As can be seen from Table 1.4, mainly elution by complexing agents is applied for lanthanide separation using ion exchange methods. In this process, the elution order of individual elements depends on the stability constants of formed complexes. The stability constant values generally increase from light lanthanides (La-Sm) to heavy lanthanides (Eu-Lu) due to the decrease of ionic radii as a result of the lanthanide contraction (Table 1.5) [72]. The difference in stability constants between lanthanides and complexing agent forms the genesis of separation: the higher the differences in the stability constants of the lanthanide complexes, the better separation can be achieved [73, 74]. The highest separation factors for neighboring lanthanides at room temperature were observed with the application of α-hydroxy-isobutyric acid (α-HIBA) as an eluent [70], which remains the most popular eluent for lanthanide separation to date.

**Figure 1.14.** Sorption and elution steps using cation-exchange chromatography. [M⁻] corresponds to the functional group fixed on the resin; [A⁺] and [D⁺] corresponds to target ions and the primary ions of the resin, respectively.
Table 1.5. Overall stability constants (log β), separation factors of the lanthanides mentioned in this work, with the use of α-HIBA as eluent and ionic radii of Ln(III) in aqueous solution. The table is adapted from Nikonorov, 2010 [74], Raut et al., 2002 [75] and D’Angelo et al., 2011 [76].

<table>
<thead>
<tr>
<th>Lanthanide</th>
<th>Ionic radii, Å</th>
<th>log β</th>
<th>Separation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gadolinium (Gd)</td>
<td>1.11</td>
<td>7.19</td>
<td>2.66 (Gd-Tb)</td>
</tr>
<tr>
<td>Terbium (Tb)</td>
<td>1.09</td>
<td>7.43</td>
<td></td>
</tr>
<tr>
<td>Holmium (Ho)</td>
<td>1.06</td>
<td>7.96</td>
<td>2.08 (Ho-Er)</td>
</tr>
<tr>
<td>Erbium (Er)</td>
<td>1.04</td>
<td>8.13</td>
<td></td>
</tr>
<tr>
<td>Thulium (Tm)</td>
<td>1.03</td>
<td>8.38</td>
<td>1.87 (Er-Tm)</td>
</tr>
</tbody>
</table>

Only one study on the coordination chemistry of lanthanide (III) complexes with α-HIBA was found in literature [77]. The coordination mode for Ln-HIBA (inner coordination sphere) revealed to be identical for the lanthanide series: eight-coordinated lanthanide (III) centers coordinated with six oxygen atoms from four HIBA ligands and two oxygen atoms from two bound water molecules (Figure 1.15). However, the length of the Ln-O bonds within the Ln-HIBA structure decreased across the lanthanide series as a result of the increasing charge density of the ions [78]. In general, this results in the increase of the complexation constants, making lanthanide separation possible.

Figure 1.15. Possible structure of [Er(HIBA)_2(H_2O)_2](NO_3)_2·H_2O with the disordered nitrate showed in black. Hydrogen atoms were removed for certainty. The figure adapted from Chen et al., 2012 [77].
1.5 Aim of the thesis

The thesis aimed to develop novel therapeutic radionuclides in order to make primary efforts to increase the efficacy of the existing TRNTs. In the future, this may lead to the establishment of an effective treatment for cancer patients who have no alternatives in the current standard means of therapy. In the current work, we focused on the development of production of therapeutic radiolanthanides, which are not currently in routine use in clinics, but could have an advantage over the most common clinically applied $\beta^-$-emitters. The higher therapeutic efficacy could be obtained by the application of radionuclides emitting particle radiation of high LET (Auger and conversion electrons, $\alpha$–particles) with short penetration paths. Therefore, it was aimed to reproducibly produce $\beta^-$ and conversion and Auger–electron emitter $^{161}$Tb, $\alpha$–emitter $^{149}$Tb and pure Auger–electron emitter $^{165}$Er of high purity (quality), suitable for preclinical research.

To achieve the aim of the thesis, radionuclides of interest should be progressively developed based on the steps outlined in Figure 1.16. At the beginning of the development work, an appropriate production route has to be chosen in order to obtain amounts of the radionuclide, suitable for performing preclinical research and, ideally, clinical studies. A target material should be chosen in a way to provide safety during the bombardment and different irradiation conditions (beam energy, beam current, irradiation time) should be tested to obtain the maximum production yield of the radionuclide of interest. Microamounts (or nanoamounts) of the radionuclide are produced from macroamounts of the target material. Therefore, an efficient and reproducible method to separate the radionuclide from a target material needs to be investigated. The developed purification method should provide radionuclidically, radiochemically and chemically pure product, suitable for high-specific radiolabeling, in order to facilitate preclinical studies. The quality of the product is examined by the following methods – $\gamma$–ray spectrometry, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), or radio TLC.
Terbium-161

The goal of the project was to obtain pure $\beta^-$, conversion and Auger–electron emitter $^{161}$Tb, which was proposed to be a better alternative to the clinically applied $^{177}$Lu – regarded as the “gold standard” in TRNT. To compete with commercially available no-carrier-added $^{177}$Lu, $^{161}$Tb should possess similar physical characteristics (purity, activity concentration, etc.) and one should be able to provide high activities to satisfy the needs of the hospitals. Before the start of the project, $^{161}$Tb was obtained from the bombardment of enriched $^{160}$Gd$\text{Gd(NO}_3\text{)}_3$ targets, followed by purification, as reported by Lehenberger et al. [79]. However, lanthanide nitrates are hygroscopic materials and the heating of the ampoule in the nuclear reactor can create water vapor within the ampoule, which cause overpressure and ampoule breakage. Therefore, long-term irradiation and regular production of high activities of $^{161}$Tb with the chosen target material is questionable. In order to fulfill the project goal, a target material was selected in a way to provide stability for lengthy irradiations (up to three weeks) at the nuclear reactors, as well as high production yields. After the bombardment, the development work to establish purification (chemical separation) of $^{161}$Tb has been done in order to obtain pure $^{161}$Tb product of specifications similar to $^{177}$Lu. $^{161}$Tb purification method was developed and proved to be reproducible and reliable; that was not the case for the method, developed by Lehenberger.

Erbium-165

The goal of the project was to obtain activity of pure Auger–electron emitter $^{165}$Er, which was proposed as a candidate for Auger–electron therapy, enough to perform preclinical research. Several production routes for $^{165}$Er were to be considered, developed and tested at both research and medical cyclotrons to determine the most
reasonable way of $^{165}$Er production as, so far, experimental research to obtain $^{165}$Er remains exceptionally poor. The corresponding purification method was to be established for each chosen production route, followed by the quality control of the obtained product.

**Terbium-149**

Production of $\alpha$–emitter $^{149}$Tb, as well as the purification method was established previously. However, further research was required to decrease the activity loss when trying to extract $^{149}$Tb, implemented into the commonly-used catcher material – Zn coated gold foils. In addition, the formulation of $^{149}$Tb in low concentrated HCl would be advantageous for preclinical use as the radiolabeling technique, in this case, is well established. Therefore, an attempt to change the material for $^{149}$Tb implementation after the radionuclide production was made. After changing the material, the purification method should be adjusted accordingly. Therefore, we performed development work to establish a reproducible purification method as well as proposed another alternative to decrease the time of the process.
Chapter 2

Medically interesting radiolanthanide $^{161}$Tb – from production towards clinical application

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Author contributions:

Nadezda Gracheva developed the production and separation process, prepared ampoules for the irradiation, performed the separation experiments of $^{161}$Tb, did the characterization of the final product, performed radiolabeling and the stability experiments, analyzed the data and drafted the manuscript. Stephan Heinitz supported the development of the separation process on bench, helped with the recycling of the irradiated targets and characterization of the recycled material. Cristina Müller supported with the radiolabeling experiments. Ulli Köster and Jan Rijn Zeevaart were responsible for the irradiation of $^{150}$Gd targets at ILL and Necsa, respectively. Alexander Vögele was responsible for the irradiation of $^{150}$Gd targets at PSI, as well as the logistics of irradiated ampoules. Roger Schibli reviewed the manuscript. Nicholas van der Meulen supervised the study, organized irradiations and reviewed and revised the manuscript.
2.1 Introduction

The use of the $\beta^-$-emitter $^{177}$Lu ($E_{\beta-av}$=134 keV (100%), $T_{1/2}$=6.7 d) [80], in combination with somatostatin analogues (e.g., DOTA-TOC, DOTA-TATE), is considered a promising tool for the treatment of neuroendocrine tumors (NET). It has been extensively utilized in clinics, which recently resulted in the approval of Lutathera® ($^{[177]Lu}$Lu-DOTA-TATE), by the U.S. Food and Drug Administration (FDA) [9, 81, 82]. Treatment with $^{[177]Lu}$Lu-DOTA-TATE resulted in longer progression-free survival time (65.2% at Month 20, compared to 10.8% in the control group); nevertheless, partial remission remained at $\leq$50% in the assessable patients, with the complete response being $\leq$12% [9, 83-86]. The radiolanthanide $^{161}$Tb shows similar decay characteristics ($E_{\beta-av}$=154 keV (100%), $T_{1/2}$=7.0 d [80, 87]) and coordination chemistry to $^{177}$Lu. $^{161}$Tb can, therefore, be stably coordinated with a DOTA chelator and be used in combination with a number of small molecules, peptides, and antibodies currently employed with $^{177}$Lu. $^{161}$Tb may show an increased therapeutic efficacy over $^{177}$Lu, due to the co-emission of a substantially larger number of conversion and Auger electrons at a favorable energy range ($\sim$12 e$^-$, $\sim$36 keV per decay for $^{161}$Tb and $\sim$1 e$^-$, $\sim$1.0 keV per decay for $^{177}$Lu, respectively) [40, 88]. The possibility of using $^{161}$Tb as an alternative to $^{177}$Lu was first proposed by Lehenberger et al. and, subsequently, corroborated by Müller et al. by comparison of in vitro and in vivo studies using a DOTA-folate conjugate labeled with $^{161}$Tb and $^{177}$Lu [40]. The enhanced anti-tumor effect, as well as higher average survival time, was found in mice treated with $^{[161]Tb}$Tb-folate over those which received $^{[177]Lu}$Lu-folate. In a preliminary therapy study using $^{[161]Tb}$Tb-PSMA-617, PSMA-positive PC-3 PIP tumor-bearing mice demonstrated significant tumor-growth delay, as compared to the control group, without causing early side effects [89]. Better therapeutic efficacy was also observed for a $^{161}$Tb-labeled radioimmunoconjugate in an ovarian cancer model when compared to the $^{177}$Lu-radioimmunoconjugate counterpart [90]. The low-energy conversion and Auger–electron emission from $^{161}$Tb contribute 25.9–88.3% to the total absorbed dose (compared to 10.0–33.9% for $^{177}$Lu), depending on the tumor size, which could be associated to its enhanced therapeutic efficacy over that of $^{177}$Lu [91]. The doses delivered by $^{161}$Tb or $^{177}$Lu to 10 mm-diameter spheres were calculated to be comparable for both radionuclides, however, for 100 µm-diameter and 10 µm-diameter spheres $^{161}$Tb could deliver 1.8 and 3.6 times higher dose than $^{177}$Lu, respectively,
making $^{161}\text{Tb}$ the more appropriate candidate for treating micrometastases [92]. Also, the co-emission of 48.9 keV and 74.6 keV $^{161}\text{Tb}$ $\gamma$-rays allows for the acquisition of single photon emission computed tomography (SPECT) images for dosimetry determination before administration of the therapeutic dose, comparable to that performed with $^{177}\text{Lu}$ [40, 90, 93]. In addition, $^{161}\text{Tb}$ could be used in combination with diagnostic radioisotopes, namely, $^{152}\text{Tb}$ (PET) or $^{155}\text{Tb}$ (SPECT) as a matched pair towards the concept of theragnostics [94, 95].

The $^{161}\text{Tb}$ production route was proposed by Lehenberger et al. via the $^{160}\text{Gd}(n,\gamma)^{161}\text{Gd} \rightarrow ^{161}\text{Tb}$ nuclear reaction, which provided no-carrier-added radiolanthanide at high specific activities (~4 TBq/mg) [79]. Enriched $[^{160}\text{Gd}]\text{Gd(NO}_3\text{)_3}$ targets (ampoules) were prepared by dissolving $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ in nitric acid and evaporating to dryness. Lanthanide nitrates are hygroscopic materials and the heating of the ampoule in the nuclear reactor can create water vapor within the ampoule. The vapor could create overpressure, resulting in ampoule breakage and radioactivity release in the reactor. The $^{161}\text{Tb}$ separation method from $[^{160}\text{Gd}]\text{Gd(NO}_3\text{)_3}$ targets was previously developed at Paul Scherrer Institut (Villigen-PSI, Switzerland), but the radiolabeling capability of the $^{161}\text{Tb}$ product was three times lower than the commercial no-carrier-added $^{177}\text{Lu}$ [79]. This implied that the $^{161}\text{Tb}$ product contained undesired environmental impurities at the end of separation (EOS), thereby, compromising the capability of reproducible routine production.

Herein, we report on the large-scale $^{161}\text{Tb}$ production from $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ target material, suitable for introduction into a process in accordance with Good Manufacturing Practice (GMP) and, thereafter, clinical application. The $^{161}\text{Tb}$ purification method was improved by optimization of the Tb/Gd separation process, followed by characterization of the final product ($[^{161}\text{Tb}]\text{TbCl}_3$). The $^{161}\text{Tb}$ purity was compared with no-carrier-added $^{177}\text{Lu}$ (EndolucinBeta), as currently produced by ITG GmbH, Germany, for worldwide clinical application.

### 2.2 Material and methods

#### 2.2.1 Target preparation for the production of $^{161}\text{Tb}$

Gadolinium oxide ($[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$, 98.2% enrichment, Isoflex, USA) was used as target material for the production of no-carrier-added $^{161}\text{Tb}$, as previously reported [79]. To prepare the targets for irradiation at the spallation-induced neutron source
(SINQ, Paul Scherrer Institut, $4.10^{13}$ n.cm$^{-2}$.s$^{-1}$), 80–95 mg $[^{160}\text{Gd}]\text{Gd(NO}_3\text{)}_3$ were placed in a quartz glass ampoule (Suprasil, Heraeus, Germany) and sealed. Ampoules containing 7–33 mg $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ were prepared in a similar manner and sent for irradiation to two research nuclear reactors (SAFARI-1, South African Nuclear Energy Corporation, $2.10^{14}$ n.cm$^{-2}$.s$^{-1}$; and RHF ILL, Institut Laue–Langevin, $1.10^{15}$ n.cm$^{-2}$.s$^{-1}$). The mass of the target material, required for the irradiation at the chosen facility, was calculated using the ChainSolver code [96].

### 2.2.2 Determination of the neutron fluxes of the irradiation facilities with $^{59}\text{Co}$ monitors

In order to monitor neutron fluxes at ILL, SAFARI-1 and SINQ, quartz ampoules containing $^{59}\text{Co}$ as standard ($^{59}\text{Co}$ in 2% w/w HNO$_3$, Sigma-Aldrich, USA) with 33 ng–2 µg $^{59}\text{Co}$ (mass determined based on the volume of the standard solution pipetted) were prepared. Ampoules were dried at 80°C, to ensure water evaporation, and sealed. Ampoules with $^{59}\text{Co}$ standard were placed and sealed in the same Al container as the ampoules containing $^{160}\text{Gd}$ target material, along with empty ampoules (used as references) for the irradiation process. $^{59}\text{Co}$ masses were calculated to produce 50–100 kBq $^{60}\text{Co}$ activity via $^{59}\text{Co}(n,\gamma)^{60}\text{Co}$ nuclear reaction, depending on the reactor neutron flux. The $^{60}\text{Co}$ activities in the ampoules were measured after irradiations using a high-purity germanium (HPGe) detector (Canberra, France), in combination with the InterWinner software package (version 7.1, Itech Instruments, France), until 3σ uncertainty was below 5%. The total activities of $^{60}\text{Co}$ in the ampoules, after irradiation at the facility in question, were calculated by subtraction $^{60}\text{Co}$ activities of the reference (empty) ampoules from the $^{60}\text{Co}$ activities of the ampoules containing $^{59}\text{Co}$ standard. Based on these $^{60}\text{Co}$ activity values, the average neutron flux ($\phi_{th}$) of each irradiation was calculated using the following equation:

$$\phi_{th} = \frac{A_0}{\sigma_n N \left(1 - e^{-\lambda t_B}\right)} \quad (2.1)$$

where $A_0$ is the $^{60}\text{Co}$ activity at the end of bombardment, $\sigma_n$ is neutron capture cross section (37.18±0.06 barns [97]), $N$ is number of target atoms, $\lambda$ is the radioactive decay constant and $t_B$ is the irradiation time.
2.2.3 Development of the procedure for $^{161}$Tb purification process on bench

A chromatographic column (10 mm x 170 mm) was prepared using Sykam macroporous cation exchange resin (Sykam Chromatographie Vertriebs GmbH, Germany; particle size 12–22 µm, NH$_4^+$ form). The parameters to separate Tb from the Gd target material and side products were first optimized by means of bench experiments with the use of radioactive tracers. Long-lived radioactive tracers ($^{22}$Na, $^{65}$Zn, $^{152}$Eu, $^{153}$Gd, $^{160}$Tb, $^{192}$Ir) were provided by the Laboratory of Radiochemistry (PSI) (Table 2.1). $^{59}$Fe and $^{51}$Cr were obtained by neutron activation of the OPTIFERV material (Saarschmiede GmbH, Germany) at SINQ (PSI). The required mass of the steel (383 mg) was placed inside the plastic capsule for irradiation and bombarded for 1 h at the SINQ spallation source (NAA position). After the irradiation, the steel was dissolved in 2.0 mL 37% hydrochloric acid (HCl, Normapur, VWR Chemicals, USA) and evaporated until dryness at 80°C under gas flow. $^{65}$Ni tracer was obtained by 1 h neutron irradiation of 12 mg Nickel (II) nitrate hexahedrate (N$_2$NiO$_6$·6H$_2$O, Sigma-Aldrich, USA) at NAA position (SINQ), followed by the same dissolving procedure as for $^{59}$Fe and $^{51}$Cr.

Table 2.1. Long-lived radioactive tracers used for bench experiments.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>$^{22}$Na</th>
<th>$^{51}$Cr</th>
<th>$^{59}$Fe</th>
<th>$^{65}$Ni</th>
<th>$^{65}$Zn</th>
<th>$^{152}$Eu</th>
<th>$^{153}$Gd</th>
<th>$^{160}$Tb</th>
<th>$^{169}$Yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$</td>
<td>2.6 y</td>
<td>27.7 d</td>
<td>44.5 d</td>
<td>2.5 h</td>
<td>244.3 d</td>
<td>13.5 y</td>
<td>239.5 d</td>
<td>72.3 d</td>
<td>32.0 d</td>
</tr>
</tbody>
</table>

The radioactive tracers were mixed with 2.0 mL 0.1 M ammonium nitrate (NH$_4$NO$_3$, prepared from 25% Suprapur NH$_3$ and 65% Suprapur HNO$_3$, Merck, Germany) and loaded onto the prepared Sykam resin column by means of Ismatec peristaltic pump (Cole-Parmer Instrument Company LLC, USA). Subsequently, 0.13 M (pH 4.5) $\alpha$-hydroxyisobutyric acid ($\alpha$-HIBA, Sigma-Aldrich GmbH, Germany) was passed through the column until Tb was eluted. The concentration of $\alpha$-HIBA was then increased to 0.175 M and 1.0 M, respectively, in order to elute the impurities in question. Samples (6–12 mL fractions) of $\alpha$-HIBA were collected and measured using a high-purity germanium (HPGe) detector (Canberra, France) in combination with the InterWinner software package (version 7.1, Itech Instruments, France) until 3σ uncertainty was below 10%. In several experiments, $^{153}$Gd tracer was mixed with 8–140 mg natGd$_2$O$_3$ (Research chemicals, Division of Rhone-Polenc Inc., USA).
The Tb-containing fractions, eluted from the Sykam resin, were loaded onto the bis(2,4,4-trimethyl-1-pentyl)phosphinic acid extraction resin (LN3, Triskem International, France) column (6 mm x 5 mm) in order to concentrate Tb. After loading, the resin was rinsed with 5 mL MilliQ water, followed by the final elution of Tb in 0.05 M HCl. The investigated purification method was applied towards the separation of an aliquot of reactor-produced $^{161}$Tb (230 MBq).

**2.2.4 The purification procedure for the processing of high activities of $^{161}$Tb in the hot cell**

Bench experiments resulted in the design and construction of a chemical separation module, such that high activities (GBq) of the radionuclide can be processed in the hot cell. The quartz glass ampoule with the $[^{156}\text{Gd}]\text{Gd}_2\text{O}_3$ target material, delivered from the irradiation facility, was placed in a plastic target tube, crushed and attached to the module inside the hot cell with the aid of manipulators. The target material from the ampoule was dissolved in 2.0 mL 7.0 M nitric acid (HNO$_3$, Suprapur, Merck, Germany), followed by evaporation at 80°C under nitrogen flow. The residue was taken up in 0.1 M NH$_4$NO$_3$ and loaded onto the cation exchange resin column. The $^{161}$Tb separation from the target material and impurities was performed with the use of 0.13 M (pH 4.5) $\alpha$-HIBA as eluent. Concentration of $^{161}$Tb was performed using LN3 extraction resin column (6 mm x 5 mm), followed by the elution of the final product ($[^{161}\text{Tb}]\text{TbCl}_3$) in 500 µL 0.05 M HCl. The pH of the final product was determined using pH indicator strips (Merck, Germany).

**2.2.5 Characterization of the $^{161}$Tb product after purification**

**Radionuclidic purity:** The identification and radionuclidic purity of the $^{161}$Tb were examined by $\gamma$–ray spectrometry using the HPGe detector mentioned above. The aliquot of the final product, containing 5–10 MBq of $^{161}$Tb, was measured on HPGe until the 3σ uncertainty was below 5%.

**Radiochemical purity:** The radiochemical purity of the final product was determined by means of radio thin layer chromatography (radio TLC) using a procedure established for $^{177}$Lu [98]. The aliquot of $[^{161}\text{Tb}]\text{TbCl}_3$ (2 µL, ~100 kBq) was deposited on TLC silica gel 60 F$_{254}$ plates (Merck, Italy) and placed in the chamber with 0.1 M sodium citrate (pH 5.5; Merck, Germany) mobile phase. After elution, the plate was
dried and analyzed using a radio TLC scanner instrument (Raytest Isotopenmessgeräte GmbH, Germany). The results were interpreted with the MiniGita Control software package (version 1.14, Raytest Isotopenmessgeräte GmbH, Germany).

