EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

Addendum to the ISOLDE and Neutron Time-of-Flight Committee

Terbium-149 for targeted alpha therapy

11 January 2023

Pascal Grundler[1], Karl Johnston[2], Ulli Köster[3], Cristina Müller[1], Zeynep Talip[1], Nick van der Meulen[1]

[1] Paul Scherrer Institut, Villigen – ETH Zürich, Switzerland
[2] CERN, ISOLDE
[3] Institut Laue-Langevin, Grenoble, France

Spokespersons: Karl Johnston (karl.johnston@cern.ch)
Nick van der Meulen (nick.vandermeulen@psi.ch)
Local contact: Ulli Köster (koester@ill.eu)

CERN-INTC-2023-025 / INTC-P-593-ADD-1
11/01/2023

Abstract

We request additional shifts to continue the programme of IS688 for the preclinical studies of ^{149}Tb for Targeted Alpha Therapy. Excellent progress has been made in the past two campaigns, especially with the production and separation efficiency of this radionuclide. Radionuclidic purities >99.9% of the final ^{149}Tb product can now be reproducibly achieved. Although initial studies showed delayed tumour growth in mice treated with ^{149}Tb-based somatostatin analogues, there is a need for additional preclinical studies to obtain proper data of a systematic investigation of this nuclide. This presents the objective of this addendum. With regard to the characterization of the radioisotope, labelling at higher molar activities will be attempted. In preclinical studies, the effect of ^{149}Tb will be investigated using additional studies including radiobiological investigations. The tolerability of ^{149}Tb-based somatostatin analogues will be investigated as potential undesired side effects in radiosensitive organs such as the kidneys and bone marrow of mice should be determined and analysed.

Requested shifts: 10 (split into 2-3 runs over 2 years)

Introduction

Terbium is a unique element in that it includes a quadruplet of radioisotopes suitable for diagnostics and therapy in nuclear medicine [1]. By harnessing the characteristics of the various isotopes, it can contribute to the theragnostic concept, where one can use chemically identical radioligands based on different Tb (diagnostic vs. therapeutic) radioisotopes for diagnosis and therapy, respectively. Much success has been gained from the Paul Scherrer Insitute (PSI)-ISOLDE collaboration, with the collection and purification of ^{149}Tb (α-emitter, $T_{1/2}$ = 4.1 h), used for preclinical therapy studies [2, 3] and PET imaging [4], and ^{152}Tb (β^+-emitter, $T_{1/2}$ = 17.5 h), for preclinical [5] and clinical [6] PET imaging, respectively. The versatility of the Tb isotopes have led to their being dubbed the "Swiss army knife for nuclear medicine", as shown in Figure 1 [1]. However, although ISOLDE can produce all four radioisotopes, the interest in this addendum remains focused on ^{149}Tb.

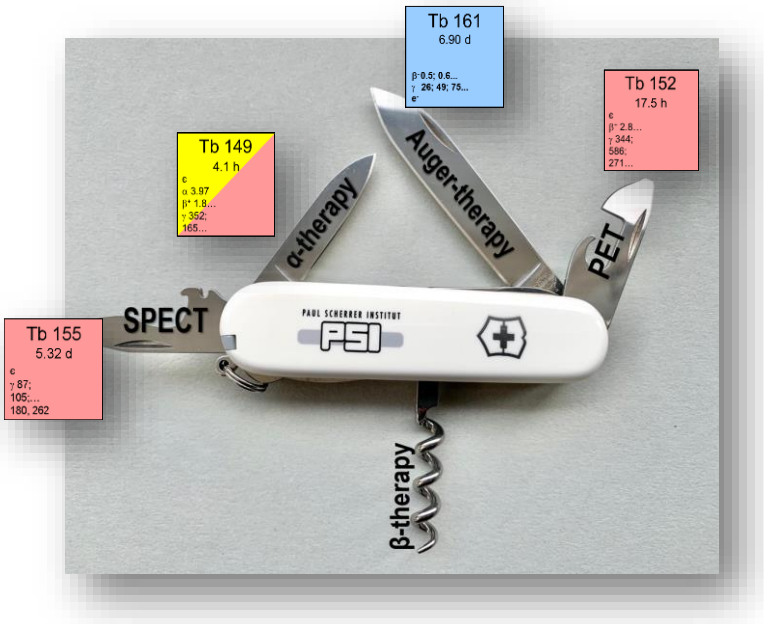

Figure 1 The quadruplet of theragnostic terbium radioisotopes.