**Radiolabeling yield:** Radiolabeling of DOTA-NOC (ABX GmbH, Germany) at a molar activity of 180 MBq/nmol (1-to-4 nuclide-to-peptide molar ratio) was performed in order to evaluate the success of the purification process. Sodium acetate (Alfa Aesar, Germany; 0.5 M, pH 8) was added to $[^{161}\text{Tb}]{\text{TbCl}_3}$ solution (~200 MBq) to adjust pH to ~4.5. The relevant quantity of DOTA-NOC was subsequently added from a 1mM stock solution. The reaction solution was incubated for 10 min at 95°C. The radiolabeling yield was determined by reverse-phase high performance liquid chromatography (HPLC, Merck Hitachi LaChrom) with a radioactivity detector (LB 506, Berthold, Germany) and a C-18 reverse-phase column (150 mm x 4.6 mm; Xterra™ MS, C18; Waters). Trifluoroacetic acid 0.1% (Sigma-Aldrich, USA) in MilliQ water (A) and acetonitrile (VWR Chemicals, USA; HPLC grade) (B) were used as mobile phase with a linear gradient of solvent A (95–5% over 15 min) in solvent B at a flow rate of 1 mL/min. The sample for the analysis was prepared by diluting ~0.3 MBq aliquot of the radiolabeling solution in 100 µL MilliQ water containing sodium diethylenetriamine pentaacetic acid (Na-DTPA, 50 µM). The radiolabeling yield of $[^{161}\text{Tb}]{\text{Tb}}$-DOTA-NOC was determined by integration of the product peak from the obtained HPLC chromatogram in relation to the sum of all radioactive peaks (the radiolabeled product, potentially released $^{161}\text{Tb}$ as well as degradation products of unknown structure), which were set to 100%.

### 2.2.6 Determination of the radiolabeling yield of $[^{161}\text{Tb}]{\text{Tb}}$- and $[^{177}\text{Lu}]{\text{Lu}}$-DOTA over a two-week period

In order to assess the change of the molar activity of $[^{161}\text{Tb}]{\text{Tb}}$-DOTA at different DOTA-to-nuclide molar ratios over time (2 weeks after EOS) and to compare it with the molar activity of $[^{177}\text{Lu}]{\text{Lu}}$-DOTA, thin layer chromatography (TLC) analysis was performed. The required DOTA solutions (1–500 pmol DOTA) were obtained by dilution of the initial 1 mM DOTA solution (CheMatech, France) with 0.5 M sodium acetate (pH 4.5). The prepared DOTA dilutions were mixed with 2.5–4.0 MBq $^{161}\text{Tb}$ (corresponding to 3.1–5 pmol), $^{177}\text{Lu}$ (no-carrier-added, ITG GmbH, Germany) or $^{177}\text{Lu}$ (carrier-added, IDB Holland bv, the Netherlands) at different
DOTA-to-nuclide molar ratios (160:1 to 1:1). The reaction solutions were incubated for 20 min at 95°C and 2 µL of each solution were deposited on TLC silica gel 60 F254 plates, which served as a stationary phase. The mixture of 10% ammonium acetate (Sigma-Aldrich, USA) and methanol (Merck, Germany) was used as mobile phase (ratio 1:1 (v/v), pH 5.5). After elution, the phosphor screen (Multisensitive, Perkin Elmer Inc., USA) was illuminated with the TLC plate and analyzed with a Cyclon Phosphor Imager (Perkin Elmer Inc., USA). The peaks corresponding to the unlabeled $^{161}\text{Tb}$ or $^{177}\text{Lu}$ ($R_f=0$) and to the $[^{161}\text{Tb}]\text{Tb}$- or $[^{177}\text{Lu}]\text{Lu}$-DOTA compounds ($R_f=0.4$) were integrated with the OptiQuant image analysis software (version 5.0, Perkin Elmer Inc., USA) and the radiolabeling yield determined. Based on the data obtained, the DOTA-to-nuclide molar ratios were plotted against the radiolabeling yield using Origin software, fitted with a Boltzmann’s sigmoidal modified equation. Experiments were repeated three times for $^{161}\text{Tb}$ and $^{177}\text{Lu}$ (no-carrier-added) and once for $^{177}\text{Lu}$ (carrier-added). The average DOTA-to-nuclide molar ratios, corresponding to 50% labeling efficiency of DOTA with $^{161}\text{Tb}$ (no-carrier-added) and $^{177}\text{Lu}$ (carrier-added) at different time points (Day 3 to Day 14 after EOS), were determined and compared with each other for statistical significance by an unpaired t test using Graph Pad Prism (version 7.00).

2.2.7 $[^{161}\text{Tb}]\text{Tb}/[^{177}\text{Lu}]\text{Lu}$-DOTA-NOC and $[^{161}\text{Tb}]\text{Tb}/[^{177}\text{Lu}]\text{Lu}$-DOTA-TOC stability studies

The radiolabeling of DOTA-NOC and DOTA-TOC with no-carrier-added $^{161}\text{Tb}$ or $^{177}\text{Lu}$ at 50 MBq/nmol (300 MBq $^{161}\text{Tb}$ activity in total) was performed as described above, in the absence or in the presence of L-ascorbic acid (2.9 mg, Sigma-Aldrich, USA). The radiolabeling yield was determined by means of HPLC immediately after preparation of $[^{161}\text{Tb}]\text{Tb}/[^{177}\text{Lu}]\text{Lu}$-DOTA-NOC ($[^{161}\text{Tb}]\text{Tb}/[^{177}\text{Lu}]\text{Lu}$-DOTA-TOC). The radioactivity concentration of the labeling solutions was adjusted to 250 MBq/500 µL with saline and radiolytic stability of the radioligands was determined over time (1 h, 4 h and 24 h) by means of HPLC. The values of the determined formation yield were compared for $[^{161}\text{Tb}]\text{Tb}$- and $[^{177}\text{Lu}]\text{Lu}$-DOTA-NOC (DOTA-TOC) for statistical significance by an unpaired t test using Graph Pad Prism (version 7.00).
2.2.8 Determination of the influence of Gd and Dy on the $^{161}$Tb-DOTA-NOC radiolabeling yield

The radiolabeling of DOTA-NOC with no-carrier-added $^{161}$Tb was performed at 50 or 100 MBq/nmol molar activity (based on the amount of the received activity of the total $^{161}$Tb fraction) as described above. Briefly, $^{161}$TbCl$_3$ solution (70–200 MBq) was mixed with sodium acetate, DOTA-NOC and relevant amount of Dysprosium or Gadolinium (prepared from 1000 mg/L ICP standards in 2% nitric acid of Dy or Gd (Sigma-Aldrich, USA)) to obtain different Dy-to-$^{161}$Tb or Gd-to-$^{161}$Tb mass ratios (from 0.1-to-1 to 10-to-1). The reaction solution was incubated for 10 min at 95°C and the radiolabeling yield was analyzed by HPLC as previously described. The values of the determined $^{161}$Tb-DOTA-NOC formation yield for the same Dy-to-$^{161}$Tb and Gd-to-$^{161}$Tb mass ratios were compared for statistical significance by an unpaired t test using Graph Pad Prism (version 7.00).

2.2.9 The recycling of spent enriched $^{160}$Gd target material

Recycling procedure: The aliquot (10 mL) from the target recycling solution (900 mL, ~1100 mg in 1 M α-HIBA) was measured using HPGe detector for the determination of $^{153}$Gd content until 3σ uncertainty was below <5%. The target recycling solution was mixed with 100 mL 30% HCl to destroy complex with α-HIBA, then loaded onto the AG MP-50 column (10 mm x 170 mm, cation exchange resin, Bio-Rad Laboratories AG, USA) and eluted with 20.0 mL 7 M HNO$_3$. This solution was evaporated until dryness at 80°C under gas flow, mixed with 6.0 mL 0.1 M NH$_4$NO$_3$ and divided to three part for the three recycling repetitions. Each part (2.0 mL 0.1 M NH$_4$NO$_3$, containing ~367 mg) was loaded onto the prepared Sykam resin column (10 mm x 270 mm) by means of Ismatec peristaltic pump. Gd separation from the impurities of the target recycling solution (Dy, Tb, Eu, Co) was performed with the use of 0.13 M (pH 4.5) α-HIBA as eluent and 0.6 mL/min flow rate. Samples (6–18 mL fractions) of α-HIBA were collected and measured using HPGe detector until the 3σ uncertainty was below 10%. Fractions, containing Gd were collected, loaded onto AG MP-50 resin column (10 mm x 70 mm), eluted with 20.0 mL 7 M HNO$_3$ and evaporated until dryness at 80°C under gas flow. The residue was afterwards heated at > 600°C to transfer $^{160}$Gd(NO$_3$)$_3$ to $^{160}$GdGd$_2$O$_3$ form.
ICP-MS analysis of the recycled material: The obtained $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ was analyzed by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS), using a Thermo Fisher Element 2 Sector Field ICP-MS in low and medium mass resolution setting with a hot plasma. The sample was prepared as following: 1.0 mg of $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ was dissolved in 1.0 mL 7 M HNO$_3$, evaporated until dryness at 80°C and taken with 0.3 M HNO$_3$ to obtain 0.5 ppb sample of Gd. Two dilutions of a gravimetrically prepared custom multi-element-solution that comprises approximately equal concentrations of all elements was analyzed at ca. 0.5 and ca. 3 ppb to obtain a standard regression line. Machine background was corrected by analyzing the sample matrix solution (0.3 M HNO$_3$) before each standard or sample analysis and subtracting the respective count rates obtained on pure acid dilutions from those obtained for standards and samples.

2.3 Results
2.3.1 $^{161}\text{Tb}$ production yield (theoretical versus experimental)

No-carrier-added $^{161}\text{Tb}$ was produced by neutron irradiation of enriched $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ (98.2% enrichment) targets via the $^{160}\text{Gd}(n,\gamma)^{161}\text{Gd}\rightarrow^{161}\text{Tb}$ nuclear reaction (Figure 2.1). The mass of the target material had to be calculated precisely in order to ensure that the $^{161}\text{Tb}$ activity allowed for international transportation was not exceeded. As $^{161}\text{Tb}$ has not yet been recognized by International Air Transport Agency (IATA) for transport purposes, the A2 value (activity value of radioactive material) according to the “Basic Radionuclide Values For Unknown Radionuclides Or Mixtures” was applied [99]. As a result, a maximum of 20 GBq (0.02 TBq) $^{161}\text{Tb}$ can currently be transported internationally (in this case, shipping from ILL and SAFARI-1 research reactors to PSI) [99].
Masses of the $^{160}\text{Gd}$ target material, required to produce 20 GBq $^{161}\text{Tb}$ after bombardment at the irradiation facilities, were calculated with the ChainSolver code. Two-week irradiations at ILL (8 mg $^{160}\text{Gd}$Gd$_2$O$_3$, 1.10$^{15}$ n.cm$^{-2}$.s$^{-1}$, 1 day cooling) and at SAFARI-1 (32 mg $^{160}\text{Gd}$Gd$_2$O$_3$, 2.10$^{14}$ n.cm$^{-2}$.s$^{-1}$, 1 day cooling) would theoretically result in 20 GBq $^{161}\text{Tb}$. At PSI’s neutron source facility (SINQ, 4·10$^{13}$ n.cm$^{-2}$.s$^{-1}$) each irradiation cycle is three weeks, which was calculated to provide 17.2 GBq $^{161}\text{Tb}$ after the bombardment of 100 mg $^{160}\text{Gd}$Gd(NO$_3$)$_3$. The masses of the target material could be adapted to operator/user requirements based on the ChainSolver code calculations and neutron fluxes, calculated from the measured $^{60}\text{Co}$ activity values of the $^{59}\text{Co}$ monitors (Table 2.2). Three ampoules with $^{59}\text{Co}$ were bombarded at SAFARI-1 nuclear reactor and one ampoule each at ILL and at SINQ irradiation facilities (together with the $^{160}\text{Gd}$ ampoules), respectively. The measured values of the effective perturbed neutron fluxes in the samples irradiated at the ILL and SAFARI-1 nuclear reactors scaled as expected with the unperturbed neutron flux values reported by the facility in question.
Table 2.2. Measured neutron fluxes of the irradiation facilities used for $^{161}$Tb production.

<table>
<thead>
<tr>
<th>Facility</th>
<th>Irradiation time, d</th>
<th>$^{60}$Co activity, kBq</th>
<th>Measured perturbed neutron flux, n.cm$^{-2}$.s$^{-1}$</th>
<th>Nominal unperturbed neutron flux, n.cm$^{-2}$.s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILL</td>
<td>10</td>
<td>33.5</td>
<td>$7.4 \times 10^{14}$</td>
<td>$1.0 \times 10^{15}$</td>
</tr>
<tr>
<td>SAFARI-1</td>
<td>13–16</td>
<td>41.0–54.0</td>
<td>$1.8 \times 10^{14}$</td>
<td>$2.0 \times 10^{14}$</td>
</tr>
<tr>
<td>SINQ</td>
<td>21</td>
<td>92.3</td>
<td>$1.8 \times 10^{13}$</td>
<td>$4.0 \times 10^{13}$</td>
</tr>
</tbody>
</table>

In practice, one-to-two-week irradiations of $^{160}$Gd target ampoules using SAFARI-1 (22–33 mg $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$) and ILL (7–13 mg $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$) research reactors resulted in production of 10–20 GBq of $^{161}$Tb (Table 2.3). The calculated neutron flux at the PNA irradiation position of the SINQ facility was determined experimentally to be only ~50% of its originally reported value (Table 2.2), which resulted in 6–9 GBq $^{161}$Tb after three weeks irradiation of the enriched $[^{160}\text{Gd}]\text{Gd(NO}_3)_3$ target material (Table 2.3). This was due to the fact that the spallation target had recently been replaced and was being operated at a lower beam current.

Table 2.3. Production yields of several $^{161}$Tb batches, obtained from the irradiation facilities.

<table>
<thead>
<tr>
<th>Facility</th>
<th>Irradiation time, d</th>
<th>Target material</th>
<th>Mass of the target material, mg</th>
<th>Measured $^{161}$Tb activity (EOB), GBq</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILL</td>
<td>5</td>
<td>$[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$</td>
<td>12.5</td>
<td>11.6</td>
</tr>
<tr>
<td>ILL</td>
<td>10</td>
<td>$[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$</td>
<td>7.3</td>
<td>16.7</td>
</tr>
<tr>
<td>SAFARI-1</td>
<td>14</td>
<td>$[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$</td>
<td>33.3</td>
<td>19.6</td>
</tr>
<tr>
<td>SAFARI-1</td>
<td>7</td>
<td>$[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$</td>
<td>32.5</td>
<td>11.9</td>
</tr>
<tr>
<td>SINQ</td>
<td>21</td>
<td>$[^{160}\text{Gd}]\text{Gd(NO}_3)_3$</td>
<td>94.9</td>
<td>8.8</td>
</tr>
<tr>
<td>SINQ</td>
<td>21</td>
<td>$[^{160}\text{Gd}]\text{Gd(NO}_3)_3$</td>
<td>86.4</td>
<td>6.0</td>
</tr>
</tbody>
</table>
2.3.2 Radiochemical isolation of $^{161}$Tb from the target material and accumulated impurities

By performing bench experiments, using a Sykam cation exchange resin column of 10 mm x 170 mm dimension and long-lived radioactive tracers, conditions for the efficient separation of Tb from up to 140 mg of Gd$_2$O$_3$ and the presence of various impurities were established (Figure 2.2 a, b). Initially, $^{153}$Gd and $^{160}$Tb tracers were used to determine conditions for appropriate Tb/Gd separation on the Sykam resin column with the use of $\alpha$-HIBA as eluent (Experiment 1; Figure 2.2 a). The $^{152}$Eu, present in the experiment, was a contaminant of the provided $^{153}$Gd solution; however, this allowed one to understand the behavior of Eu as a possible impurity of the target material for $^{161}$Tb production (Table 2.4). After the experimental conditions for appropriate Tb/Gd separation were determined (0.13 M $\alpha$-HIBA, pH 4.5; 0.6 mL/min flow rate) with the corresponding tracers, similar conditions were applied for the Tb separation of up to 140 mg of natGd$_2$O$_3$ (Experiment 2; Figure 2.2 b). Even in the presence of 140 mg of Gd$_2$O$_3$ in the system (Experiment 2), Tb was effectively separated from nat/153Gd (Figure 2.2 b). With the increase of the natGd mass, a significant tailing of the Gd peak was observed due to the expanded longitudinal occupation of the column volume. This zone broadening had no effect on the purity of the Tb peak, however.
Figure 2.2. Elution profile of Tb/Gd separation from the target material (10 mm x 170 mm Sykam resin column, 0.6 mL/min eluent flow rate). Experiment 1 (a) was run without addition of Gd$_2$O$_3$, while Experiment 2 (b) was performed with the addition of 140 mg Gd$_2$O$_3$. 
The next step was to study the behavior of various radioactive tracers in order to simulate the presence of potential impurities (Table 2.4), coming from the target material, in the $^{161}$Tb chemical separation system (Figure 2.3 a, b). The purpose of Experiment 3, shown in Figure 2.3 a, was to understand the behavior of Zn and Na (or K) in simulated Tb production conditions, while the purpose of Experiment 4 (Figure 2.3 b) was to evaluate the behavior of Fe, Cr and Ni on the Sykam resin column – also simulating Tb production conditions. In Experiment 4, the concentration of $\alpha$-HIBA was increased from 0.13 M to 0.175 M before Tb was eluted from the Sykam column, such that one could speed up the elution of $^{65}$Ni (its short 2.5 h half-life made detection problematic in the performed time-consuming experiment). This resulted in the overlapping of Tb and Gd peaks.

Table 2.4. Chemical admixtures of 1000 mg $[^{160}$Gd]$\text{Gd}_2\text{O}_3$, provided by the supplier (Isoflex, USA).

<table>
<thead>
<tr>
<th>Element</th>
<th>K</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Al</th>
<th>Si</th>
<th>Cr</th>
<th>Ni</th>
<th>Cu</th>
<th>Pb</th>
<th>Sb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (ppm)</td>
<td>&lt;50</td>
<td>&lt;20</td>
<td>&lt;50</td>
<td>&lt;3</td>
<td>&lt;50</td>
<td>&lt;3</td>
<td>&lt;50</td>
<td>&lt;5</td>
<td>&lt;1</td>
<td>&lt;10</td>
<td>13</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Element</th>
<th>Sn</th>
<th>Pt</th>
<th>Sm</th>
<th>Ho</th>
<th>Dy</th>
<th>Eu</th>
<th>Nd</th>
<th>Tb</th>
<th>Er</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (ppm)</td>
<td>&lt;1</td>
<td>20</td>
<td>13</td>
<td>6</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
The separation of Tb from the possible impurities of the target material on the Sykam resin column showed that Cr species were eluted at different concentrations of α-HIBA and resulted in the presence of Cr in Tb fraction (Experiment 4; Figure 2.3 b). Cr ions form oligomers (monomers to tetramers) in aqueous solution, which may
interact individually with the eluent [100]. Unfortunately, the complexation mechanisms and complexation constants of such Cr species with $\alpha$-HIBA were not determined and it is difficult to explain why traces of Cr were eluted in every fraction throughout the separation process. Nevertheless, Cr was not retained on the LN3 resin column (Column 2 of the purification process, 6 mm x 5 mm), which allowed efficient Tb separation from Cr as an impurity in the $^{161}$Tb final product (Figure 2.4). LN3 extraction resin was reported to have low affinity for Tb ions in low concentrated acids [101], which allowed $^{161}$Tb to be eluted in a small volume of 0.05 M HCl.

![Figure 2.4](image)

Figure 2.4. Elution profile of Tb separation from Cr as a possible radioactive impurity in the final $^{161}$Tb product (6 mm x 5 mm LN3 resin column, 0.6 mL/min eluent flow rate).

Subsequently, the established experimental conditions (Sykam resin; 0.13 M $\alpha$-HIBA, pH 4.5; 0.6 mL/min eluent flow rate) were applied towards the purification of the reactor-produced $^{161}$Tb (230 MBq). During the irradiation, side products were co-produced from the impurities of the target material ($^{46}$Sc, $^{124}$Sb, $^{141}$Ce, $^{147}$Nd, $^{153}$Gd, $^{153}$Sm, $^{152/155/156}$Eu, $^{169}$Yb) and the ampoule material ($^{65}$Zn, $^{60}$Co, $^{192}$Ir). Despite this impurity formation, the established method demonstrated effective $^{161}$Tb separation from Gd and accumulated impurities using the Sykam resin column (Figure 2.5). $^{161}$Tb was eluted from the Sykam resin in ~20 mL $\alpha$-HIBA, followed by the concentration of the radionuclide on the LN3 resin column (6 mm x 5 mm).
Figure 2.5. Elution profile of $^{161}$Tb separation from the irradiated target material and side products (10 mm x 170 mm Sykam resin column, 8 mg $[^{160}$Gd]$\text{Gd}_2\text{O}_3$, 0.6 mL/min eluent flow rate).

Based on the developed two-column purification method (combination of Sykam and LN3 resin columns), a $^{161}$Tb purification module was designed (Figure 2.6). The module was constructed and introduced to the hot cell, making it possible to perform separations under more extreme conditions with high activities (up to 20 GBq) of the reactor-produced $^{161}$Tb. The established procedure for $^{161}$Tb purification process on the designed module resulted in the elution of the final product ($[^{161}$Tb]$\text{TbCl}_3$) in a small volume (500 µL) of 0.05 M HCl, with an activity concentration of 11–21 MBq/µL. Using the developed purification method, 80–90% separation yield was achieved at EOS. Losses of 10–20% of $^{161}$Tb activity were observed in the target dissolving/column loading and final elution steps.
2.3.3 Characteristics of the $^{161}$Tb product

$^{161}$Tb, obtained after the purification process, was characterized to provide a product specification (Table 2.5). The identification of the product was confirmed by the $^{161}$Tb-characteristic $\gamma$–lines (Figure 2.7). The content of long-lived $^{160}$Tb ($T_{1/2}=72.3$ d), produced by the $^{159}$Tb(n,$\gamma$)$^{160}$Tb nuclear reaction due to the presence of $^{159}$Tb impurity in the target material (as sold by the vendor), was determined after the decay of $^{161}$Tb and did not exceed 0.007% of the total $^{161}$Tb activity at EOS (Figure 2.8). The absence of the long-lived $^{153}$Gd ($T_{1/2}=239.5$ d) in the decayed product, produced via the $^{152}$Gd(n,$\gamma$)$^{153}$Gd nuclear reaction, emphasizes the efficient $^{161}$Tb separation from the Gd target material.
**Table 2.5.** Product data specification of $[^{161}\text{Tb}]\text{TbCl}_3$, developed in this work, compared to the commercially-available $[^{177}\text{Lu}]\text{LuCl}_3$.

<table>
<thead>
<tr>
<th>Test</th>
<th>Resulted Specification of $[^{161}\text{Tb}]\text{TbCl}_3$</th>
<th>Resulted Specification of $[^{177}\text{Lu}]\text{LuCl}_3$ (EndolucinBeta)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity concentration</td>
<td>11–21 MBq/µL</td>
<td>36–44 MBq/µL</td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear and colorless solution</td>
<td>Clear and colorless solution</td>
</tr>
<tr>
<td>pH</td>
<td>1–2</td>
<td>1–2</td>
</tr>
<tr>
<td>Radiolabeling yield</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>HPLC based on radiolabeling with $^{161}\text{Tb}$ of DOTA-NOC, molar ratio 1:4 (180 MBq/nmol)</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Identity $^{161}\text{Tb}$ (γ–ray spectrometry)</td>
<td>48.9 keV γ–line</td>
<td>113 keV γ–line</td>
</tr>
<tr>
<td></td>
<td>74.6 keV γ–line</td>
<td>208 keV γ–line</td>
</tr>
<tr>
<td>Radionuclidic purity (γ–ray spectrometry)</td>
<td>$^{160}\text{Tb} \leq 0.007%$</td>
<td>$^{175}\text{Yb} \leq 0.01%$</td>
</tr>
<tr>
<td>Radiochemical purity (radio-TLC)</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

* Specification from the EndolucinBeta certificate of analysis (ITG)

**Figure 2.7.** Gamma spectrum of $^{161}\text{Tb}$, obtained after the purification process. The radionuclidic impurity $^{160}\text{Tb}$ is not visible due to the negligible activity as compared to that of $^{161}\text{Tb}$ at EOS.
Figure 2.8. Gamma spectrum of the decayed product ([\(^{161}\text{Tb}\)]\text{TbCl}_3), used for the determination of \(^{160}\text{Tb}\) radionuclidic impurity in the total \(^{161}\text{Tb}\) fraction. No other radionuclides, other than \(^{160}\text{Tb}\), were found.

The radiochemical purity of \(^{161}\text{Tb}\) solution, determined by radio TLC, was >99%. The absence of the \(R_f\) 0 fraction (which would indicate Tb species not forming complexes with citrate, e.g., colloids) on the radio TLC chromatogram (Figure 2.9) indicates the existence of only \([^{161}\text{Tb}]\text{Tb}^{3+}\) in the final product. The radiolabeling yield of \([^{161}\text{Tb}]\text{Tb-DOTA-NOC}\) showed >99% efficiency at 180 MBq/nmol molar activity, which corresponds to 1-to-4 nuclide-to-peptide molar ratio (Figure 2.10).
Figure 2.9. Radio TLC chromatogram of $^{161}\text{Tb}]\text{TbCl}_3$ solution in 0.1 M sodium citrate (pH 5.5) for the determination of $^{161}\text{Tb}$ radiochemical purity.

Figure 2.10. HPLC chromatogram of $^{161}\text{Tb}]\text{Tb-DOTA-NOC}$ (2 min retention time would indicate “free” non-labeled $^{161}\text{Tb}$ and 8.2 min indicates $^{161}\text{Tb]}\text{Tb-DOTA-NOC}$).
2.3.4 Comparison of the $^{161}$Tb and $^{177}$Lu quality, based on the [$^{161}$Tb]Tb- and [$^{177}$Lu]Lu-DOTA molar activities over a two-week period

Radiolabeling of DOTA with $^{161}$Tb (no-carrier-added) and $^{177}$Lu (either carrier-added or no-carrier-added) was performed at different DOTA-to-nuclide molar ratios in order to monitor the quality change of the radiolanthanides of interest over a two-week period after EOS. DOTA could be complexed with $^{161}$Tb and $^{177}$Lu (no-carrier-added) at 15:1 and 13:1 DOTA-to-nuclide molar ratios, respectively, with $>90\%$ radiolabeling yield at Day 14 after EOS (Figure 2.11 a, b). This indicates the possibility of using the prospective drug product (e.g., DOTA peptides radiolabeled with $^{161}$Tb) for up to two weeks after the chemical separation. With carrier-added $^{177}$Lu, 90% radiolabeling yield was only achieved when using a much higher DOTA-to-nuclide ratio (32:1) at the two-week time point (Figure 2.11 c).

Based on the results shown in Figure 2.11, the average DOTA-to-nuclide molar ratios corresponding to 50% labeling efficiency of DOTA with $^{161}$Tb and $^{177}$Lu, were determined at specific time points (Day 3, Day 7, Day 10 and Day 14 – Table 2.6). The values allow an estimation of the possible radiolabeling yield of different biomolecules conjugated with a DOTA chelator, labeled with the radionuclide of interest, over a certain decay period, as well as comparison of the radiolabeling capability of the radionuclides of interest. When DOTA was radiolabeled with carrier-added $^{177}$Lu, 50% labeling efficiency was obtained at higher DOTA-to-nuclide molar ratios at each time point as compared to no-carrier-added $^{161}$Tb and $^{177}$Lu, respectively. The 50% labeling efficiency of [$^{161}$Tb]Tb-DOTA was found to be comparable with that of no-carrier-added $^{177}$Lu at Day 3 (p=0.13), while slight increase of the values was observed for Day 7, Day 10 and Day 14 (p<0.05), respectively.
Figure 2.11. Comparison of the radiolabeling yield of (a) no-carrier-added $^{161}$Tb (SAFARI-1), (b) no-carrier-added $^{177}$Lu (ITG) and (c) carrier-added $^{177}$Lu (IDB) in combination with DOTA over time at different DOTA-to-nuclide molar ratios.
Table 2.6. DOTA-to-nuclide molar ratios, corresponding to 50% labeling efficiency of \(^{161}\text{Tb}\)-DOTA and both carrier-added and no-carrier-added \(^{177}\text{Lu}\)-DOTA over a two-week decay period.

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{161}\text{Tb}) (PSI)</td>
<td>No-carrier-added</td>
<td>4.4±0.3</td>
<td>5.7±0.4</td>
<td>7.6±0.5</td>
</tr>
<tr>
<td>(^{177}\text{Lu}) (ITG)</td>
<td>No-carrier-added</td>
<td>3.8±0.1</td>
<td>4.2±0.1</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td>(^{177}\text{Lu}) (IDB)</td>
<td>Carrier-added</td>
<td>5.8*</td>
<td>7.5*</td>
<td>9.2*</td>
</tr>
</tbody>
</table>

*Statistically different to no-carrier-added \(^{161}\text{Tb}\) and \(^{177}\text{Lu}\) (p<0.05)

2.3.5 Radiolytic stability of DOTA-TOC and DOTA-NOC labeled with no-carrier-added \(^{161}\text{Tb}\) or \(^{177}\text{Lu}\)

In order to determine whether the conversion and Auger electrons from \(^{161}\text{Tb}\) may cause additional radiolytic degradation of radiolabeled somatostatin analogues, stability tests were performed using DOTA-TOC or DOTA-NOC. The preparation of the any clinically-applied radiopharmaceutical (e.g., \(^{177}\text{Lu}\)-DOTA-TOC), requires the use of a stabilizer (e.g., L-ascorbic acid, gentisic acid) in order to inhibit peptide autoradiolysis [102-104] [73, 105, 106]. The stability studies of DOTA-TOC and DOTA-TOC, radiolabeled with carrier-added \(^{161}\text{Tb}\) or \(^{177}\text{Lu}\), were performed with and without addition of L-ascorbic acid to determine whether conversion and Auger electrons have an extra effect on radiolytic degradation of the compound with and without the presence of a stabilizer. The analogue in question was radiolabeled with \(^{177}\text{Lu}\) and \(^{161}\text{Tb}\) at high activity concentration (250 MBq/500 µL) over a 24-hour period. \(^{161}\text{Tb}\)- and \(^{177}\text{Lu}\)-DOTA-TOC were stable over 1 hour (>99% intact product) in the absence of L-ascorbic acid, but both showed radiolytic degradation after an incubation period of 24 hours. After 4 hours, slight degradation of both \(^{161}\text{Tb}\)-DOTA-TOC (78% intact product) and \(^{177}\text{Lu}\)-DOTA-TOC (85% intact product) was observed (Figure 2.12 a, b). The disintegration of \(^{161}\text{Tb}\)- and \(^{177}\text{Lu}\)-DOTA-NOC was observed already at 1 hour time point with the difference in the degree of radiolysis between \(^{161}\text{Tb}\)-DOTA-NOC and \(^{177}\text{Lu}\)-DOTA-NOC at 1 hour (p=0.02) and 4 hour time points (p=0.01). \(^{161}\text{Tb}\)- and \(^{177}\text{Lu}\)-DOTA-NOC and \(^{161}\text{Tb}\)- and \(^{177}\text{Lu}\)-DOTA-TOC were stable over 24 hours’ incubation in the presence of a stabilizer (L-ascorbic acid) and showed >94% radiolabeling yield at the 24-hour time point.
Figure 2.12. Stability of the radiopeptides over time in the presence and absence of ascorbic acid; (a) graph showing the % intact $^{161}$Tb$^-$ and $^{177}$Lu$^-$DOTA-TOC over a time period of 24 h (b) graph showing the % intact $^{161}$Tb$^-$ and $^{177}$Lu$^-$DOTA-NOC over a time period of 24 h (in the presence of ascorbic acid $^{161}$Tb$^-$ and $^{177}$Lu$^-$DOTA-TOC curves overlap, making impossible to visualize both).

Interestingly, the disintegration rate of $^{177}$Lu$^-$DOTA-NOC in the absence of the stabilizer was slightly lower, compared to $^{161}$Tb, indicating the possible influence of conversion and Auger electrons on the ligand stability. Shpinkova et al. reported the hypothesis about the influence of the electron capture after-effects on the integrity of radiometal complexes with organic ligands [107]. When the decay process is accompanied by the emission of conversion and Auger electrons, the molecules gain positive charge, followed by Coulomb repulsion and the sharing of repulsion energy between a number of atoms, causing the breaking of bonds and molecule degradation [107, 108]. This could explain the slightly higher DOTA-NOC disintegration, when it was labeled with $^{161}$Tb as compared to $^{177}$Lu. The preparation of therapeutic radiopharmaceuticals for clinical application, however, involves the use of quenchers such as ascorbic acid or gentisic acid in order to prevent radiolysis [109]. Therefore, the results of the stability studies in the presence of ascorbic acid are of main interest.
2.3.6 Radiolabeling competition of $^{161}$Tb with the target material (Gd) and decay product (Dy)

No influence of the neighboring lanthanides (Dy as the decay product of $^{161}$Tb or Gd as a target material) on the $[^{161}$Tb]$\text{[Tb-DOTA-NOC]}$ formation yield was observed until 3-to-1 lanthanide-to-$^{161}$Tb mass ratio. The radiolabeling yield of $[^{161}$Tb]$\text{Tb-DOTA-NOC}$ decreased at 3-to-1 mass ratio to 91.6% (Gd) and 84.8% (Dy), indicating the start of the radiolabeling competition of $^{161}$Tb with the neighboring lanthanide. Three repetitions of the experiments were carried out without significant difference between the formation yield of $[^{161}$Tb]$\text{Tb-DOTA-NOC}$ for the same Dy-to-$^{161}$Tb and Gd-to-$^{161}$Tb mass ratios (p<0.05) (Figure 2.13). This result confirmed the reproducibility of the $[^{161}$Tb]$\text{TbCl}_3$ product purity, regarding Dy and Gd content, indicating the reliability of the investigated purification method. Furthermore, the result indicates the possibility of using $[^{161}$Tb]$\text{TbCl}_3$ up to three weeks EOS based on the Dy and Gd content (assuming the content of these impurities is zero after the purification process).

![Figure 2.13. Radiolabeling competition of $^{161}$Tb with the neighboring lanthanides (Gd and Dy) at different lanthanide-to-$^{161}$Tb mass ratios.](image)

2.3.7 Target recycling

Irradiated $^{160}$Gd targets require recycling due to the high cost of the $[^{160}$Gd]$\text{Gd}_2\text{O}_3$ material (98.2% enrichment, $\$5900$ for $1000$ mg, Isoflex, USA). The
recycling procedure includes separation of the irradiated $^{160}$Gd from the impurities, coming from the target material or co-produced during the ampoule bombardment. The gamma spectrum of the aliquot of the recycling solution is shown in Figure 2.14. The critical point of the recycling is the separation of $^{160}$Gd from $^{159}$Tb and $^{160}$Tb isotopes, present in the recycling solution after irradiation of the target. $^{159}$Tb is a cold impurity in the target material (as sold by the vendor) (Table 2.4) and undesirable contaminant in the $^{161}$Tb product after the purification process. Furthermore, during the $^{160}$Gd irradiation, $^{159}$Tb is a source of the long-lived isotope $^{160}$Tb ($T_{1/2}=72.3$ d), produced by the $^{159}$Tb(n,γ)$^{160}$Tb nuclear reaction (Figure 2.15). $^{160}$Tb decay is accompanied by the emission of high energy γ–rays and β–particles, which may result in the additional dose to the patient (Table 2.7).

**Figure 2.14.** Gamma spectrum of the recycling solution, before processing. $^{159}$Dy and $^{160}$Tb is not visible due to the negligible activity as compared to the present long-lived radionuclides.
Figure 2.15. Production of $^{160}$Tb via $^{159}$Tb(n,γ)$^{160}$Tb nuclear reaction.

Table 2.7. High energy γ-rays and electron emissions of $^{160}$Tb [110].

<table>
<thead>
<tr>
<th>$E_\gamma$ (Intensity %)</th>
<th>298 keV (26)</th>
<th>879 keV (30)</th>
<th>962 keV (10)</th>
<th>966 keV (25)</th>
<th>1178 keV (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_\beta$ (Intensity %)</td>
<td>129 keV (5)</td>
<td>143 keV (10)</td>
<td>176 keV (45)</td>
<td>255 keV (7)</td>
<td>287 keV (28)</td>
</tr>
</tbody>
</table>

During the irradiation of $^{160}$Gd ampoules in the nuclear reactor, long-lived $^{153}$Gd ($T_{1/2}=239.5$ d) is co-produced by the $^{152}$Gd(n,γ)$^{153}$Gd nuclear reaction (Figure 2.16), which allows to monitor the behavior of Gd during the recycling process. For example, 14 days irradiation of 30 mg $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ at SAFARI-1 nuclear reactor may theoretically result in the production of 5.7 MBq $^{153}$Gd at the end of bombardment (EOB) (ChainSolver code calculation [96]).

Figure 2.16. Production of $^{153}$Gd via $^{152}$Gd(n,γ)$^{153}$Gd nuclear reaction.

The recycling procedure was primarily established on bench with the use of Sykam resin (10 mm x 220 mm), long-lived radioactive $^{153}$Gd, $^{160}$Tb and $^{152}$Eu tracers (Table 2.1) and addition of 300 mg nat-Gd$_2$O$_3$ (Figure 2.17). $^{153}\text{nat}$Gd was effectively separated from $^{160}$Tb with 18.0 mL gap between these neighboring lanthanides. However, $^{153}\text{nat}$Gd and $^{152}$Eu peaks overlapped with the purity of Gd peak being 96.5%. The content of $^{152}$Eu in the $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ target is not critical in terms of purity of the $[^{161}\text{Tb}]\text{TbCl}_3$ final product, as Tb is effectively separated from Gd and Eu during the
investigated $^{161}$Tb purification process (Figure 2.2). Nevertheless, the presence of $^{152}$Eu ($\sim$1 kBq/mg) in the $^{160}$Gd target material can be an issue with regards to the ampoule preparation for irradiation in the nuclear reactor.

![Figure 2.17. Elution profile of the $^{153}$natGd (300 mg) separation from the neighboring lanthanides, which could be present in the target recycling solution (10 mm x 220 mm Sykam resin column, 0.6 mL/min eluent flow rate).](image)

Therefore, the length of the column for the recycling was increased from 220 mm to 270 mm and $\sim$1100 mg [$^{160}$Gd]Gd$_2$O$_3$ were processed (3 repetitions, $\sim$367 mg per experiment) from the year 2015 (Figure 2.18). The long-lived $^{159}$Dy ($T_{1/2}$=144.4 d) was found in the target recycling solution due to the neutron capture reaction $^{158}$Dy(n,$\gamma$)$^{159}$Dy and was efficiently separated from Gd (as well as $^{160}$Tb) under chosen experimental conditions. However, Gd and Eu peaks overlapped even more compared to bench tests. The possible explanation is the presence of cold impurities (environmental and from the target material) in the recycling solution, which additionally occupy column volume and decrease the separation efficiency of Gd/Eu pair. Nevertheless, pure Gd fractions in 0.15 M $\alpha$-HIBA (after the separation on the Sykam column) were transferred to the [$^{160}$Gd]Gd$_2$O$_3$ form as described in 2.2.9.
Figure 2.18. Elution profile of the $^{153/160}$Gd (~367 mg) separation from the impurities in the target recycling solution (10 mm x 270 mm Sykam resin column, 0.6 mL/min eluent flow rate). $^{154}$Eu and $^{155}$Eu isotopes have the same behavior as $^{152}$Eu, therefore, not shown in the elution profile. $^{60}$Co and $^{139}$Ce were eluted later with 1 M $\alpha$-HIBA.

In total, 975 mg of $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ was recycled, corresponding to 88.6% yield of the recycling process. Another 125 mg of $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ (11.4%), that were eluted together with $^{152}$Eu, could be recycled during the future repetition of the process. The obtained $^{160}$Gd-oxide was analyzed by means of ICP-MS in order to investigate the isotopic composition of the obtained $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ after the recycling process and to determine the content of different lanthanides, especially $^{159}$Tb. No $^{159}$Tb or other lanthanides were found in the recycled material, indicating the efficiency of the investigated method. The isotopic composition of the recycled $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ was determined to be similar to the $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$, provided by the supplier (Table 2.8). Two bombardments of the recycled $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ were performed (one at ILL and one at SAFARI-1), providing $[^{161}\text{Tb}]\text{TbCl}_3$ of the specification equal to those obtained after the irradiation of commercial $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ (Isoflex, USA) (Table 2.5).
Table 2.8. Isotopic composition of $^{160}\text{Gd}\text{Gd}_2\text{O}_3$, used for the $^{161}\text{Tb}$ production

<table>
<thead>
<tr>
<th>Isotopic composition (%) in the recycled $^{160}\text{Gd}\text{Gd}_2\text{O}_3$</th>
<th>$^{152}\text{Gd}$</th>
<th>$^{154}\text{Gd}$</th>
<th>$^{155}\text{Gd}$</th>
<th>$^{156}\text{Gd}$</th>
<th>$^{157}\text{Gd}$</th>
<th>$^{158}\text{Gd}$</th>
<th>$^{160}\text{Gd}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.6</td>
<td>&lt;0.01</td>
<td>1.3</td>
<td>98.1</td>
<td></td>
</tr>
</tbody>
</table>

| Isotopic composition (%) in $^{160}\text{Gd}\text{Gd}_2\text{O}_3$ sold by the vendor (Isoflex, USA) | <0.01 | 0.01 | 0.18 | 0.36 | 0.25 | 1 | 98.2 |

2.4 Discussion

In the present study, the development of a reproducible chemical separation to produce no-carrier-added $^{161}\text{Tb}$ from enriched $^{160}\text{Gd}$ targets and the characterization of the final product ($^{161}\text{Tb}\text{TbCl}_3$) is reported. Gd(NO$_3$)$_3$, previously used as target material [79], is not suitable for large-scale $^{161}\text{Tb}$ production as lanthanide nitrates are hygroscopic materials, which begin to decompose at 70°C [111, 112]. The pressure change due to the water evaporation and release of the gases at higher temperatures inside the ampoule may result in the ampoule cracking, loss of the material and radioactive contamination of the nuclear reactor. The use of oxide targets (melting point 2420°C) eliminates the potential issues described and will give rise to large-scale $^{161}\text{Tb}$ production in future (TBq activity). The ChainSolver code allows quick calculations of the required masses of $^{160}\text{Gd}$ targets for the production of the desired $^{161}\text{Tb}$ activity, based on irradiation times and neutron fluxes, obtained with $^{59}\text{Co}$ monitors.

Recently, Brezovcsik et al. reported a Tb separation procedure from massive Gd targets (>100 mg), indicating no mass influence of Gd on the 20 cm long analytical column (GE) [113]. The efficiency of Tb separation from Gd was only 85%, however, showing significant overlapping of Tb and Gd peaks, which would result in the presence of “cold” Gd in the Tb final product and interfere with radiolabeling. The purification method described in this work provides an effective $^{161}\text{Tb}$ separation from the Gd$_2$O$_3$ target material with mass up to 140 mg (higher masses were not studied). This is a valuable result for possible future commercial application of the developed method for $^{161}\text{Tb}$ separation from massive Gd$_2$O$_3$ targets (>100 mg). For example, 2 weeks irradiation of 140 mg $^{160}\text{Gd}$$\text{Gd}_2\text{O}_3$ target at ILL nuclear reactor could theoretically result in the production of 0.5 TBq $^{161}\text{Tb}$ (ChainSolver Code calculations [96]), which...
can be efficiently purified with the investigated method. $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ target material contains various trace elements (Table 2.4), which may show similar chemical behavior to $^{161}\text{Tb}$ on the resin in question and result in the elution of Tb and the impurities in the same fraction. It was demonstrated that, despite the impurities that could be produced via activation of trace elements in the target material or in the quartz ampoule, the established purification method effectively separated $^{161}\text{Tb}$ from potential impurities present in the system, based on the combination of Sykam and LN3 resin columns. The investigated recycling process resulted in the obtaining of 98.1% enriched $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ with lower $^{159}\text{Tb}$ content, compared to the commercial $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$ (Isoflex, USA). The recycled material can be used for future irradiations and may result in the $[^{161}\text{Tb}]\text{TbCl}_3$ product of higher radionuclidic purity compared to $[^{161}\text{Tb}]\text{TbCl}_3$ obtained after bombardment of the commercial $[^{160}\text{Gd}]\text{Gd}_2\text{O}_3$.

The two-column $^{161}\text{Tb}$ purification process proposed by Lehenberger et al. [79] was adopted as the baseline for this study towards further development. The cation exchange resin of the first column, column dimension and pump flow rate were changed, while the concentration and pH of the eluent remained the same (0.13 M α-HIBA pH 4.5). The resin used for the second column previously reported was changed from AG 50W-X8 (cation exchange resin) to LN3 (extraction resin). These modifications played a vital role in obtaining the final product ($[^{161}\text{Tb}]\text{TbCl}_3$) in purity comparable to that of the commercially-available no-carrier-added $[^{177}\text{Lu}]\text{LuCl}_3$ (EndolucinBeta). The radiolabeling capability of $^{161}\text{Tb}$ in this work was similar to no-carrier-added $^{177}\text{Lu}$ and three times higher than that of $^{161}\text{Tb}$ obtained by Lehenberger et al. Somatostatin analogues could be labeled with no-carrier-added $^{161}\text{Tb}$ (this work) at 1-to-4 nuclide-to-peptide molar ratio (Figure 2.10) immediately after the purification process with >99% radiolabeling yield. Quantitative formation of $[^{161}\text{Tb}]\text{Tb}$-DOTA-TATE, reported by Lehenberger et al., was possible only at 1-to-12 nuclide-to-peptide molar ratio.

The radiolabeling capability of $^{161}\text{Tb}$ was higher than the radiolabeling capability of carrier-added $^{177}\text{Lu}$ at each time point over a two-week decay period, indicating that $^{161}\text{Tb}$ has a superior radiolabeling capability. The faster drop of $^{161}\text{Tb}$ radiolabeling capability as compared to no-carrier-added $^{177}\text{Lu}$ at Day 7, Day 10 and Day 14 after purification (Table 2.6) could be explained by the lower radioactivity concentration of the final product obtained (11–21 MBq/µL for $[^{161}\text{Tb}]\text{TbCl}_3$ vs 36–44 MBq/µL for $[^{177}\text{Lu}]\text{LuCl}_3$). This implies that the mass ratio between $^{161}\text{Tb}$ and the
impurities (Zn, Fe, Co, Pb etc.), which can be introduced during product analysis and post-processing, is lower as compared to $^{177}$Lu. This results in the potentially stronger interference from environmental impurities during radiolabeling of DOTA with $^{161}$Tb than with $^{177}$Lu [114]. Nevertheless, complexation of $^{161}$Tb with DOTA was possible over a two-week period after EOS, at 1-to-15 nuclide-to-peptide molar ratio (corresponding to 48 MBq/nmol molar activity), with >90% radiolabeling yield (Figure 2.11). This ratio would be appropriate when using DOTA-functionalized targeting agents, such as peptides for peptide receptor radionuclide therapy (PRRNT) [73, 82]. These excellent achievements indicate the possible clinical use of [$^{161}$Tb]TbCl$_3$ for a period of up to 2 weeks after EOS. Moreover, clinically-applied DOTA-TOC (DOTA-NOC) radiolabeled with $^{161}$Tb was stable over 24 hours at high radioactivity concentration, indicating that storage and transportation of $^{161}$Tb-labeled somatostatin analogues would be feasible, as is the case for their $^{177}$Lu-labeled counterparts.

### 2.5 Conclusion

A new method to separate $^{161}$Tb from the enriched [$^{160}$Gd]Gd$_2$O$_3$ target material and co-produced impurities was developed, with the use of cation exchange and extraction chromatography, respectively. The method resulted in radionuclidically and radiochemically pure product ([$^{161}$Tb]TbCl$_3$), comparable to commercially available, no-carrier-added $^{177}$Lu. The quantity and quality of $^{161}$Tb is suitable for high-specific radiolabeling, potentially useful for the GMP production of radioligands towards future clinical application.
Chapter 3

Production and purification process of $^{165}$Er

3.1 Development of $^{165}$Er – a candidate for Auger–electron cancer therapy – using $^{\text{nat}}$Ho(p,n)$^{165}$Er nuclear reaction

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The chapter will be published in a modified version.

Author contributions:

Nadezda Gracheva designed and carried out the experiments, analyzed and interpreted the data and wrote the chapter. Saverio Braccini and Tommaso Carzaniga performed irradiations at the IBA Cyclone (Bern University Hospital (Inselspital)). Roger Schibli reviewed the manuscript. Nicholas van der Meulen oversaw and supervised the study and revised the manuscript.
3.1.1 Introduction

The use of radiolanthanides for the targeted radionuclide therapy (TRNT) has been intensively investigated during the last years and resulted in the approval of several new drugs by US Food and Drug Administration (FDA). Among them are e.g., Quadramet® (Lantheus Medical Imaging, Inc., USA), based on $^{153}$Sm and recently approved Lutathera®, containing $^{177}$Lu [115]. The radionuclides, emitting $\beta^-$–particles (LET $\sim$0.2 keV/µm) [116], have been mostly utilized for clinical application due to the availability (production in large quantities of GBq–TBq amounts), suitable decay characteristics (e.g., half-life) and variable range of the applicable energies [54, 117]. The penetration paths of the therapeutic $\beta^-$–emitters (between 0.1–10 mm [118]) are relevant for treatment of large metastases or tumors due to the crossfire effect [119, 120]. However, radionuclides with high LET, such as Auger–electron emitters, could be preferable for killing micrometastases or single cancer cells. This kind of emitter possesses 4–26 keV/µm energy deposition per unit length, allowing controlled travel paths in water of only $\sim$0.5 µm [16, 54]. The extremely short range of Auger electrons makes them highly toxic in the vicinity of the DNA, meaning the internalization of the labeled compounds to the cell nucleus is required [116, 121]. For this reason, Auger–electron emitters remain of low toxicity to the normal surrounding tissues while decaying outside the nucleus [122]. The developing of the strategies to deliver Auger–electron emitters into the DNA are currently under development and already showed promising results [123-125].

The radiolanthanide $^{165}$Er ($T_{1/2}$=10.4 h) decays by electron capture (EC), followed by the emission of Auger electrons with energies of 5.3 keV (65.6%) and 38.4 keV (4.8%) and low energetic X-rays [110]. The energies of Auger electrons, emitted by $^{165}$Er, are similar to those of the most intensively studied $^{125}$I ($T_{1/2}$=59.4 d), which showed extremely high cytotoxicity when incorporated into DNA [126, 127]. The decay of $^{165}$Er is accompanied by no gamma radiation that allows to avoid additional patient doses. Based on the characteristics, mentioned above, $^{165}$Er could be proposed as a promising candidate for the Auger–electron therapy.

$^{165}$Er can be produced via several production routes by charged particle induced reactions (Table 3.1.1) [39, 128-130]. The highest cross-section values were reported for indirect production routes $^{166}$Er(p,2n)$^{165}$Tm$\rightarrow^{165}$Er (Route 1) and $^{166}$Er(d,3n)$^{165}$Tm$\rightarrow^{165}$Er (Route 2). $^{165}$Er production via Route 1 requires proton beam
energies in the range of 16–23 MeV (with the highest cross-section at 21 MeV) that is not optimal for the production at generally available 18 MeV small medical cyclotrons (SMC) (approximate number – 1050 [131]). $^{165}\text{Er}$ production via Route 2 requires deuteron beam energies in the range of 22–29 MeV, necessitating the use of high-energy cyclotrons with deuteron capability, which are not commercially available. The direct production route via $^{\text{nat}}\text{Ho}(p,n)^{165}\text{Er}$ nuclear reaction may allow production of $^{165}\text{Er}$ at SMC with the use of natural Holmium ($^{\text{nat}}\text{Ho}$) targets, which are of low-cost due to the 100% natural abundance of $^{\text{nat}}\text{Ho}$.

Table 3.1.1. Possible $^{165}\text{Er}$ production routes using charged particle induced reactions.

<table>
<thead>
<tr>
<th>Projectile particle</th>
<th>Production route</th>
<th>Theoretical cross-section [mbarn]</th>
<th>Projectile energy [MeV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton (p)</td>
<td>$^{\text{nat}}\text{Ho}(p,n)^{165}\text{Er}$</td>
<td>172 ± 19*</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>$^{166}\text{Er}(p,2n)^{165}\text{Tm}→^{165}\text{Er}$</td>
<td>1263.3</td>
<td>21</td>
</tr>
<tr>
<td>Deuteron (d)</td>
<td>$^{\text{nat}}\text{Ho}(d,2n)^{165}\text{Er}$</td>
<td>753.6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>$^{164}\text{Er}(d,n)^{165}\text{Tm}→^{165}\text{Er}$</td>
<td>120.4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$^{166}\text{Er}(d,3n)^{165}\text{Tm}→^{165}\text{Er}$</td>
<td>1523.9</td>
<td>25</td>
</tr>
</tbody>
</table>

*The value corresponds to the experimentally-measured cross-section [128], while other values show theoretical cross-sections, calculated by TALYS-1.2 (Equilibrium and Pre-Equilibrium) code [39].

Herein, we report on the production of $^{165}\text{Er}$ at the Injector 2 PSI research cyclotron or IBA Cyclone (Bern University Hospital). The method for the separation of $^{165}\text{Er}$ from the macroamounts of the target material (up to 200 mg $^{\text{nat}}\text{Ho}_2\text{O}_3$) has been investigated, followed by the concentration of the final product ([$^{165}\text{Er}]\text{ErCl}_3$) in a low concentrated HCl. The developed purification method allowed obtaining radionuclidically pure $^{165}\text{Er}$.

### 3.1.2 Material and Methods

#### 3.1.2.1 Irradiation of natural Holmium targets for the production of $^{165}\text{Er}$

$^{165}\text{Er}$ was produced at the Injector 2 PSI research cyclotron or at the IBA Cyclone (Bern University Hospital (Inselspital)) via $^{\text{nat}}\text{Ho}(p,n)^{165}\text{Er}$ nuclear reaction. For the bombardment at the PSI cyclotron, 120–200 mg Holmium oxide targets ($^{\text{nat}}\text{Ho}_2\text{O}_3$, Sigma-Aldrich, USA) (Table 3.1.2) were pressed with 2 t of pressure to have
a dimensions of ~0.4 mm thickness and a diameter of 13 mm. The prepared targets were placed inside the target holder system before introduction into the irradiation facility (Figure 3.1 a). The initial proton energy of 72 MeV from the Injector 2 cyclotron at PSI was reduced by a Niobium degrader of 3.0–3.5 mm thickness to provide different entering proton beam energies 8.6–16.2 MeV (calculated by SRIM (The Stopping and Range of Ions in Matter software)) to determine the energy value for obtaining the highest $^{165}$Er production yield [132]. Afterwards, the production of $^{165}$Er from via $^{\text{nat}}$Ho(p,n)$^{165}$Er nuclear reaction was performed by 8 h bombardment of 200 mg $^{\text{nat}}$Ho$_2$O$_3$ targets at 13.4 MeV proton beam with 20 μA beam intensity.

For the bombardment at the IBA Cyclone, a $^{\text{nat}}$Ho disc (200 mg $^{\text{nat}}$Ho foil, 10 mm diameter, 400 μm thickness) (Alfa Aesar, USA) was placed into the coin (Figure 3.1 b), designed by the Bern Medical Cyclotron Laboratory to optimize $^{165}$Er production. The coin has no material in front of the target, allowing to receive higher impinging proton energy on the target and efficient heat dissipation since the Helium cooling acts directly on the $^{\text{nat}}$Ho target material. Two prepared targets were irradiated for 10 h with either 10 μA or 15 μA beam intensity at 13.5 MeV proton beam energy.

![Figure 3.1.1](image)

**Figure 3.1.1.** (a) $^{\text{nat}}$Ho$_2$O$_3$ target inside the Aluminum capsule for the positioning of the target, used for at Injector 2 PSI; (b) $^{\text{nat}}$Ho metal foil target inside the target coin, used at IBA Cyclone

<table>
<thead>
<tr>
<th>Element</th>
<th>Al</th>
<th>Bi</th>
<th>Ca</th>
<th>Cd</th>
<th>Ir</th>
<th>Mn</th>
<th>Mo</th>
<th>Ni</th>
<th>Zn</th>
<th>REE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (ppm)</td>
<td>34</td>
<td>16.9</td>
<td>18.1</td>
<td>0.5</td>
<td>27.4</td>
<td>1.9</td>
<td>1.7</td>
<td>7.6</td>
<td>0.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>

**Table 3.1.2.** Chemical impurities of $^{\text{nat}}$Ho$_2$O$_3$ (99.999%), provided by the supplier (Sigma-Aldrich, USA)

### 3.1.2.2 Development of the procedure for $^{165}$Er purification process

The purification method for $^{165}$Er was established, based on the previously reported 2-column procedure, developed to obtain radiochemically and
radionuclidically pure $^{161}\text{Tb}$ [133] and described in Chapter 2 of this thesis. The method was primarily developed by means of bench experiments, using $^{165}\text{Er}$ ($T_{1/2}=10.4$ h) and $^{166}\text{Ho}$ ($T_{1/2}=26.8$ h) radioactive tracers, obtained by short irradiations (10–30 min) of 120 mg or 200 mg of $^{nat}\text{Ho}_2\text{O}_3$ with 8.6–16.2 MeV proton beam at the PSI cyclotron. The target material ($^{nat}\text{Ho}_2\text{O}_3$) was dissolved in 2.0 mL 2 M HNO$_3$ at 80°C with magnetic stirring. The separation of $^{165}\text{Er}$ from the target material of various masses (120 mg or 200 mg) was performed on the Sykam cation exchange resin column with the use of $\alpha$-HIBA (pH 4.5) as eluent. Different column lengths (10 mm x 140 mm or 10 mm x 160 mm dimension), flow rates (0.5–0.7 mL/min) and eluent concentrations (0.07 M–0.09 M $\alpha$-HIBA) were applied to determine optimal conditions for the sufficient Er/Ho separation. During the separation process, each 5–10 mL fractions were collected and measured using HPGe detector in combination with the InterWinner software package to determine the presence, identity and activity of the tracers. The measurements were performed until the 3σ uncertainty was below 5%. The fractions, eluted from the Sykam column and containing $^{165}\text{Er}$ tracer, were afterwards loaded onto LN3 resin column for the radionuclide concentration, followed by the elution of the final product ($[^{165}\text{Er}]\text{ErCl}_3$) in 500–700 µL 0.1 M HCl. Once the purification method was developed on bench, the purification module was constructed, introduced to the hot-cell and the described above purification procedure was applied for the separation of 0.9–1.6 GBq (EOB) cyclotron-produced $^{165}\text{Er}$ (PSI cyclotron or IBA Cyclone) from the target material and co-produced side products.

3.1.2.3 Post-process characterization of the obtained $^{168}\text{Er}$

Radionuclidic purity: The identification and radionuclidic purity of $[^{165}\text{Er}]\text{ErCl}_3$ were examined by $\gamma$–ray spectrometry using HPGe detector. The aliquot of the final product, containing 0.1–0.5 MBq of $^{165}\text{Er}$, was measured on HPGe until the 3σ uncertainty was below 5%.

Chemical purity: The content of the environmental impurities in the final $[^{165}\text{Er}]\text{ErCl}_3$ product or contaminants, coming from the target material, was determined using inductively coupled plasma optical emission spectroscopy (ICP-EOS, OPTIMA 3000, PerkinElmer, USA). After the production of $[^{165}\text{Er}]\text{ErCl}_3$, two $[^{165}\text{Er}]\text{ErCl}_3$ fractions (Experiment 1 and Experiment 2; 600 µL and 700 µL $^{165}\text{Er}$ in 0.1 M HCl, respectively) were left to decay and after the decay were diluted with 2% HNO$_3$ in order
to obtain the suitable volume of the sample (4.2 mL) in a matrix, acceptable for the ICP-OES analysis. The calibration solutions with various concentrations (0.1 ppm, 1.0 ppm, 5 ppm, 10 ppm and 50 ppm) were prepared by dilution of the 1000 ppm ICP standards (in 2% nitric acid, Sigma-Aldrich, USA) of the element of interest (Al, Ca, Co, Cr, Cu, Er, Fe, Ho, Ni, Pb, Tm and Zn).

Radiolabeling yield: Radiolabeling of DOTA-NOC (ABX GmbH, Germany) at a molar activity of 5–20 MBq/nmol was performed in order to evaluate the success of the purification process. The radiolabeling was performed as described in detail in Chapter 2 of this thesis.

3.1.2.4 Analysis of the natHo2O3 target material for natEr content

natHo2O3 provided by the supplier and used as a target material was analyzed for the content of natEr by means of ICP-EOS (5110 ICP-OES, Agilent Technologies, USA). Two samples for ICP-OES analysis were prepared in a following way: 1.0–1.2 mg of natHo2O3 were dissolved in 1.0 mL 3 M HNO3 and evaporated until dryness at 80°C. The residue was taken with 30 mL 0.3 M HNO3 to obtain natHo solution with the concentration of 30–35 ppm. The calibration solutions with various concentrations (0.01 ppm, 0.05 ppm, 0.10 ppm, 1 ppm, 5 ppm, 10 ppm and 50 ppm) were prepared by dilution of the 1000 ppm ICP Er and Ho standards (in 2% nitric acid, Sigma-Aldrich, USA).

3.1.3 Results

3.1.3.1 Production yields of 165Er, obtained via natHo(p,n)165Er nuclear reaction

Irradiations of 200 mg natHo2O3 target material at 8.6–16.2 MeV proton beam energy (PSI cyclotron) resulted in the production of 165Er activity (EOB) ranged between 51 and 923 MBq. The experimentally-obtained production yields were 2–44 times lower as compared to the theoretically achievable 165Er activity values (Table 3.1.3). The theoretical values were calculated by taking into account the cross section for the natHo(p,n)165Er nuclear reaction at the specific projectile energy (σ) [128], the proton flux (ϕ), areal density of target natHo atoms (N), the irradiation time (tB) and the radioactive decay constant (λ) (Equation 3.1.1).
\[ A_{EOB} = \sigma \cdot \phi \cdot N \cdot \left(1 - e^{-\lambda t_B}\right) \] (3.1.1)

The highest production yield of \(^{165}\)Er (923 MeV) was obtained at 13.4 MeV projectile energy with the difference with the theoretical value of a factor of 2.2. These conditions were considered to be preferable for the following bombardments of the \(^{nat}\)Ho\(_2\)O\(_3\) targets at the Injector 2 PSI research cyclotron. At EOB, \(^{166}\)Ho was detected with the \(^{165}\)Er-to-\(^{166}\)Ho ratio being 70±5-to-1.

Bombardment of the two \(^{nat}\)Ho foil targets (200 mg) at the IBA Cyclone at 13.5 MeV proton beam energy resulted in the production of 534 MBq and 1577 MBq \(^{165}\)Er at 10 µA and 15 µA beam intensity, respectively. Theoretical values under these irradiation conditions were calculated (Equation 3.1.1) to be 1392 MeV (10 µA) and 2089 MeV (15 µA), indicating a good correlation with the experimental values.

Table 3.1.3. Experimental production yields of \(^{165}\)Er obtained by 8 h proton irradiation of 200 mg \(^{nat}\)Ho\(_2\)O\(_3\) target material at PSI cyclotron, compared to the theoretically-calculated values.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>8.6</td>
<td>135 ± 15</td>
<td>75</td>
<td>3356</td>
</tr>
<tr>
<td>10.3</td>
<td>151 ± 17</td>
<td>200</td>
<td>3753</td>
</tr>
<tr>
<td>11.8</td>
<td>119 ± 14</td>
<td>660</td>
<td>2958</td>
</tr>
<tr>
<td>13.4</td>
<td>83.3 ± 9.4</td>
<td>923</td>
<td>2063</td>
</tr>
<tr>
<td>16.2</td>
<td>41.3 ± 4.8</td>
<td>51</td>
<td>1019</td>
</tr>
</tbody>
</table>

3.1.3.2 Development of the \(^{165}\)Er separation from the target material

In order to efficiently separate \(^{165}\)Er from \(^{nat}\)Ho (target material), various experimental conditions (column length, flow rate, eluent concentration) were applied for bench tests. Primary, gradient elution (0.07 M–1 M α-HIBA, 0.5 mL/min flow rate) of Er and Ho from the Sykam resin column (10 mm x 140 mm) was performed in order to understand the elements’ behavior under chosen conditions (Figure 3.1.2). Er can be separated from 120 mg \(^{nat}\)Ho\(_2\)O\(_3\) with ~30 mL gap between the neighboring lanthanides. After Er was eluted from the column (8 h process time), the concentration of α-HIBA
was increased to 0.11 M and 1 M in order to speed up Ho elution. The experiment was repeated with 0.6 mL/min eluent flow rate that resulted in $^{165}$Er elution after 7 h.

Based on these primary experiments, the interval of the eluent concentration, which can be applied for the Er/Ho separation, was determined: 0.07 M–0.09 M α-HIBA. Isocratic elution with 0.09 M α-HIBA was performed (Figure 3.1.3) to understand whether the sufficient separation of the lanthanides of interest can be achieved under chosen experimental conditions with shortening the time of the process. Er elution from the Sykam column started after 3.5 h from the start of the experiment, however, overlapping with Ho peak was observed. Therefore, these experimental conditions were considered inappropriate for the obtaining radionuclidically and chemically pure Er.

![Figure 3.1.2. Elution profile of Er separation from 120 mg $^{165}$Ho$_2$O$_3$ (10 mm x 140 mm Sykam resin column, 0.5 mL/min eluent flow rate, gradient elution).](image)
Figure 3.1.3. Elution profile of Er separation from 120 mg nat HoO₃ (10 mm x 140 mm Sykam resin column, 0.5 mL/min eluent flow rate, isocratic elution).

For further experiments, 0.08 M α-HIBA was used as eluent, the mass of the nat HoO₃ was increased to 200 mg and γ-emitting tracer 167Tm (T₁/²=9.3 d, provided by the Laboratory of Radiochemistry (PSI)) was added to the system to insure the separation of 165Er from the possible Tm impurities, present in the target material (Table 3.1.2, Figure 3.1.4 a). Under chosen experimental conditions, Er can be efficiently separated from Ho and Tm and eluted after 7 h from the start of the experiment.

Once the purification method was developed with kBq activities of the tracers, it was applied for the separation of low-activities cyclotron-produced 165Er (4.6 MBq) from the target material and side products (Figure 3.1.4 b). However, the column length was increased from 140 mm to 160 mm what allowed to make the Tm/Er gap wider (~7 mL and ~15 mL gap, respectively). The eluent flow rate was changed from 0.6 mL/min to 0.7 mL/min, leaving the process time of 7 h. The reproducibility of the separation process was confirmed by performing two more repetitions on bench.

166/167/168Tm isotopes were measured during the separation due to the activation of the 166/167/168Er natural isotopes, present in the target material, via ⁵Er(p,n)⁷Tm nuclear reaction. The amount of these radionuclides is negligible (~1 kBq) and has no influence on the 165Er purification based on the established method.
Figure 3.1.4. Elution profile of Er separation from 200 mg $^{164}$Ho$_2$O$_3$ (a) 10 mm x 140 mm Sykam resin column, 0.6 mL/min eluent flow rate (b) 10 mm x 160 mm Sykam resin column, 0.7 mL/min eluent flow rate.

After the separation from the target material and co-produced impurities, $^{165}$Er was eluted in ~70 mL volume of 0.08 M $\alpha$-HIBA and loaded on LN3 resin column (6 mm x 5 mm) for the concentration of the radionuclide. The final product was eluted in 500 µL of 0.1 M HCl.

Based on the developed two-column method, $^{165}$Er purification module was
designed (Figure 3.1.5). The module was constructed and introduced to the hot cell, making it possible to perform separations under investigated experimental conditions with higher activities of $^{165}$Er (up to 1.6 GBq). Seven $^{165}$Er/natHo productions were performed at the purification module.

![Figure 3.1.5. Schematic diagram of $^{165}$Er purification module.](image)

### 3.1.3.3 Quality control of the $[^{165}\text{Er}]\text{ErCl}_3$ product

The final product ($[^{165}\text{Er}]\text{ErCl}_3$) was collected in the product vial and measured using HPGe detector until the 3σ uncertainty was below 5% to identify $^{165}$Er and to determine radionuclidic purity of the product (Figure 3.1.6). Absence of any radioactive impurities indicated high radionuclidic purity of the product (>99% as $^{165}$Er). The final $^{165}$Er product after two independent purifications (80 MBq (EOS) Experiment 1 and 191 MBq (EOS) Experiment 2) was analyzed using ICP-OES for the content of natural Er, which was determined in ng quantities in both fractions (Table 3.1.4). natEr-to-$^{165}$Er ratio in the final $^{165}$Er product was 60-to-1 and 51-to-1 in Experiment 1 and Experiment 2, respectively, making impossible radiolabeling of DOTA-NOC with the obtained $^{165}$Er even at low molar activities (e.g., 5.4% $[^{165}\text{Er}]\text{Er}$-DOTA-NOC radiolabeling yield.
at 5 MBq/nmol molar activity).

![Gamma spectrum of $^{165}$Er, obtained after the purification process.](image)

**Figure 3.1.6.** Gamma spectrum of $^{165}$Er, obtained after the purification process.

**Table 3.1.4.** Comparison of the $^{165}$Er and $^{\text{nat}}$Er content in the final $[^{165}\text{Er}]\text{ErCl}_3$ product (Experiment 1 and Experiment 2)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mass of the obtained $^{165}$Er (EOS)</th>
<th>Mass of $^{\text{nat}}$Er in the $^{165}$Er fraction</th>
<th>$^{\text{nat}}$Er-to-$^{165}$Er mass ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2 ng (corresponds to 80 MBq of the activity)</td>
<td>72.0 ng</td>
<td>60-to-1</td>
</tr>
<tr>
<td>2</td>
<td>2.8 ng (corresponds to 191 MBq of the activity)</td>
<td>142.4 ng</td>
<td>51-to-1</td>
</tr>
</tbody>
</table>

$^{\text{nat}}$Er isotopes in the final product could be present as an impurity of the $^{\text{nat}}$Ho$_2$O$_3$ target material, produced during the irradiation via $^{\text{nat}}$Ho(p,2n)$^{164}$Er nuclear reaction and/or be present due to decay of $^{166}$Ho to “cold” $^{166}$Er during the purification process. The mass of $^{166}$Ho at EOB was calculated to be 0.1 ng and 0.2 ng for Experiment 1 and Experiment 2, respectively. Therefore, the presence of 72 ng and 142.4 ng of $^{\text{nat}}$Er in the final $^{165}$Er product due to $^{166}$Ho decay during the separation process was excluded.
ICP-OES analysis of natHo₂O₃ target material showed the presence of 0.2±0.02 ppm of natEr, corresponding to 40±4 ng of natEr in 200 mg natHo₂O₃. The obtained value are 1.8–3.6 times lower as compared to the amount of natEr, detected in the ¹⁶⁵Er final product (Table 3.1.4), indicating the formation of ¹⁶⁴Er during the irradiation via natHo(p,2n)¹⁶⁴Er nuclear reaction (~250 mbarn cross section at 14 MeV proton beam energy) [39]. The theoretical mass of the obtained ¹⁶⁴Er under irradiation conditions, chosen for ¹⁶⁵Er production at 13.4 MeV proton beam energy, was calculated to be 64 ng based on Equation 3.1.1 and the radioactive decay law. The summary of the mass values of natEr, coming from the natHo₂O₃ target material and formed during the irradiation process, is in good agreement with natEr masses determined in the final ¹⁶⁵Er product (Table 3.1.4). Other cold impurities, detected in the final ¹⁶⁵Er fractions, were Cobalt (~2 ng), Iron (~1 ng) and Zinc (~2 ng).

3.1.4 Discussion

Activities of ¹⁶⁵Er in the range of ~1–2 GBq (EOB) could be regularly obtained by proton irradiation of the natural Holmium targets either at research cyclotrons or prevalent 18 MeV small medical cyclotrons via natHo(p,n)¹⁶⁵Er nuclear reaction. The obtained ¹⁶⁵Er activity values were 2–44 times lower (at different proton beam energies) as compared to the theoretically calculated values when bombardments were performed at the Injector 2 PSI research cyclotron. The discrepancies between experimental and theoretical results in this work could be explained by the large energy degradation (using Nb degrader) of the original 72 MeV proton beam to 8.6–16.2 MeV, resulting in a beam with a broad spread of energies and by the lack of beam diagnostics closer than 80 cm from the target. Much better correlation was observed between theoretical and experimental values (1.3–2.6 factor of difference) once ¹⁶⁵Er was produce at the IBA Cyclone, which is equipped with the so-called universal beam monitor (UniBEaM) to perform precise beam diagnostic during the entire bombardment [134]. Nevertheless, irradiations at the Injector 2 PSI research cyclotron give an advantage of performing required radionuclide purification directly after the production due to the availability of the hot cells on side.

After the bombardment, ¹⁶⁵Er/natHo separation procedure was developed based on the ¹⁶¹Tb purification, described in Chapter 2 of this thesis, and translated to the designed module constructed by Radionuclide-Production and Maintenance Group of
PSI. The developed Er/Ho purification method provides an effective $^{165}\text{Er}$ separation from the $^{\text{nat}}\text{Ho}$ target material with mass up to 200 mg (higher masses were not studied). The purification method required in total ~10 h processing time, which correspond to one half-life of $^{165}\text{Er}$ ($T_{1/2}=10.4$ h). Therefore, the activity of the final $^{165}\text{Er}$ product would be twice lower as compared to the $^{165}\text{Er}$ activity at EOB, once the radionuclide is produced on side and followed by the immediate purification. In this case, after 8 h irradiation of 200 mg $^{\text{nat}}\text{Ho}_2\text{O}_3$ target ~500 MBq $^{165}\text{Er}$ could be obtained at EOS. This amount of activity is doubtful to be appropriate for the clinical research, however, could be sufficient for the preclinical studies of the Auger–electron effect on cancer cells once $^{165}\text{Er}$ possesses a required purity.

The investigated $^{165}\text{Er}$ purification method allowed obtaining radionuclidically pure $^{165}\text{Er}$ (Figure 3.1.6). The absence of $^{166}\text{Ho} \gamma$–lines on the spectrum of the final $^{165}\text{Er}$ product ($^{165}\text{Er}\cdot\text{ErCl}_3$) confirmed that the developed method provided efficient $^{165}\text{Er}$ separation from the macroamounts of the $^{\text{nat}}\text{Ho}$ targets. Nevertheless, the radiolabeling yield of $^{165}\text{Er}\cdot\text{DOTA-TOC}$ at 5 MBq/nmol molar activity was only 5.4%, indicating the presence of cold impurities in the final product. The detected Zinc impurity in the final product ($^{165}\text{Er}\cdot\text{ErCl}_3$) is known to be a strong competitor with REEs for the radiolabeling of DOTA derivatives, dropping the radiolabeling yield to <1% already at 0.5 Zn-to-$^{165}\text{Er}$ molar ratio [114]. The results of the ICP-OES measurements showed that Zn-to-$^{165}\text{Er}$ molar ratio in $^{165}\text{Er}\cdot\text{ErCl}_3$ solution was ~5, making impossible quantitative formation of $^{165}\text{Er}\cdot\text{Er-DOTA-TOC}$. Zinc, present in the $^{\text{nat}}\text{Ho}_2\text{O}_3$ target material (Table 3.1.2) or introduced to the system before loading Sykam resin column is efficiently separated from $^{165}\text{Er}$ on Sykam column due to the Zn elution only with $>0.5$ M $\alpha$-HIBA (Figure 2.5). Therefore, Zn is present in the final $^{165}\text{Er}$ product as an environmental impurity either from 0.1 M HCl, used for the elution of the final product, or coming from the quality control experiments, performed after the final elution of $^{165}\text{Er}\cdot\text{ErCl}_3$ (taking aliquot for radiolabeling, radionuclidic purity check etc.). In this case, only more careful work (e.g., cleaning pipet tips with HNO$_3$ and MilliQ water, using gloves which do not contain Zn etc.) could decrease the amount or eliminate Zn from the $^{165}\text{Er}\cdot\text{ErCl}_3$ final product.

Another impurity, detected in the final product at 50–60 times higher amounts as compared to $^{165}\text{Er}$, was $^{\text{nat}}\text{Er}$. Natural Erbium was originally present as an impurity of the $^{\text{nat}}\text{Ho}_2\text{O}_3$ target material and “cold” Er isotope ($^{164}\text{Er}$) was probably being produced during the proton irradiation of $^{\text{nat}}\text{Ho}_2\text{O}_3$ via $^{\text{nat}}\text{Ho}(p,2n)^{164}\text{Er}$ nuclear reaction.
Sadeghi et al. suggested proton energies <9 MeV for the production of no-carrier-added $^{165}$Er based on theoretical calculations of the reaction cross-sections with TALYS 1.2 code (Figure 1.9) [39]. However, 8 h irradiation of 200 mg $^{nat}$Ho$_2$O$_3$ at 8.6 MeV proton beam resulted in only 75 MBq $^{165}$Er (EOB). This amount of radioactivity is not sufficient even for performing $^{165}$Er quality control after the purification process, which takes ~10 h. Therefore, 13.4 MeV proton beam energy was chosen for the $^{165}$Er production, allowing at the same time to crosscheck whether the theoretical predictions for $^{164}$Er co-production, reported on literature, are reasonable. After analyzing [$^{165}$Er]ErCl$_3$ final product by means of ICP-OES, the content of $^{nat}$Er was comparable to the amount of $^{164}$Er, calculated based on the reported theoretical cross-section [39], summarized with the amount of $^{nat}$Er, coming from the $^{nat}$Ho$_2$O$_3$ target material. For this reason, the production of $^{165}$Er at 13.4 MeV proton beam energy is doubtful to give access to no-carrier-added radionuclide. Unfortunately, $^{165}$Er production at lower beam energies at the Injector 2 PSI research cyclotron, followed by the separation of $^{165}$Er from the target material with the developed method, is not feasible for providing high activities of the radionuclide.

Nevertheless, in order to make a final statement about the nature of $^{nat}$Er in the final [$^{165}$Er]ErCl$_3$ product, the authors recommend to perform recycling of the irradiated and purified $^{nat}$Ho$_2$O$_3$, based on the scheme shown in Figure 3.1.7. For this, after $^{165}$Er elution from the Sykam resin column (Figure 3.1.4 b) during the purification process, the concentration of $\alpha$-HIBA should be increased to 0.11 M and $^{nat}$Ho should be eluted to the separate vessel/bottle for the recycling process. Afterwards, $^{nat}$Ho solution could be loaded onto AG MP-50 resin column (10 mm x 140 mm) and eluted with 7.0 M HNO$_3$ (Figure 3.1.7). Obtained $^{nat}$Ho(NO$_3$)$_3$ could be transferred to $^{nat}$Ho$_2$O$_3$ by heating with >600°C.
Figure 3.1.7. Schematic illustration of the natHo recycling procedure.

The recycled \textsuperscript{nat}Ho\textsubscript{2}O\textsubscript{3} material should be analyzed for the \textsuperscript{nat}Er content before the bombardment (ideally no \textsuperscript{nat}Er is detected in the recycled \textsuperscript{nat}Ho\textsubscript{2}O\textsubscript{3}), followed by irradiation under the chosen conditions (200 mg, 8 h irradiation time, 13.4 MeV proton beam) and by the purification and analysis of the final product by means of ICP-MS. This procedure would allow to make a confident statement about the source of \textsuperscript{nat}Er in the final \textsuperscript{165}Er\textsubscript{Cl\textsubscript{3}} product and conclude whether production of no-carrier-added \textsuperscript{165}Er via direct route at 13.4 MeV proton beam energy is possible. The recycling of \textsuperscript{nat}Ho\textsubscript{2}O\textsubscript{3} before the irradiation could also result in obtaining higher molar activities during the radiolabeling process. Therefore, the experiments are currently ongoing at PSI.

3.1.5 Conclusion

Production of \textsuperscript{165}Er via \textsuperscript{nat}Ho(p,n)\textsuperscript{165}Er nuclear reaction, followed by the developed purification process allows obtaining radionuclidically pure \textsuperscript{165}Er in \textasciitilde10 h time. The amount of the obtained radionuclide is reasonable for preclinical research (\textasciitilde500 MBq). Analyzed \textsuperscript{165}Er\textsubscript{Cl\textsubscript{3}} final product contained \textsuperscript{nat}Er at \textsuperscript{nat}Er-to-\textsuperscript{165}Er mass ratio being 50-to-1, therefore preventing radiolabeling of DOTA-NOC with the obtained \textsuperscript{165}Er even at low molar activities. In order to determine, whether higher molar activities could be achieved, the recycling method of \textsuperscript{nat}Ho\textsubscript{2}O\textsubscript{3} targets was proposed and
developed on bench. Recycling method implemented before radionuclide production could overcome the shortcoming in the $^{165}$Er production process by removing $^{nat}$Er impurities. In this case $^{nat}$Er/$^{nat}$Ho separation should be performed before the preparation of $^{nat}$Ho$_2$O$_3$ target for the bombardment at cyclotron. Separation method was developed on bench and experiments are currently ongoing at PSI. In order to determine whether produced $^{165}$Er activity could be scale up for potential clinical application, we examined $^{166}$Er(p,2n)$^{165}$Tm$\rightarrow$$^{165}$Er production route (described in Chapter 3.2 of the thesis). In this case, higher amounts of $^{165}$Er activity could be obtained due to 10 times higher cross-section values of $^{166}$Er(p,2n)$^{165}$Tm nuclear reaction ($\sim$1.2 barn for 21 MeV proton beam) as compared to $^{nat}$Ho(p,n)$^{165}$Er nuclear reaction ($\sim$0.12 barn for 12 MeV proton beam). Theoretical cross-section values for both reactions are available in literature, nevertheless, $^{nat}$Ho(p,n)$^{165}$Er nuclear reaction theoretical cross-section values were experimentally confirmed at the Laboratory for High Energy Physics (LHEP) of the University of Bern by performing irradiation of the full mass of a thin $^{nat}$Ho target by a proton beam with a flat profile. Experiments for the cross-section determination of $^{166}$Er(p,2n)$^{165}$Tm nuclear reaction are currently ongoing at the LHEP.
3.2 A new purification process to obtain no-carrier-added $^{165}$Er, produced from enriched Erbium targets

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The chapter will be published in a modified version.

Author contributions:

Nadezda Gracheva designed and carried out the experiments, analyzed and interpreted the data and wrote the chapter. Roger Schibli reviewed the manuscript. Nicholas van der Meulen supervised the study and revised the manuscript.
3.2.1 Introduction

General knowledge about the main principles and mechanisms of action of Auger–electron emitters remained elusive before the 1960s, however, curiosity resulting in extensive studies over the next few decades. Auger–electron emitters possess <50 keV energy, which is deposited in a small area (2 nm to 500 nm) and result in significant ionizing effects [135, 136]. It was discovered that the fragmentation of a biomolecule to which Auger–electron emitter is bound was caused not only by radiation but also by transmutation effects (local charge effects). Based on this, the possible mechanism of the DNA damage was described: the direct radiation damage of the sugar-phosphate group, leading to the single-strand breakage, and the rupture of the second strand due to the local radiolysis and charge redistribution [137]. A clear increase of the cytotoxic effects of $^{125}$I (~21 e$^-$, 12.2 keV per decay) was observed with the decrease of the distance of the radionuclide from the cell DNA on the example of NaI (extracellular localization), iododihydrorhodamine (cytoplasmic localization) and 5-halo-2'-deoxyuridine (I UdR, DNA localization) [138]. Important conclusion has been made: the radiobiological effects strongly depend on the location of the Auger–electron emitter. The internalization of $^{125}$IUdR, $^{123}$IUdR and $^{77}$BrUdR showed that the number of decays, required to produce the same V79 cell survival level was inversely proportional to the local energy deposition. The lack of the shoulder on the survival curves for $^{77}$BrUdR and $^{125}$IUdR indicated a possibly not repairable damage caused by Auger–electron emitters, which possess a toxicity comparable to the 5 MeV α–particles [137, 139]. These important findings from 1980s and promising results for the application in the TRNT of the radionuclides, emitting short-range electrons, raised the interest of researchers to produce novel Auger–electron emitters. Bernhardt et al. discovered supplementary obligations to the Auger–electron emitters, which could be applied for TRNT by performing computer simulations to evaluate the relation between the TND (tumor-to-normal-tissue mean absorbed dose-rate ratio) and electron energy. It was discovered that Auger–electron emitters with the half-life in between 30 min and 10 days and energies of the emitted Auger electrons ≤40 keV, will be most suitable for treatment of the single cells [38].

Nowadays, Auger–electron emitters were proposed as appropriate candidates for treatment of single cells and micrometastases based on their ability to provide a high energy density in the immediate vicinity of the decay site, while sparing surrounding
tissues [37, 38, 140, 141]. As was mentioned in the previous chapter, a pure Auger–electron emitter $^{165}\text{Er}$ ($T_{1/2}=10.4\text{ h}$) is one of the possible radionuclides, suitable for targeted cancer therapy. $^{165}\text{Er}$ decays by electron capture and emission of Auger electrons of energies ≤40 keV (5.3 keV (65.6%) and 38.4 keV (4.8%)) [110]. Theoretical and experimental cross-sections of $^{165}\text{Er}$ were studied for several production routes, being the highest (~1.2 barn for 21 MeV proton beam) for the “indirect” production route via $^{166}\text{Er}(p,2n)^{165}\text{Tm\rightarrow}^{165}\text{Er}$ nuclear reaction [39, 128-130]. The required beam energies in the range 16–23 MeV are not ideal for the radionuclide production at widely available 18 MeV medical cyclotrons [39, 129], however, they may allow production of sufficient amounts of no-carrier-added $^{165}\text{Er}$. Certainly, this statement needs to be proved as, so far, no one has produced $^{165}\text{Er}$ from the enriched $^{166}\text{Er}$ targets. The indirect production route requires two chemical separations – $^{166}\text{Er}/^{165}\text{Tm}$ separation directly after the irradiation and $^{165}\text{Tm}/^{165}\text{Er}$ separation after ingrowth of $^{165}\text{Er}$ activity from $^{165}\text{Tm}$ ($T_{1/2}=30.1\text{ h}$) decay (after ~24 h). High abundance of 5.3 keV electrons might require internalization of the radionuclide into cell nucleus [142]. This could be achieved by using nuclear localizing peptide sequences (NLSs) to deliver peptides or antibodies, radiolabeled with Auger–electron emitter, to the cell nucleus [143].

Herein, we report on the production of no-carrier-added $^{165}\text{Er}$ via $^{166}\text{Er}(p,2n)^{165}\text{Tm\rightarrow}^{165}\text{Er}$ nuclear reaction in quantities suitable for preclinical studies. The purification method was developed such that radionuclidically and radiochemically pure $^{165}\text{Er}$ could be obtained. Radiolabeling of DOTA-NOC with $^{165}\text{Er}$ was performed immediately after the purification at 30 MBq/nmol molar activities with $\geq99\%$ radiolabeling yield.

### 3.2.2 Material and methods

#### 3.2.2.1 Preparation of the $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ targets for irradiation at the PSI research cyclotron

$^{165}\text{Er}$ was obtained by proton irradiation of enriched Erbium oxide ($[^{166}\text{Er}]\text{Er}_2\text{O}_3$, 98.1% enrichment, Isoflex, USA) target material via the indirect production route $^{166}\text{Er}(p,2n)^{165}\text{Tm\rightarrow}^{165}\text{Er}$. To prepare the target, 60±1 mg of $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ were pressed with 2 t of pressure, resulting in a 6.0 mm diameter target, 0.4–0.5 mm thick. The target was placed into the aluminum capsule, which has a slot 0.5 mm in depth and 6.0 mm
diameter (Figure 3.2.1). The encapsulated $^{166}$Er$\text{Er}_2\text{O}_3$ target was introduced into the target holder system, before the bombardment at IP2 target station using the Injector 2 PSI research cyclotron.

![Figure 3.2.1](image)

Figure 3.2.1. Aluminum capsule for the positioning of the target: (a) side view; (b) top view with the outline of the place for the 6 mm target.

The $^{166}$Er$\text{Er}_2\text{O}_3$ target was irradiated with 20 $\mu$A for 8 h with 22.8 MeV proton beam. The initial Injector 2 cyclotron proton beam energy of 72 MeV was reduced by applying a niobium degrader of 2.4 mm thickness as shown in Figure 3.2.2. The proton energy on the target surface in this case was calculated with the Stopping and Range of Ions in Matter (SRIM) software [132].

![Figure 3.2.2](image)

Figure 3.2.2. Schematic illustration of the reduction of proton beam energy at the Injector 2 PSI research cyclotron. Reduction of the initial Injector 2 cyclotron proton beam energy of 72 MeV occurs due to the target window (Al), cooling water, Nb degrader and Al target lid.

3.2.2.2 Production of the radioactive tracers for the application in bench experiments

Production of Tm and Er tracers for the bench experiments (investigation of the purification method) were performed under the same irradiation conditions as described in the previous section, however, the target material was changed to nat$\text{Er}_2\text{O}_3$ (Research chemicals, Division of Rhone-Polenc Inc., USA) due to the lower cost as compared to the enriched material. Irradiation time was decreased to 0.5–1 h. The long-lived $^{171}$Tm
(T1/2=1.9 y) was provided by the Laboratory of Radiochemistry (PSI). \(^{171}\text{Er}\) (T1/2=26.8 h) was obtained by neutron activation of Erbium (III) nitrate (Er(NO\(_3\))\(_3\)) at SINQ (PSI). The required volume (602 \(\mu\)L, corresponds to 0.6 mg) of the 1000 ppm Erbium ICP standard (2% w/w HNO\(_3\), Sigma-Aldrich, USA) was placed inside the plastic capsule for irradiation, evaporated until dryness at 80°C under gas flow and bombarded for 3 h at the SINQ neutron source (NAA position).

### 3.2.2.3 Development of the \(^{165}\text{Er}\) purification process on bench

Initially, conditions for the effective Er/Tm separation were determined by performing bench experiments, using Sykam cation exchange resin columns and \(^{165}\text{Tm}\) or \(^{171}\text{Tm}\) and \(^{165}\text{Er}\) or \(^{171}\text{Er}\) tracers.

First, appropriate conditions for the efficient separation of \(^{165}\text{Tm}\) from up to 200 mg of \(^{nat}\text{Er}_2\text{O}_3\) were established on Column 1 (Sykam resin column, 10 mm x 160 mm). After the bombardment at SINQ, Er(NO\(_3\))\(_3\) (containing 4 MBq \(^{171}\text{Er}\)) was dissolved in 2.0 mL 0.1 M NH\(_4\)NO\(_3\) (pH 3.0). An aliquot of 20 \(\mu\)L, containing 40 kBq \(^{171}\text{Er}\), was mixed with 200 mg Er(NO\(_3\))\(_3\) (\(^{nat}\text{Er}_2\text{O}_3\) dissolved in 7 M HNO\(_3\) and evaporated until dryness at 80°C) and ~10 kBq \(^{171}\text{Tm}\). Afterwards, Thulium(III) nitrate (Tm(NO\(_3\))\(_3\) in 2% w/w HNO\(_3\), Sigma-Aldrich, USA) was added to the solution to simulate the presence of 5 GBq of \(^{165}\text{Tm}\). The resulted solution was mixed with 2.0 mL 0.1 M NH\(_4\)NO\(_3\) and loaded onto the prepared Sykam resin column by means of an Ismatec peristaltic pump. Subsequently, 0.07 M (pH 4.5) \(\alpha\)-HIBA was passed through the column until Tm was eluted. The concentration of \(\alpha\)-HIBA was then increased to 0.11 M, 0.13 M and 1.0 M, respectively, in order to elute Er. Samples (8–16 mL fractions) of \(\alpha\)-HIBA were collected and measured using HPGe detector in combination with the InterWinner software package (version 7.1, Itech Instruments, France) until 3\(\sigma\) uncertainty was below 10\%. The Tm-containing fractions were loaded onto Column 2 (LN3 resin column, 6 mm x 5 mm) in order to concentrate Tm. After loading, the resin was rinsed with 2.0 mL MilliQ water, followed by the elution of Tm in 2.0 mL 0.1 M HCl. The experiment was repeated three more times with the irradiated \(^{nat}\text{Er}_2\text{O}_3\) pellets (60 mg±1) in order to check the relevance of the method towards the real production.

Second, experimental conditions for the efficient separation of \(^{165}\text{Tm}\) from \(^{165}\text{Er}\) were established on Column 3 (Sykam resin column, 6 mm x 50 mm). For this, irradiated \(^{nat}\text{Er}_2\text{O}_3\) was dissolved in 1.0 mL 7.0 M nitric acid (HNO\(_3\), Normapur, VWR
Chemicals, USA), evaporated until dryness at 80°C and left for 1–3 days for the ingrowth of $^{165}$Er ($T_{1/2}=10.4$ h) activity from the obtained $^{165}$Tm ($T_{1/2}=30.1$ h). After the sufficient decay time, the activity was taken with 2.0 mL 0.1 M HCl, mixed with 8.0 mL MilliQ water and 5.0 mL 0.5 M NH$_4$NO$_3$ and was loaded onto the prepared Sykam resin column. During the experiment, the concentration of α-HIBA was increased from 0.04 M to 0.06 M. Samples (6 mL fractions) of α-HIBA were collected and measured using HPGe detector until $3\sigma$ uncertainty was below 10%. The Er-containing fractions were loaded onto Column 4 (LN3 resin column, 6 mm x 5 mm) in order to concentrate Er. After loading, the resin was rinsed with 2.0 mL MilliQ water, followed by the elution of Er in 500 µL 0.1 M HCl.

### 3.2.2.4 Established procedure for $^{165}$Er purification using a specially-designed module

The irradiated target ([$^{166}$Er]Er$_2$O$_3$, 60 mg) was dissolved in 1.0 mL 2.0 M HNO$_3$, mixed with 30.0 mL of MilliQ water and loaded onto Column 1. The $^{165}$Tm separation from the target material was performed with the use of 0.07 M (pH 4.5) α-HIBA as eluent. Concentration of $^{165}$Tm was performed on Column 2 and $^{165}$Tm was eluted in 2.0 mL 0.1 M HCl, which were mixed with 8.0 mL MilliQ water and 5.0 mL 0.5 M NH$_4$NO$_3$. The resulted solution was loaded onto Column 3 (Sykam resin column, 9 mm x 50 mm) after 17–20 h decay of $^{165}$Tm to $^{165}$Er. The $^{165}$Er/$^{165}$Tm separation was performed with the use of 0.04 M and 0.06 M (pH 4.5) α-HIBA as eluent, respectively. $^{165}$Er was loaded onto Column 4 (LN3 resin column, 6 mm x 5 mm), followed by the elution of the final product ([$^{165}$Er]ErCl$_3$) in 500 µL 0.1 M HCl. The rest of $^{165}$Tm activity was eluted into a falcon tube, left for the 20–22 h decay to $^{165}$Er and separation on Column 3 and elution of [[$^{165}$Er]ErCl$_3$ from Column 4 was repeated.

### 3.2.2.5 Characterization of the $^{165}$Er product

**Radionuclidic purity:** The identification and radionuclidic purity of the obtained $^{165}$Er were examined by γ–ray spectrometry using the HPGe detector mentioned above. The aliquot of the final product, containing 0.5–1 MBq of $^{165}$Er, was measured on HPGe until the $3\sigma$ uncertainty was below 5%.

**Radiochemical purity:** The radiochemical purity of the final product was determined by means of radio thin layer chromatography (radio TLC) using a procedure...
Radiolabeling yield: Radiolabeling of DOTA-NOC (ABX GmbH, Germany) at a molar activity of 30 MBq/nmol was performed in order to evaluate the success of the purification process. The radiolabeling was performed as described in detail in Chapter 2 of this thesis. The radiolabeling procedure was repeated after 1.5T\textsubscript{1/2} decay time.

3.2.3 Results

3.2.3.1 Experimental yields of \(^{165}\text{Er}\), produced via \(^{166}\text{Er}(p,2n)^{165}\text{Tm}\rightarrow^{165}\text{Er}\) nuclear reaction, and co-produced impurities

Irradiation of the 60±1 mg \(^{166}\text{Er}\)\textsubscript{2}O\textsubscript{3} targets under the chosen conditions (8 h irradiation time, 22.8 MeV proton beam energy, 20 \(\mu\)A beam intensity) resulted in the production of \(~1.5–3\) GBq \(^{165}\text{Tm}\). The experimentally obtained production yields are \(~4–7\) times lower as compared to the theoretically calculated (Equation 3.1.1). The radionuclides, determined after 10 days EOB of the \(^{166}\text{Er}\)\textsubscript{2}O\textsubscript{3} target, are shown in Figure 3.2.3. The long waiting time after EOB was introduced due to the high dose rates, measured on the target at EOB (~100 mSv/h). The co-produced short-lived Tm isotopes – \(^{164}\text{Tm}\) (T\textsubscript{1/2}=2.0 min) and \(^{166}\text{Tm}\) (T\textsubscript{1/2}=7.7 h) were not detected due to the long waiting time after EOB as compared to the radionuclide’s half-life. However, Tm isotopes with longer half-life (\(^{167}\text{Tm}\) (T\textsubscript{1/2}=9.3 d) and \(^{168}\text{Tm}\) (T\textsubscript{1/2}=93.1 d)), were determined at \(^{165}\text{Tm}\)-to-\(^{167}\text{Tm}\) and \(^{165}\text{Tm}\)-to-\(^{168}\text{Tm}\) ratio being 3600-to-1 and 300000-to-1, respectively, at EOB (activities were corrected based on the radioactive decay law). The mentioned Tm isotopes could be eluted during the purification process from Column 1 together with \(^{165}\text{Tm}\) and lead to the contamination of \(^{165}\text{Er}\) final product with stable \(^{167/168}\text{Er}\) due to the decay of these Tm isotopes to stable Er isotopes. The masses of the stable Er isotopes, arising from \(^{167}\text{Tm}\) and \(^{168}\text{Tm}\) after 30 h waiting time after EOB (corresponds to the obtaining \(^{165}\text{Er}\) after EOB) are presented in Table 3.2.1. The masses were calculated based on the radioactive decay law and Equation 3.2.1, taking into account the number of atoms of the parent radionuclide \(N_1^0\) (at t=0), the decay time (t=30 h) and the radioactive decay constant (\(\lambda_1\)) of the parent radionuclide.

\[
N_2 = N_1^0\left(1 - e^{-\lambda_1 t}\right) \quad (3.2.1)
\]
Table 3.2.1. Possible stable isotopes of Er, arising from the co-produced Tm isotopes during the bombardment of $^{166}\text{Er}\text{Er}_2\text{O}_3$ targets under chosen irradiation conditions. The mass of 500 MBq $^{165}\text{Er}$, obtained after the purification process, corresponds to 7.4 ng.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$T_{1/2}$</th>
<th>Activity (EOB)</th>
<th>Decay product</th>
<th>Mass of the decay product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{167}\text{Tm}$</td>
<td>9.3 d</td>
<td>3 MBq</td>
<td>$^{167}\text{Er}$ (stable)</td>
<td>0.1 ng</td>
</tr>
<tr>
<td>$^{168}\text{Tm}$</td>
<td>93.1 d</td>
<td>0.1 MBq</td>
<td>$^{168}\text{Er}$ (stable)</td>
<td>0.01 ng</td>
</tr>
</tbody>
</table>

Figure 3.2.3. Gamma spectrum of the irradiated $^{166}\text{Er}\text{Er}_2\text{O}_3$ target material 10 days after EOB. Peaks with energies ≤60 keV correspond to X-rays from the detected radiolanthanides and from $^{165}\text{Er}$.

3.2.3.2 Development of the Er/Tm separation procedure

The idea of the $^{165}\text{Er}$ purification, produced via $^{166}\text{Er}(p,2n)^{165}\text{Tm} \rightarrow ^{165}\text{Er}$ nuclear reaction, is shown in Figure 3.2.4. The chosen indirect production route requires two separations: Separation 1 – ng amounts of $^{165}\text{Tm}$ from mg amounts of $^{166}\text{Er}$ and Separation 2 – ng amounts of $^{165}\text{Tm}$ from ng amounts of $^{165}\text{Er}$. Therefore, two Sykam resin columns of different dimensions, 10 mm x 160 mm (Column 1) or 6 mm x 50 mm (Column 3) (chosen based on the amount of the lanthanide used in the experiments) were applied for the particular separation. In between Column 1 and Column 3, a small
LN3 resin column of 6 mm x 5 mm dimension (Column 2) was introduced in order to concentrate $^{165}\text{Tm}$ and to obtain it in the chemical form suitable for loading onto the cation exchange resin column – Column 3. An LN3 resin column of the same dimension (Column 4) was applied at the end of the process for the concentration of the final product ($[^{165}\text{Er}]\text{ErCl}_3$) and elution in a small volume of dilute acid, appropriate for preclinical research.

![Diagram of purification process](image)

**Figure 3.2.4.** Schematic illustration of the $^{165}\text{Er}$ purification process.

Initially, appropriate conditions for the separation of Tm from up to 200 mg of $^{\text{nat}}\text{Er}_2\text{O}_3$ were established on Column 1. Gradient elution with the use of 0.07–0.13 M (pH 4.5) $\alpha$-HIBA as eluent resulted in the efficient separation of Tm from Er (Figure 3.2.5). Tm was eluted with 0.07 M $\alpha$-HIBA in ~7 h from the start of the purification process, with 40 mL gap between the neighboring Tm and Er lanthanides. Afterwards, $\alpha$-HIBA concentration was gradually increased until 0.11 M and 0.13 M, respectively, in order to study the behavior of Er under chosen experimental conditions. $^{166}\text{Ho}$ was a co-produced impurity from the neutron activation of $^{165}\text{Ho}$, coming from Er(NO$_3$)$_3$, via $^{165}\text{Ho}(n, \gamma)^{166}\text{Ho}$ nuclear reaction and was eluted with 1 M $\alpha$-HIBA.
After the conditions for appropriate Tm/Er separation were determined (0.07 M α-HIBA, pH 4.5; 0.8 mL/min flow rate) with the corresponding tracers, these conditions were applied for the Tm separation from the irradiated natEr2O3 target (60 mg) (Figure 3.2.6). The experiment was performed in order to simulate the load step onto Column 1 for the real 165Er production (Tm and Er are taken with 1.0 mL 2.0 M HNO3, mixed with 30.0 mL of MilliQ water, pH~1.5), which is different from the load step of the bench test described above (Tm and Er were taken with 2.0 mL 0.1 M NH4NO3, pH~3). Nevertheless, Tm was effectively separated from 60 mg natEr2O3. Due to the fact that natEr2O3 contains several natEr isotopes with the highest abundance of 166Er (33.5%), 167Er (22.9%) and 168Er (26.9%), the corresponding Tm isotopes were produced via XEr(p,n)X−1Tm or XEr(p,2n)X−1Tm nuclear reactions after the proton irradiation of the natEr2O3 target. In Figure 3.2.6, only 165Tm is outlined as the isotope of interest in the current work. A significant tailing of the Er peak was observed due to the decay of 165Tm to 165Er during the separation process. However, this zone broadening had no effect on the purity of the Tm peak.

Figure 3.2.5. Elution profile of 171/natTm separation from 171/natEr (200 mg) and co-produced impurity 166Ho on Column 1 (10 mm x 160 mm Sykam resin column, 0.8 mL/min eluent flow rate).
Elution profile of $^{165/166/167/168}$Tm separation from $^{165}$Er (60 mg) on Column 1 (10 mm x 160 mm Sykam resin column, 0.8 mL/min eluent flow rate). Before loading Column 3, $^{165}$Tm should be left for 17–25 h to decay (corresponds to 32.4–43.8% decay of the $^{165}$Tm activity) for the ingrowth of the $^{165}$Er daughter radionuclide (Figure 3.2.7). The maximum $^{165}$Er activity value will be obtained after 24 h waiting time (Equation 3.2.1). After the sufficient decay time,
\(^{165}\text{Er}/^{165}\text{Tm}\) separation can be performed on Column 3 in order to obtain radionuclidically pure \(^{165}\text{Er}\) final product.

\[
t_{\text{max}} = \frac{\ln(\lambda_2/\lambda_1)}{\lambda_2 - \lambda_1} \quad (3.2.1)
\]

Figure 3.2.7. Ingrowth of \(^{165}\text{Er}\) activity from \(^{165}\text{Tm}\) over a 40-h period. Activities were calculated based on the radioactive decay law.

After loading the Tm/Er solution on Column 3, the next step was to establish experimental conditions to achieve efficient separation of \(^{165}\text{Er}\) from \(^{165}\text{Tm}\). The dimensions of the Column 3 were significantly decreased as compared to Column 1 due to the decrease of Er mass, required for the purification (from 60–200 mg of Er to ng amounts (e.g., 1.0 GBq of \(^{165}\text{Er}\) corresponds to 14.8 ng)). Gradient elution with the use of 0.04–0.06 M (pH 4.5) \(\alpha\)-HIBA as eluent resulted in the efficient separation of Er from Tm (Figure 3.2.8). Er fractions were loaded on Column 4 and eluted with 500 \(\mu\)L 0.1 M HCl.
Successful performance of the experiments on bench allowed to establish procedure for the $^{165}$Er purification process, which was translated to a designed purification module. The module was introduced to the hot cell and high $^{165}$Tm/$^{165}$Er activities (GBq amounts) were processed. During the first production and purification on the module inside the hot cell, $^{165}$Er was eluted with a radionuclidic purity of only 92% (8% of $^{165}$Tm in the final product). For this reason, the dimensions of Column 3 were changed from 6 mm x 50 mm to 9 mm x 50 mm. This allowed to obtain $^{165}$Er with radionuclidic purity being >99%.

### 3.2.3.3 $[^{165}\text{Er}]\text{ErCl}_3$ product data

The successful performance of the bench experiments ensured the establishment of a procedure for the $^{165}$Er purification process, which was translated to a specially-designed purification module. The module was introduced to the hot cell and high $^{165}$Tm/$^{165}$Er activities (GBq amounts) were processed. During the first production and purification on the module inside the hot cell, $^{165}$Er was eluted with a radionuclidic purity of only 92% (8% of $^{165}$Tm in the final product). For this reason, the dimensions of Column 3 were changed from 6 mm x 50 mm to 9 mm x 50 mm. This allowed to obtain $^{165}$Er with radionuclidic purity of >99% (Figure 3.2.9). The radiolabeling yield
of $[^{165}\text{Er}]\text{Er-DOTA-NOC}$ showed $\geq 99\%$ efficiency at 30 MBq/nmol (Figure 3.2.10).

**Figure 3.2.9.** Gamma spectrum of $^{165}\text{Er}$, obtained after the purification process.

**Figure 3.2.10.** HPLC chromatogram of $[^{165}\text{Er}]\text{Er-DOTA-NOC}$ (2.3 min retention time would indicate “free” non-labeled $^{165}\text{Er}$ and 9.3 min indicates $[^{165}\text{Er}]\text{Er-DOTA-NOC}$).
3.2.4 Discussion

Production of $^{165}\text{Er}$ via $^{166}\text{Er}(p,2n)^{165}\text{Tm}\rightarrow^{165}\text{Er}$ nuclear reaction was performed at 22.8 MeV proton beam ($\sim$1 b cross-section) based on the excitation function reported by Sadeghi et al. [39]. The highest cross-section of 1.2 b was reported at 21 MeV proton beam energy, however, at the Injector 2 PSI cyclotron the reduction of the beam energy within the 20–23 MeV beam energy window is only possible to 22.8 MeV. At the chosen proton beam energy, $^{167}\text{Tm}$ and $^{168}\text{Tm}$ isotopes are co-produced together with $^{165}\text{Tm}$ and decay to stable $^{167}\text{Er}$ and $^{168}\text{Er}$, respectively. Due to the relatively long half-life of these Tm isotopes, they are eluted together with $^{165}\text{Tm}$ from Column 1 of the purification module, followed by the decay to the stable Er isotopes. As a result, stable Er isotopes will be present in the $^{165}\text{Er}$ final product. However, the amount of $^{167}\text{Er}$ and $^{168}\text{Er}$ in the final product is $\sim$0.1 ng as compared to $\sim$7 ng of $^{165}\text{Er}$ at EOS and will not dramatically decrease the formation yield of $[^{165}\text{Er}]\text{DOTA-NOC}$ during the radiolabeling process. At the chosen proton energy beam, the co-production of $^{164}\text{Tm}$ ($T_{1/2}=2.0$ min) is possible, which decays to stable $^{164}\text{Er}$. In order to avoid contamination of $^{165}\text{Tm}$ and as a result of the final $^{165}\text{Er}$ product with $^{164}\text{Er}$, the short waiting time after EOB should be introduced before the performance of Tm/Er separation. In the developed purification process, the waiting time between these two steps was $\sim$1 h due to the target dissolving and the loading of the column. The waiting time of 1 h decreased $^{164}\text{Tm}$ activity $10^9$ times and allows achieving appropriate Tm/Er separation.

In order to obtain pure $^{165}\text{Er}$, the purification method was developed based on the $^{161}\text{Tb}$ experience, described in Chapter 2 of this thesis as well as in [133]. However, due to the necessity of two chemical separations as a result of the indirect production route ($^{166}\text{Er}(p,2n)^{165}\text{Tm}\rightarrow^{165}\text{Er}$), the method was further developed to a 4-column method (Figure 3.2.4). The established purification method is based on the combination of cation exchange (separation of the neighboring lanthanides Er/Tm) and extraction chromatography (concentration of the product) and ensured radionuclidically and radiochemically pure $^{165}\text{Er}$ after $\sim$30 h EOB. The drawback of the purification process is the application of the cation exchange resin column as Column 3, serving for $^{165}\text{Er}^{165}\text{Tm}$ separation after 17–25 h activity ingrowth of $^{165}\text{Er}$ from $^{165}\text{Tm}$. Lanthanides are eluted from the cation exchange resin column in order of decreasing the atomic number [72, 133], meaning $^{165}\text{Tm}$ will be eluted from the Column 3 before $^{165}\text{Er}$. However, after 17–25 h waiting time, $\sim$60% of $^{165}\text{Tm}$ is still left. In order to be able to
use the remained $^{165}$Tm activity, the radionuclide should be transported to the separate vessel, afterwards loaded on Column 3 and $^{165}$Er/$^{165}$Tm separation should be performed one more time (~5 h time of process). To avoid the repetition of the loading, LN3 extraction resin was thought to be applied as Column 3 that would allow elution of $^{165}$Er in a small volume of low concentrated HCl before $^{165}$Tm. Therefore, the column could be used as a generator for milking $^{165}$Er after the sufficient decay of $^{165}$Tm. However, the elution of radionuclidically pure $^{165}$Er from the LN3 resin column (46 mm x 6 mm) was only possible in ~100 mL volume of 0.04 M HCl [144]. The $^{165}$Er/$^{165}$Tm separation based on the Szilard-Chalmers effect in macrocyclic ligands (e.g., DOTA) [145, 146] could be a good option to use Column 3 as a generator. This would allow the shortening of the 4-column purification method to 3-column method with the use of Column 3 as a generator.

Due to the relatively high costs of the enriched $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ target material (98.1% enrichment, $\$2150$ for $1000 \text{ mg}$, Isoflex, USA), the recycling procedure would be beneficial. The co-production of $^{164}$Tm during the bombardment of $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ targets and its decay to “cold” $^{164}$Er would insignificantly decrease the level of the target enrichment. For example, after the recycling of 1000 mg of the irradiated $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ target material, the original mass of $^{164}$Er (20 mg as determined by the supplier) would increase to ~20.2 mg (theoretical calculations based on Equation 3.1.1 and radioactive decay law). The recycling of the irradiated $[^{166}\text{Er}]\text{Er}_2\text{O}_3$ targets was not performed so far, however, the experiments are ongoing. Preliminary research in order to determine the radioactive impurities within the target recycling solution has been done. After $^{165}$Tm elution from Column 1 to Column 2, ~80 mL 1.0 M $\alpha$-HIBA (pH 4.5) and 20 mL 4.0 M HCl were passed through Column 1 to the bottle for collection of the recycling solution. After 2 weeks waiting time after the production of $^{165}$Er, 10 mL aliquot from ~400 mL recycling solution (collected after 4 $^{165}$Er productions in total) was measured on HPGe until the 3σ uncertainty was below 7% (Figure 3.2.11). The determined radionuclides with the corresponding activities are shown in Table 3.2.1. In order to understand, whether the recycled material, containing few kBq amount of activity is allowed to handle for the target preparation and irradiation, the discussion and necessary calculations should be performed in future. Nevertheless, elution of $^{166}$Er from Column 1 with 0.11/0.13 M HIBA (Figure 3.2.5) would be an option to eliminate the presence of the radionuclides in the recycling solution.
Figure 3.2.11. Gamma spectrum of the $^{166}$Er recycling solution (90 min measurement time; 1 cm distance from the detector). Peaks with energies ≤60 keV correspond to X-rays from the detected radiolanthanides.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>$^{147}$Eu</th>
<th>$^{148}$Eu</th>
<th>$^{167}$Tm</th>
<th>$^{56}$Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$</td>
<td>24.6 d</td>
<td>55.6 d</td>
<td>9.3 d</td>
<td>77.3 d</td>
</tr>
<tr>
<td>Activity</td>
<td>7.2 kBq</td>
<td>2.4 kBq</td>
<td>5.2 kBq</td>
<td>0.52 kBq</td>
</tr>
<tr>
<td>(2 weeks EOS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.5 Conclusion

The production of $^{165}$Er from 98.1% enriched $[^{166}$Er]$\text{Er}_2\text{O}_3$ targets (60 mg) via $^{166}$Er(p,2n)$^{165}$Er nuclear reaction provided ~1.5–3 GBq of $^{165}$Tm which decays with a half-life of 30.1 h to $^{165}$Er. Due to the indirect route of the production of $^{165}$Er, two separations are required which were successfully performed with a developed 4-column purification method. The resultant purification process ensured a yield of ~500 MBq radionuclidically and radiochemically pure $^{165}$Er ~30 h after EOB. The radiolabeling of DOTA-NOC with $^{165}$Er after EOS at 30 MBq/nmol molar activity was >99%. The amount and quality of the obtained $^{165}$Er is sufficient for starting in vitro experiments in order to determine the ability of pure Auger–electron emitter $^{165}$Er to kill cancer cells.
Chapter 4

Purification of $^{149}$Tb samples from Isotope Separation Online (ISOL) collections


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The chapter will be published in a modified version.

Author contributions:

Nadezda Gracheva designed and carried out the experiments, analyzed and interpreted the data and wrote the chapter. Ulli Köster and Karl Johnston supervised the isobar collections. Roger Hasler supported with the purification of $^{149}$Tb in the hot cell and contributed to the writing of the manuscript. Zeynep Talip contributed to the writing of the manuscript. Roger Schibli reviewed the manuscript. Cristina Müller supervised the radiolabeling for preclinical work and co-led the project. Nicholas van der Meulen co-led the project, supervised the study, supported with the handling and purification of the radionuclide in the hot cell and revised the manuscript.
4.1 Introduction

Targeted Radionuclide Therapy (TRNT) is increasing in popularity towards cancer treatment in the 21st century. While β−-therapy is predominantly used, interest in the use of α–emitters (so-called targeted alpha-therapy (TAT)) is on the rise. Alpha-emitters possess short tissue penetration ranges (40–100 µm) and high linear energy transfer (LET; ~80 keV/µm), which results in relative biological effectiveness (RBE) 3–7 times higher as compared to β−-emitters [16, 30, 147]. These characteristics make α−-emitting radionuclides attractive for treatment of single cells or tumor clusters [148].

Several α–emitters have been already developed to the stage of Phase I/Phase II clinical trials (e.g., 225Ac, 213Bi), while [223Ra]RaCl2 (Xofigo®) has been approved by the U.S. FDA and implemented into clinical practice. Xofigo® has shown success as a palliative agent in patients having prostate cancer metastasized to the bones and showed patients’ life extension as a sign of an antitumor effect [149]. Pilot studies on [225Ac]Ac-PSMA-617 showed that the therapy can be considered promising for the heavily pre-treated metastatic castration-resistant prostate cancer (mCRPC) patients and chemotherapy-naive patients with advanced metastatic prostate carcinoma [22, 150]. The therapeutic doses of [225Ac]Ac-PSMA-617 (~10 MBq) were hundred times lower as compared to [177Lu]Lu-PSMA-617 in order to observe the response to the treatment. Graf et al. showed that DNA damage and cell death caused by [225Ac]Ac-DOTA-TOC is similar to [177Lu]Lu-DOTA-TOC when applying 700 times less 225Ac activity as compared to 177Lu, indicating remarkable efficacy of α–emitters towards TRNT [151]. [213Bi]Bi-DOTA-TOC peptide receptor α−-therapy was found to be highly effective for targeting neuroendocrine tumors (NET) and metastases in 14 patients with neuroendocrine liver metastases resistant to β−-therapy [152]. 213Bi was proved to be efficient for killing small cell clusters (spheroids with 80–100 µm diameter), resulting in complete regression of such spheroids [153]. The application of α−-emitters for treatment of solid tumors is possible due to the potential role of the bystander effect and high toxicity of alpha radiation to the capillary cells of the tumor, resulting in the shutting down of capillaries and tumor regression (so-called tumor antivascular alpha therapy (TAVAT)) [147, 154, 155].

The main disadvantage of several α–emitters, however, is their decay chain, via several subsequent alphas, to its stable daughter [45]. Each α−-decay is accompanied by the alpha recoil, with the kinetic energy of the nucleus being ~100 keV [156]. As a
result, the recoil daughter will break chemical bonds with the biomolecule (binding energies <5 eV [157]) and free the radionuclide, possibly resulting in increased toxicity to normal tissue [158]. For this reason, the use of α-emitting radionuclides containing no α-emitting daughters in the decay chain is preferable. Furthermore, the maximum tolerated dose (MTD) for α-emitters which have no additional α-decay emissions is higher, which may result in higher therapeutic efficacy [159].

One of the very few radionuclides of this type is the partial α-emitting $^{149}$Tb ($T_{1/2}$=4.1 h, $E_\alpha$=3.98 MeV (16.7%), 28 µm range in tissue), which was proposed as a candidate for TAT in 1996 [31, 160]. Beyer et al. evaluated the ability of $^{149}$Tb-Tb-rituximab to kill circulating single cancer cells in a leukemia mouse model [31]. The mice showed no signs of disease during the period of study (120 days) and were found to be tumor free at dissection. Allen et al. showed the possibility of $^{149}$Tb-Tb-immunoconjugate application (c.30.6) for the treatment of colorectal cancer [161]. A preclinical pilot study using a $^{149}$Tb-labeled DOTA-folate conjugate demonstrated significant delay in tumor growth, with the average survival time of the treated mice being 43 days as compared to untreated controls, which survived 21 days on average [162]. The short half-life of $^{149}$Tb ($T_{1/2}$=4.1 h) is an advantage when targeting cells in the vascular system and lymphatic system, as these cells can be reached quickly [147, 159]. Positron emissions of $^{149}$Tb ($E_{\beta^+}\text{mean}=730$ keV (7.1%)) were found to be suitable for PET imaging, demonstrating radiotheranostic application of the radionuclide [163]. $^{149}$Tb is stably coordinated with a DOTA chelator, as was previously demonstrated for $^{149}$Tb-cm09 [162], therefore, the radionuclide can be used in combination with $^{177}$Lu targeting agents which are in clinical use (e.g., DOTA-TOC, DOTA-TATE) or clinical trials (e.g., PSMA-617).

Despite the superior characteristics and demonstrated efficacy of $^{149}$Tb, preclinical research remains poor due to the limited availability of the radionuclide. The production of $^{149}$Tb (as well as its PET diagnostic counterpart, $^{152}$Tb) remains the major problem for large-scale scientific and clinical application. Both $^{149}$Tb and $^{152}$Tb are neutron-deficient Tb radioisotopes, which are obtained by spallation of Ta targets after bombardment with ≥500 MeV proton beam, followed by online mass separation. New centers, which may offer the possibility of “exotic” radionuclide production (such as $^{149}$Tb and $^{152}$Tb) for pilot investigations in patients, however, are under construction (MEDICIS at CERN, Switzerland; ISOL@MYRRHA, Belgium; ARIEL-TRIUMF, Canada; FRIB, USA). ISOL facilities currently in operation (ISOLDE-CERN,
Switzerland; ISAC-TRIUMF, Canada) are primarily physics research facilities, where researchers from different disciplines compete for beam time; all proof-of-principle batches used to date have been produced as a result of such research proposals which explains the limited beam time available for such purposes.

Herein, we report on the production of $^{149}$Tb from collections obtained at the ISOLDE facility (CERN, Geneva, Switzerland), as well as collection and chemical separation updates, based on the purification of $^{161}$Tb and $^{152}$Tb radioisotopes previously reported by our group [133, 164]. The final product ($^{149}$Tb in chloride solution) was analyzed for radionuclidic and radiochemical purity.

4.2 Material and Methods

4.2.1 Production of $^{149}$Tb at ISOLDE facility

$^{149}$Tb was produced by proton-induced spallation of tantalum targets, using the online isotope separator facility ISOLDE at CERN (Geneva, Switzerland), as previously reported [21]. Briefly, a tantalum foil target (94 g/cm$^2$) was irradiated with 1.4 GeV protons from the CERN PS-Booster accelerator. The spallation products were released from the target material, kept at ~2100 ºC, and ionized by a resonance ionization laser ion source tuned to dysprosium, the radioactive precursor of neutron-deficient terbium isotopes [25, 26]. The singly charged ion beam was accelerated to 30 keV and mass-separated in a magnetic sector field at A=149. The isobars ($^{149}$Dy, $^{149}$Tb, $^{149}$Gd, $^{149}$Eu) and molecular pseudo-isobars ($^{133}$PrO$^+$, $^{133}$CeO$^+$ and $^{133}$LaO$^+$) were implanted into zinc-coated (prepared by Ar ion beam sputtering) gold foils (8 mm x 40 mm, 0.1 mm thick, Goodfellow Cambridge Ltd. Huntingdon, UK) or zinc foils (8 mm x 40 mm, 0.1 mm thick, 99.994%, Alfa Aesar, USA). Gold foils were used as a well-established collection material, along with the previously developed chemical separation [24]. Zn foils were tested as a part of the development process, in order to simplify the preparation of the collection foils (avoid sputtering) and prevent activity lost once $^{149}$Tb is implanted into the gold.

4.2.2 Development of the process for $^{149}$Tb purification

The $^{149}$Tb purification development was performed based on a previously-established method performed at Paul Scherrer Institute (PSI), Switzerland, as well as a method established for $^{161}$Tb production [133]. This implied the use of the Sykam
cation exchange resin column (Column 1) with the addition of a second column containing LN3 extraction resin (Column 2) for concentration purposes and final elution in the desired chemical form and volume.

When using collections with Zn-coated gold foils, the method was combined with the purification process previously developed for $^{149/152}$Tb and resulted in the following procedure. The zinc layer containing $^{149}$Tb was dissolved in 0.1 M NH$_4$NO$_3$ at 80°C. The solution was loaded onto Column 1 (6 mm × 50 mm), followed by the $^{149}$Tb separation from Zn and isobars A=149 by gradient elution with the use of 0.07–0.13 M $\alpha$-HIBA (pH 4.5). The desired $^{149}$Tb product was concentrated by passing the eluent from Column 1 through Column 2 (6 mm x 5 mm) and, finally, eluted with 500 µL 0.05 M HCl. The purification procedure was applied towards the separation of $^{149}$Tb on the constructed purification module.

In order to adapt the described method for the application of Zn foils as a catcher material, bench experiments with the use of radioactive tracers were performed. Long-lived radioactive tracers ($^{65}$Zn (T$_{1/2}$=244.3 d), $^{141}$Ce (T$_{1/2}$=32.5 d) and $^{153}$Gd (T$_{1/2}$=239.5 d) [110]) were provided by the Laboratory of Radiochemistry at PSI. $^{161}$Tb (T$_{1/2}$=6.9 d) was obtained as previously reported [133]. Activities of the tracers used for the bench experiments ranged from 1–200 kBq. Different masses of Zn(NO$_3$)$_2$ (10–660 mg) were studied in order to determine the influence of the mass of the proposed Zn collection foil on the chemical separation process.

The method, determined from the bench experiments, was tested for the separation of $^{149}$Tb on the purification module used previously. The catcher material (Zn foil) was dissolved in 4.0 mL 7.0 M HNO$_3$, followed by the addition of 5.0 mL MilliQ and 200 µL ammonia to adjust the pH to ~2. The solution was loaded onto a column (10 mm x 80 mm) containing Sykam cation exchange resin such that $^{149}$Tb could be separated from the collection material (Zn) and A=149 isobars. The column was rinsed with 20.0 mL 0.5 M NH$_4$NO$_3$ and the separation performed by gradient elution with 0.07–0.13 M $\alpha$-HIBA (pH 4.5) as eluent. The concentration of the $^{149}$Tb product was carried out using LN3 extraction resin (6 mm x 25 mm). The final product ([$^{149}$Tb]TbCl$_3$) was eluted in 650–900 µL 0.1 M hydrochloric acid (HCl, Suprapur, Merck, Germany).
4.2.3 Characterization of the purity of the $^{149}\text{Tb}$ product

Radionuclidic purity: 10–30 μL aliquots of the final product ($[^{149}\text{Tb}]\text{TbCl}_3$) was analyzed for identification and radionuclidic purity by means of $\gamma$–ray spectrometry, using a high-purity germanium (HPGe) detector (Canberra, France) in combination with the InterWinner software package (version 7.1, Itech Instruments, France) with a $3\sigma$ uncertainty below 5%.

Radiochemical purity: The radiochemical purity of the final product was determined by means of radio thin layer chromatography (radio TLC) using a procedure, described in Chapter 2 of this thesis.

Radiolabeling yield: The radiolabeling of PSMA-617 (1 mM stock solution, ABX GmbH, Germany) was performed at molar activity of 6 MBq/nmol as described in detail in Chapter 2 of this thesis.

4.3 Results

4.3.1 Characterization of $^{149}\text{Tb}$ samples after the production at ISOLDE

The production resulted in the collection of three samples (1-3) into zinc foils and two more samples (4-5) into zinc-coated gold foils. Each collection was aimed at obtaining ~200 MBq $^{149}\text{Tb}$, along with other A=149 isobars and A=133 pseudo-isobars. The radioactive isobars (Ln corresponds to lanthanides), determined in one $^{149}\text{Tb}$ sample after collection, are shown in Figure 4.1, while $^{149}\text{Tb}$-to-Ln activity ratios are reported in Table 4.1. The sample aliquot was measured 29 hours after collection due to the sample only being addressed after performing the $^{149}\text{Tb}$ chemical separation. $^{133}\text{Ce}$ ($T_{1/2}$=97 min) was not identified in the $^{149}\text{Tb}$ fraction, due to the short half-life of the radionuclide and long waiting time after the collection at the ISOLDE facility. $^{133}\text{La}$, which stems from decay of the $^{133}\text{PrO}^+$ and $^{133}\text{CeO}^+$ in the collected $^{149}\text{Tb}$ fraction, was not detected in the measured aliquot due to the low intensities of the $^{133}\text{La}$ $\gamma$–lines compared to the Compton continuum (Figure 4.1).
Figure 4.1. Gamma spectrum of $^{149}$Tb, obtained from CERN-ISOLDE after the implantation into a zinc-foil. The spectrum was obtained 29 h after the collection (1.2 h measurement time; 24 cm distance from the detector), the ratios $^{149}$Tb-to-Ln, given in Table 4.1, are based on this measurement.

Table 4.1. Information about several $^{149}$Tb batches, delivered from ISOLDE facility and separated at PSI

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>$T_{1/2}$</th>
<th>$^{149}$Tb-to-Ln ratio before the separation</th>
<th>$^{149}$Tb-to-Ln ratio at EOS*</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{149}$Tb</td>
<td>4.1 h</td>
<td>1</td>
<td>1</td>
<td>decay product</td>
</tr>
<tr>
<td>$^{149}$Gd</td>
<td>9.3 d</td>
<td>6-to-1</td>
<td>108-to-1</td>
<td>decay product</td>
</tr>
<tr>
<td>$^{145}$Eu</td>
<td>5.9 d</td>
<td>18-to-1</td>
<td>not detected</td>
<td>decay product</td>
</tr>
<tr>
<td>$^{133m}$CeO*</td>
<td>5.1 h</td>
<td>440-to-1</td>
<td>not detected</td>
<td>pseudo-isobar</td>
</tr>
</tbody>
</table>

* EOS refers to End of Separation

4.3.2 Development of the $^{149}$Tb purification process on bench

The procedure for the separation of Tb from Gd and Ce tracers (to simulate the presence of A=149 isobars and pseudo-isobars) was determined in the presence of 10–660 mg of Zn(NO$_3$)$_2$. The broad range of the masses was chosen in order to estimate the thickness of the Zn foil (8 mm x 40 mm), which can be used for $^{149}$Tb collections (e.g., 0.25 mm thick Zn foil of the abovementioned dimensions corresponds to 660 mg;
0.1 mm thick foil corresponds to 350 mg).

The separation of Tb from Gd, Ce and 10 mg of Zn on the small Sykam resin column (6 mm x 50 mm dimension), using gradient elution of 0.07–1 M α-HIBA, resulted in the efficient separation of Tb from Gd and Zn. A significant broadening of the Zn peak was observed with only 8 mL gap from the last eluted Tb fraction (Figure 4.2), however, which implied that the possibility of efficient Tb separation from higher amounts of Zn was doubtful.

![Figure 4.2. Elution profile of $^{161}$Tb separation from $^{153}$Gd, $^{141}$Ce and $^{65}$natZn (6 mm x 50 mm Sykam resin column; 10 mg natZn; 0.07–0.09 M α-HIBA at flow rate 0.6 mL/min; 0.11–1 M α-HIBA at flow rate 0.5 mL/min).](image)

The experiment was repeated with 660 mg of Zn and the Sykam column dimensions were increased (10 mm x 80 mm). After loading the mixture of tracers and zinc onto the Sykam column, 20.0 mL 0.5 M NH$_4$NO$_3$ was passed through the column. With this rinse step, 85% of the Zn was eluted before the start of the separation. The remaining Zn could be eluted with $^{153}$Gd and $^{161}$Tb, obtained by our group and containing ≤0.007% of $^{160}$Tb. Both $^{160}$Tb and $^{65}$Zn emit 1115 keV γ–line, making the precise quantification of $^{65}$Zn impossible when containing low activities of each radionuclide in one measured fraction. Only 38.2% of the total Tb activity was eluted as a pure Tb fraction (absence of Gd tracer) as a result of a significant overlapping of
Tb and Gd peaks (Figure 4.3). This implied that the separation conditions were inappropriate for Tb/Gd separation. The pure Tb fraction from the experiment was eluted from Column 1 and loaded on Column 2 (LN3 resin; 6 mm x 5 mm). Terbium breakthrough of 89.7% was observed, even though the mass of the LN3 resin (~120 mg) was determined as sufficient to retain the amount of Tb tracer used. This implied the presence of “cold” Zn in the load solution (no other natural impurities were present), which occupied the available functional groups of the resin. $^{65}\text{Zn}$ in the Tb fraction was not identified using HPGe detector probably due to the Zn activity being less as compared to $^{160}\text{ Tb}$ activity (~20 Bq). Therefore, the overlapping of 1115 keV $\gamma$–line from both $^{65}\text{Zn}$ and $^{160}\text{ Tb}$ made impossible visualization of $^{65}\text{Zn}$.

![Figure 4.3. Elution profile of $^{161}\text{Tb}$ separation from $^{153}\text{Gd}$, $^{141}\text{Ce}$ and $^{65/\text{nat}}\text{Zn}$ (10 mm x 80 mm Sykam resin column; 660 mg $^{65/\text{nat}}\text{Zn}$; 0.07–0.09 M $\alpha$-HIBA – 1 mL/min flow rate; 0.11–1 M $\alpha$-HIBA–0.8 mL/min flow rate).]

More efficient separation of Tb from Gd and 660 mg Zn using Sykam cation exchange resin could be achieved by further increasing the length of the column, however, this is an undesirable alternative as the 2-column process (target dissolving, separation on the Sykam resin column (10 mm x 80 mm), concentration on the LN3 resin and final elution) took ~5 hours to perform. The mass of zinc was decreased to 350 mg, which corresponds to the mass of a zinc foil 8 mm x 40 mm and 0.1 mm thick,
and the experiment was repeated without column elongation (Figure 4.4). An appropriate separation of Tb from Gd, Ce and Zn was achieved on the Sykam resin column under chosen experimental conditions. Zn (97.5%) was eluted with 20 mL 0.5 M NH₄NO₃ before the start of the lanthanide separation, while 1.5% was eluted with 0.07–0.11 M α-HIBA before Tb was eluted. The process was repeated a further two times in order to confirm the results. Once confirmed, these conditions were chosen for the separation of ¹⁴⁹Tb from Zn and possible isobars.

Figure 4.4. Elution profile of ¹⁶¹Tb separation from ¹⁵₃Gd, ¹⁴¹Ce and ⁶⁵natZn (10 mm x 80 mm Sykam resin column; 1 mL/min eluent flow rate; 350 mg natZn).

Fractions containing Tb tracer were loaded onto the LN3 resin column. The length of the column was increased from 5 mm to 25 mm in order to avoid breakthrough of Tb due to the presence of residual natZn (1% corresponds to 3.5 mg) in Tb fractions after the separation on the Sykam column (Figure 4.5). The gap between the fractions with the maximum Tb and Zn activities was only 2 mL, nevertheless, radionuclidically pure Tb was obtained after a 4-hour processing period.
4.3.3 Processing high activities of $^{149}$Tb and product characterization

A separation module, suitable for processing high activities of $^{149}$Tb obtained by one or another described purification methods, was designed (Figure 4.6), constructed and placed inside the hot cell. The separation of $^{149}$Tb from its isobars was performed with the use of manipulators.
Figure 4.6. Schematic diagram of the $^{149}$Tb chemical separation system. The blue arrows show the lines of the solution flow from outside the hot cell, while red arrows indicate flow inside the hot cell.

Two batches of $^{149}$Tb were implanted into Zn-coated gold foils and purified, based on the combination of the reported procedures [133, 164]. The radionuclidic purity of the final product ($[^{149}\text{Tb}]{\text{Tb}}\text{Cl}_3$) was examined by means of $\gamma$–ray spectrometry (Figure 4.7). Immediately after the purification, only $^{149}$Tb-characteristic $\gamma$–lines were detected, indicating the success of the separation process from the co-produced isobars ($^{149}$Gd and $^{133m}\text{CeO}^+$). The radiochemical purity of the $[^{149}\text{Tb}]{\text{Tb}}\text{Cl}_3$ was $>98\%$ (determined using radio-TLC), indicating the existence of only $[^{149}\text{Tb}]{\text{Tb}}^{3+}$ in the final product. The radiolabeling yield of $[^{149}\text{Tb}]{\text{Tb}}$-PSMA at 6 MBq/nmol molar activity was $\geq98\%$. Based on these characteristics, the quality of the final product ($[^{149}\text{Tb}]{\text{Tb}}\text{Cl}_3$) was determined to be sufficient for preclinical application.
Figure 4.7. Gamma spectrum of $^{149}$Tb obtained after the purification process (10 min measurement time; 1 cm distance from the detector). Peaks with energies $\leq 50$ keV correspond to X-rays of $^{149}$Gd decay product ($^{149}$Tb decays via electron capture (83.3%) to $^{149}$Gd [110]).

Three batches of $^{149}$Tb, implanted into the Zn foils, were purified with the developed procedure on the module and resulted in radionuclidically and radiochemically pure final product – $[^{149}$Tb]$\text{Cl}_3$ (~50 MBq at EOS). Unfortunately, despite superior radionuclidic and radiochemical purity of the $^{149}$TbCl$_3$, the radiolabeling yield of $[^{149}$Tb]$\text{Cl}_3$-$\text{PSMA}$-617 was $\leq 5\%$ at 6 MBq/nmol molar activity. The result could be explained by the presence of “cold” Zn in the final $^{149}$Tb product, competing with the radiolabeling of PSMA-617 with $^{149}$Tb. Unfortunately, the final $^{149}$Tb product was not analyzed for the impurity content by means of ICP-MS due to the inaccessibility of the device.

4.4 Discussion

The spallation reaction Ta(p, spall)$^{149}$Tb, followed by online isotope separation process, is suitable to produce quantities of $^{149}$Tb enough for in vivo studies as well as for the pilot studies to be performed on patients [165]. The quantities obtained at the ISOLDE facility in the present work were sufficient for performing preclinical studies. A further increase in on-line yield may be possible by improved laser ionization and higher target temperatures for accelerated release of the $^{149}$Dy precursor of $^{149}$Tb.
significant step forward is expected from the ARIEL and ISOL@MYRRHA facilities, which operate with 50 to 100 times higher proton beam current compared to ISOLDE.

After the production, followed by online mass separation, $^{149}$Tb implantation into a catcher material is required in order to transport the radionuclide of interest to the radiochemical laboratory for further processing. Different materials were previously studies/applied for the radionuclides implantation: ice (limited to short collection times), salts (e.g., NaCl) and metallic foils (Ta foils) [166]. The metallic foils were found to be advantageous over a fine gain powder material. The use of pressed salt pills (e.g., NaCl) as a catcher material is convenient for the chemical separation due to the high solubility of salts and could be applied directly for the in vivo experiments in case of the production of the high purity radionuclides ($^{142}$Sm or $^{140}$Nd). However, the use of the pressed powder materials causes a dramatic delay of most of the lanthanides due to surface adsorption effects [166]. The dissolution of Ta foils in the mixture of HNO$_3$/HF would cause precipitation of lanthanides in LnF$_3$ form, followed by the dissolution of the precipitant. The work with HF causes safety concerns and requires the use of expensive parts for the purification module, resistant to the impact of HF.

In this work, Zn coated Au foils as well as pure Zn foils were studied for the $^{149}$Tb implantation. The use of the Zn coated gold foils allows to handle during the chemical separation much lower amounts of Zn (few ten nm layer) as compared to pure Zn foils of hundreds milligrams mass. This could be advantageous for the purification process of the short-lived $^{149}$Tb as high masses of the material usually requires the use of longer columns, which elongates the time of processing. However, for several batches of Zn coatings it was observed that a fraction of the implanted beam ended up in the gold foil instead of the soluble Zn layer. To recover the full activity, the gold foils were dissolved in aqua regia (3:1 molar mixture of HCl and HNO$_3$) dropping the pH of the loaded on Sykam resin column solution to 0. Therefore, the efficiency of the $^{149}$Tb separation from isobars A=149 decreases (Figure 4.8) and the final $^{149}$Tb product may contain the $^{149}$Tb daughter – $^{149}$Gd due to the $^{149}$Tb decay during the transportation time from ISOLDE facility (~6 h) as well as due to the co-production of $^{149}$Gd via spallation of a Ta target. Taking into consideration the $^{149}$Tb-to-$^{149}$Gd ratio being 6-to-1 (at the time of the delivery to PSI) and the $^{149}$Tb therapeutic dose ~5 GBq (radioimmunotherapy) [31], insufficient $^{149}$Tb/$^{149}$Gd separation would result in ~0.8 GBq $^{149}$Gd in the final $^{149}$Tb product, which may bring additional dose to the patient. Nevertheless, in order to make a conclusion about dose contribution of $^{149}$Gd after
several hours post-separation, dosimetry studies have to be performed.

![Graph](image)

**Figure 4.8.** Elution profile of $^{160}$Tb separation from $^{153}$Gd, $^{159}$Dy and $^{65}$Zn on bench, using the previously developed purification method [164]. The Zn-coated gold foil was dissolved in 2.0 mL of aqua regia, mixed with 9.0 mL of MilliQ water. The required tracers were added and the resulted solution was loaded on the Sykam resin column (6 mm x 50 mm). The separation was performed with gradient elution:

- $0.07/0.09/0.11$ M α-HIBA – 0.6 mL/min eluent flow rate;
- $0.11/0.13$ M α-HIBA – 0.4 mL/min eluent flow rate.

Webster et al. reported the procedure for $^{155}$Tb/$^{139}$Ce separation on the UTEVA cartridge (Triskem International, France) when dissolving the Zn coated Au foils in aqua regia [167]. $^{155}$Tb was eluted in ~15 mL 8 M HNO$_3$ that would require evaporation of the product in order to be able to formulate $^{155}$Tb in the small volume of low concentrated acid for preclinical studies. Evaporation process could be time consuming, therefore, undesirable for obtaining short-lived $^{149}$Tb, and might be the source of the environmental impurities. The method was not verified for Tb separation from Gd and Eu; the $K_d$ values for Tb, Gd and Eu on the UTEVA resin are also not reported by the supplier (Triskem International, France). Furthermore, the resistant of the resins to aqua regia is questionable and needs to be studied.

Based on the number of disadvantages of using Zn coated Au foils described above, a full implantation of $^{149}$Tb into a thicker zinc layer or thin zinc foils is preferred as a dissolution in nitric acid leads to complete recovery of the activity from the implantation foil and avoiding the use of aqua regia. The method to separate $^{149}$Tb from
Zn (up to 350 mg) and A=149 isobars was developed based on the previously reported procedures for $^{161}$Tb and $^{152}$Tb isotopes, however, by changing the dissolving step and column length.

This purification procedure ensured radionuclidically and radiochemically pure $^{149}$Tb product. The chemical quality of the final product, however, was not sufficient to reach high radiolabeling yields when using Zn foils. This indicated the possible presence of Zn in the $[^{149}\text{Tb}]\text{TbCl}_3$ final product. However, in order to make the conclusion about the contaminants in the final product, ICP-MS analysis of the radioactive sample should be performed (so far was not possible at PSI due to the very limited availability of the appropriate machine).

In order to obtain chemically pure product, we propose that a DGA resin column (N,N,N',N'-tetra-n-octyldiglycolamide, Eichrom Technologies, USA) be introduced at the beginning of the purification process, which may shorten the time of the process and remove Zn in the initial load step. As reported by the Eichrom Technologies, Zn possesses low retention on DGA resin in HNO$_3$ media (distribution coefficient ($K_d$)<2), while rare-earth elements (e.g., Y) are strongly retained by the resin when using 0.1–10.0 M HNO$_3$ ($K_d$>1000) [168].

The bench experiments for the Tb/Zn separation on DGA resin were performed and demonstrated strong retention of Tb on DGA resin (>99%), while Zn showed no retention and passed through the column. From the DGA resin column, Tb was directly loaded onto the Sykam resin column (6 mm x 50 mm) and the procedure, described above, was applied. At the end of the process, Tb was efficiently separated from 350 mg of Zn and possible isobars and was formulated in 500 µL 0.05 M HCl. Using these preliminary results, a 3-column method (DGA-Sykam-LN3) is proposed, which could be used in future as a $^{149}$Tb purification process after isobaric collection using Zn foils (Figure 4.9). The time required for the entire chemical separation process on bench was ~3 h. An undoubted advantage of using DGA resin for the $^{149}$Tb purification process is its low affinity for Al(III), Cu(II), Fe(III) and Ti(IV) ions in 0.01–10.0 M HNO$_3$, which are present in Zn collection foils in ppm quantities (1–10 ppm). The use of DGA resin instead of LN3 resin as a third column is also possible and may allow additional purification of the $^{149}$Tb final product in case of a breakthrough of any non-radioactive impurities.
4.5 Conclusion

$^{149}$Tb of quality (purity), sufficient for preclinical studies, was obtained when applying the combination of the previously reported by our group purification procedures for processing $^{152}$Tb and $^{161}$Tb radioisotopes. In this case, Zn-coated gold foils were applied as a catcher material. Further development of the procedure to obtain pure no-carrier-added $^{149}$Tb (from the CERN-ISOLDE facility) was reported. The development covered the change of the collection foil, as well as the development of the purification procedure of the radionuclide of interest, accordingly. This may help to avoid additional preparation of the collection foil (e.g., sputtering of a Zn layer onto gold foils) and prevent the activity loss from the $^{149}$Tb absorption within the gold foil. The $^{149}$Tb purification procedure was developed by performing bench experiments and was translated to the hot cell for processing high activities of $^{149}$Tb. The purification resulted in radionuclidically and radiochemically pure $[^{149}\text{Tb}]\text{TbCl}_3$ final product, however, of poor chemical purity. To improve $^{149}$Tb chemical purity the use of the DGA resin column was proposed, which was successfully applied on bench and in future could be translated for processing high activities of $^{149}$Tb on the purification module.
Chapter 5

Conclusion and Outlook
The current “gold standard” for targeted radionuclide therapy (TRNT) is $\beta^-$-emitter $^{177}\text{Lu}$. However, recent research showed higher therapeutic efficacy of another $\beta^-$-emitter $^{161}\text{Tb}$ due to the co-emission of a higher percentage of short-range conversion and Auger electrons than $^{177}\text{Lu}$. Until now, $^{161}\text{Tb}$ was not available on a regular basis, mainly due to the absence of the reliable purification method. Therefore, in the current thesis, we focused on the development of the medically interesting radiolanthanide $^{161}\text{Tb}$. The development work included the choice of the target material, irradiation parameters, establishment of the purification process, characterization of the final product (radionuclide formulated in low concentrated acid) and post-process quality assessment. As a result of the progress, $^{161}\text{Tb}$ is now constantly and reproducibly available possessing specifications, comparable to the commercially available, clinically-applied, no-carrier-added $^{177}\text{Lu}$ (EndolucinBeta). In a proof-of-concept, first-in-man study, $^{161}\text{Tb}$ was used for radiolabeling of the somatostatin analog DOTA-TOC and the corresponding radiotracer was injected into a patient, suffering from metastasized neuroendocrine tumors (Figure 5.1) at the Zentralklinik Bad Berka (Germany). The next step would be to conduct clinical trials in order to determine pharmacodynamics of $^{161}\text{Tb}$-based radiopharmaceutical and to compare the efficacy of $^{161}\text{Tb}$ compare to $^{177}\text{Lu}$ in patients. Once higher therapeutic efficacy of the radionuclide, which emits conversion and Auger electrons, is proved in clinical settings, a new era for the application of short-range emitters in TRNT would be drawn.

![Figure 5.1](image)

**Figure 5.1.** (a) The diagnostic image ($^{68}\text{Ga}$Ga-DOTA-TOC, pre-treatment) of the patient with metastasized NET. (b) Post-injection SPECT scan showing the distribution of $^{161}\text{Tb}$-DOTA-TOC in some locations of somatostatin-receptor positivity.
In order to fulfill the required specifications of the product for the routine clinical application, the chemical purity (elemental content) of $^{161}$Tb has to be characterized for each produced batch. The level of the elemental impurities in the drug product should be controlled within acceptable limits as this kind of impurities will not provide any therapeutic benefit. Therefore, the availability on the side of the machine, which allows obtaining information about chemical purity of the product (e.g., ICP-MS) and suitable for radioactive measurements, is of high value. Unfortunately, our $[^{161}\text{Tb}]\text{TbCl}_3$ samples could not be analyzed so far for the elemental content due to the poor access to the ICP-MS devices, capable of handling radioactive samples (i.e., at PSI, University of Mainz, Karlsruhe Institute of Technology). Nevertheless, the potential clinical significance of $^{161}$Tb (as proven in numerous pre-clinical studies) should facilitate access to the ICP-MS on a routine basis in the future.

Due to the increased interest for $^{161}$Tb over the last few years, the production of high amounts of activity might be required (hundreds of TBq or PBq). Therefore, the mass of the target could be significantly escalated and the purification method should be tested for higher amounts of the target material. Influence of macroamounts (e.g., gram amounts) of the target material on the separation of microamounts of the radionuclide of interest could be further investigated for Sykam resin column. The study would provide the highest mass value of the target material, which is applicable for the successful $^{161}$Tb purification and could be as well applied for the purification of other radiolanthanides under chosen experimental conditions. The drawback of the radionuclide purification after the production at nuclear reactors is the formation of high amounts of long-lived active waste, containing $^{152}$Eu ($T_{1/2}=13.5$ y), $^{155}$Eu ($T_{1/2}=4.8$ y) and $^{60}$Co ($T_{1/2}=5.3$ y). In order to minimize the amount of such waste, we recommend eluting $^{153/156}$Gd (after the purification of $^{161}$Tb), followed by the elution of the Europium isotopes and $^{60}$Co. This should be possible by using $0.13–0.15$ M $\alpha$-HIBA and $1$ M $\alpha$-HIBA. Afterwards, this long-lived waste could be loaded on the AG MP-50 resin column, eluted in $7$ M $\text{HNO}_3$ and evaporated until dryness. As a result, several liters of the long-lived radioactive waste could be transformed into small samples. In order to achieve this goal, the corresponding set-up (Figure 5.2) could be constructed and introduced to the hot cell.
An α–emitting radionuclide $^{149}$Tb ($T_{1/2}$=4.1 h, $E_\alpha$=3.98 MeV (16.7%)) with a range in the tissue of 28 µm could be a good candidate for eliminating cancer cell clusters or small micrometastases. Once $^{149}$Tb is delivered to the cell clusters, it deposited energy on a cellular level while sparing surrounding healthy tissues. $^{149}$Tb is produced by proton-induced spallation of tantalum targets via Ta(p, spall)$^{149}$Tb reaction, followed by online isotope separation process at ISOLDE facility (CERN). The amounts of the produced $^{149}$Tb are limited so far and provided $^{149}$Tb activities only in amounts suited for preclinical research, however, not for clinical studies. Nevertheless, new centers that may offer the possibility of $^{149}$Tb production in quantities, sufficient for clinical studies, are under construction. For example, ARIEL and ISOL@MYRRHA facilities, which operate with 50 to 100 times higher proton beam current compared to ISOLDE. In order to deliver the radionuclide after production to the radiochemical laboratory for the purification process, $^{149}$Tb implantation into a catcher material is required. In the current work, the attempt to use Zn foils as a catcher material was made. The fast solubility of Zn in hydrochloric acid will prevent loss of $^{149}$Tb activity due to the radionuclide implementation into the metal foil, which is the case for the currently used catcher material – Zn coated gold foils. The purification method was established and translated into the hot cell, however, the final $^{149}$Tb product was of poor chemical purity (presence of Zn in the final product). Therefore, the purification method was further developed in order to get rid of the
macroamounts of Zn already on the loading step by applying DGA resin column at the beginning of the process. As a next step of the $^{149}$Tb development, we propose the translation of this new purification method with the use of DGA resin column to a module and testing the module with high activities of $^{149}$Tb. Once the production of $^{149}$Tb is scaled up and the purification method proves to be effective and reproducible, $\alpha$–emitting radionuclide could be supplied for clinical proof-of-concept studies. In the future, the successful development of $^{149}$Tb could provide patients with the only available $\alpha$–emitter, decaying without $\alpha$–emitting daughters. Such characteristic could reduce the side effects known form currently applied $\alpha$–emitting compounds (e.g., $^{[223]$Ra$]RaCl_2$, $^{[225]$Ac$]Ac$-PSMA-617).

Finally, the pure Auger–electron emitter $^{165}$Er was prepared with the aim to irradiate tumors on the subcellular level and eliminate circulating cancer cells. Reproducible production and purification of no-carrier-added $^{165}$Er has never been described before, giving our group and other research groups at CRS the opportunity to pioneer studies with the use of $^{165}$Er. We evaluated two possibilities of $^{165}$Er production on cyclotron: direct via $^{n}\text{at}\text{Ho}(p,n)^{165}$Er nuclear reaction and indirect via $^{166}$Er$(p,2n)^{165}$Tm$\rightarrow^{165}$Er nuclear reaction. Based on our data, the direct production route was considered inappropriate for obtaining high yields of no-carrier-added $^{165}$Er due to the long purification process after the production and due to the presence of natural Er impurities in the original target material (200 mg 99.999% $^{\text{nat}}$Ho$_2$O$_3$). The indirect production route, which possesses higher cross-section values as compared to the direct route, was experimentally performed for the first time ever. The obtained production yield of $^{165}$Tm was $\sim$4–7 times lower as compared to the theoretically calculated, probably due to the off-centered beam (84% of the beam did not reach the target) (Figure 5.3). This implies that improved beam diagnostics is crucial. We propose to install the diagnostics closer than 80 cm before the target. Furthermore, we proposed to enlarge the diameter of the $^{[166]$Er$]$Er$_2$O$_3$ target from 6 mm to 12 mm that will also increase $\sim$2–3 times the mass of the target (up to 120 – 200 mg) and therefore, significantly increase the production yield. This would be the next step in scaling up $^{165}$Tm activity at EOB. We also suggest to compare the $^{165}$Tm production yields at the Injector 2 cyclotron at PSI and at the IBA Cyclone (18 MeV medical cyclotron in Bern University Hospital and in the future at the ETHZ) at $\sim$18 MeV proton beam energy. The cross-section for $^{165}$Tm production at 18 MeV beam energy remains high ($\sim$1 barn) and the experiment would give an answer whether the production of $^{165}$Er via the
indirect route is possible at generally available 18 MeV small medical cyclotrons (approximate number – 1050).

**Figure 5.3.** Schematic illustration of the encapsulated target material (6 mm diameter) and possible upcoming beam position (5.5±0.2 mm diameter).

The established purification method gave access to the radionuclidically and radiochemically pure $^{165}$Er. Nevertheless, the method could be further developed to the concept of the $^{165}$Tm/$^{165}$Er generator based on the Szillard-Chalmers effect, using $[^{165}\text{Tm}]$Tm-DOTA. Szillard-Chalmers effect would lead to a rupture of the chemical bond between the radioactive mother isotope $^{165}$Tm and the DOTA chelator while liberating the daughter nuclide $^{165}$Er (Figure 5.4). Kinetic inertness of DOTA chelator will inhibit the formation of $[^{165}\text{Er}]$Er-DOTA complex at room temperature.

**Figure 5.4.** Schematic illustration of the Szillard-Chalmers effect, useful for the concept of $^{165}$Er/$^{165}$Tm generator.

The idea to apply Szillard-Chalmers effect for the purification of $^{165}$Er was proposed and described by Severin et al. 2014 [146] and could be further optimized by the combination with the purification method, developed by our group (Figure 5.5). $^{166}$Er/$^{165}$Tm separation could be performed on the Sykam resin column (Column 1) as
described in Chapter 3.2 of this thesis, followed by concentration of \( {^{165}}\text{Tm} \) on small LN3 resin column (Column 2) and elution of the radionuclide to the glass vial for radiolabeling purposes. The radiolabeling of DOTA with \( {^{165}}\text{Tm} \) could be performed using the procedure described in Chapter 2 of this thesis. The radiolabeling solution is loaded afterwards on Column 3 (C-18 cartridge), which will retain polar molecules. After the sufficient waiting time for the \( {^{165}}\text{Er} \) activity in-growth, \( {^{165}}\text{Er} \) “milking” could be performed from Column 3 with citrate or DTPA as eluent [169]. Nevertheless, the most suitable experimental conditions for elution of the final \( {^{165}}\text{Er} \) product from Column 3 have to be investigated by performing bench tests as well as the stability of the \( {^{165}}\text{Tm}/^{165}\text{Er} \) generator system has to be examined. The concept of generator will decrease the time of processing due to a fast elution of \( {^{165}}\text{Er} \) from the generator in a small volume of eluent, avoiding six hours separation of the developed purification method. In this case, the \( {^{165}}\text{Er} \) elution process could be repeated several times until \( {^{165}}\text{Tm} \) decay completely. Multiple \( {^{165}}\text{Er} \) elutions are challenging with the developed purification method due to the time consuming \( {^{165}}\text{Tm}/^{165}\text{Er} \) separation on the cation-exchange resin column, which is also followed by the time-consuming cleaning of the system and preparation of the column for the next separation.

**Figure 5.5.** Schematic illustration of the possible \( {^{165}}\text{Er} \) purification process with a \( {^{165}}\text{Tm}/^{165}\text{Er} \) generator concept.

Production of pure radiolanthanides, possessing different LET and tissue penetration paths, could give an advantage of combining different types of TRNT, e.g., \( \beta^- \)-therapy with Auger electron therapy. In this case, the treatment of large metastases
could be carried with $^{161}$Tb-DOTA-TOC (as an example), followed by the $^{165}$Er-NLS-DOTA-TOC injection (nucleus localization) to eliminate circulating single cancer cells. The combination of therapies, acting on multicellular, cellular and subcellular levels might be an option to provide better therapeutic efficacy of the existing TRNT. A combination of diagnostic and therapeutic isotopes of the same radiolanthanide, which can be used for radiolabeling of the same biomolecule, might be applied as a theragnostics approach in TRNT. In this case, visualization of potential targets with a diagnostic agent (e.g., $^{155}$Tb-DOTA-TOC as γ–ray emitting radiopharmaceutical) could help to predict if a patient may benefit from the upcoming treatment with the corresponding therapeutic agent (e.g., $^{161}$Tb-DOTA-TOC as β−–emitting radiopharmaceutical) and perform precise dosimetry calculations.

To conclude, novel radiolanthanides are attractive candidates for the application in TRNT due to the suitable and variable decay characteristics and elementary coordination chemistry. The choice of the radiolanthanide, possessing appropriate characteristics (LET, tissue range) for a particular clinical situation, would give an opportunity to selectively damage only cancerous cells and diminish side effects. Furthermore, the theragnostics approach would be an important and valuable step towards personalized medicine.
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Publications and Scientific Contributions