IS688 was initially recommended for the approval of 10 shifts at the 66th meeting of the INTC in 2021. The goals were to build on the success of a previous experiment IS528, which ran for several years, but with the focus on exploiting ^{149}Tb for Targeted Alpha Therapy (TAT). This is the only radiolanthanide that can be considered for alpha therapy and, among the attractions of exploiting this radionuclide, is the fact that it is trivalent. Trivalent lanthanides have a well-known labelling chemistry, forming *in vivo* stable compounds with the macrocyclic DOTA chelator. Furthermore, experience from the clinically-established ^{177}Lu can be directly applied to ^{149}Tb and due to its positron branch, it is the only alpha-emitting radionuclide for which PET can be carried out in parallel to therapy. This would allow *in vivo*

distribution to be directly followed using nuclear imaging methodologies. To date, the therapeutic potential of ^{149}Tb has been demonstrated using radiolabelled antibodies targeting a leukaemia model [7], radiolabelled DOTA-folates targeting KB tumour xenografts [1,2] and radiolabelled PSMA-targeting prostate cancer cells [3].

In addition, the potential for the therapeutic use of ^{149}Tb is gaining traction, with several large-scale facilities being considered for its exploitation in the future, among them the TATTOOS (as part of the IMPACT project) facility at PSI. However, in the meantime, ISOLDE is the only facility worldwide capable of producing this radionuclide in quantities adequate for preclinical studies.

Since then, two successful experimental campaigns have been carried out, building on the experience gained in previous years. Many of the goals of the original proposal have been achieved, but more preclinical research has to be conducted to understand this novel, interesting radionuclide. More shifts are, therefore, required to understand the various subtleties of applying ^{149}Tb for therapy.

Implantation method and purification

The required neutron-deficient lanthanides have been produced by 1.4 GeV proton-induced spallation in tantalum foil targets. The cumulative yield of ^{149}Tb is boosted by resonantly laser-ionising the ^{149}Dy precursor. The mass-separated beam will be ion-implanted into a temporary matrix (e.g. Zn-coated gold foils), and shipped in shielded containers to PSI. In addition to ^{149}Tb, many impurities due to e.g. oxides are also collected which can lead to high ambient dose rates. The design and implementation of a dedicated compact chamber, which can be well shielded, has considerably eased concerns about these high doses during implantation and the redevelopment of the GLM area has simplified the logistics of carrying out such collections at ISOLDE. The compact chamber and an example of the Zn-coated foil are shown in Figure 2.

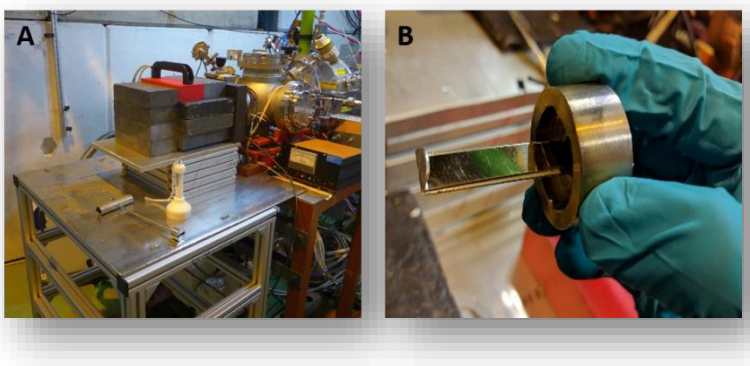

Figure 2 The compact chamber where the collections are performed (A), and the Zn coated Au foil into which the beam is deposited (B).

Upon arrival at PSI, the temporary matrix is dissolved and the desired radionuclide separated from its isobaric contaminants in a hot cell, as shown in Figure 3. The product will be used for the labelling of the aforementioned tumour-targeting agents. These served for preclinical *in vitro* and *in vivo* studies using prostate, ovarian and breast cancer cell lines, as well as a pancreatic cancer cell line and the corresponding tumour mouse models.

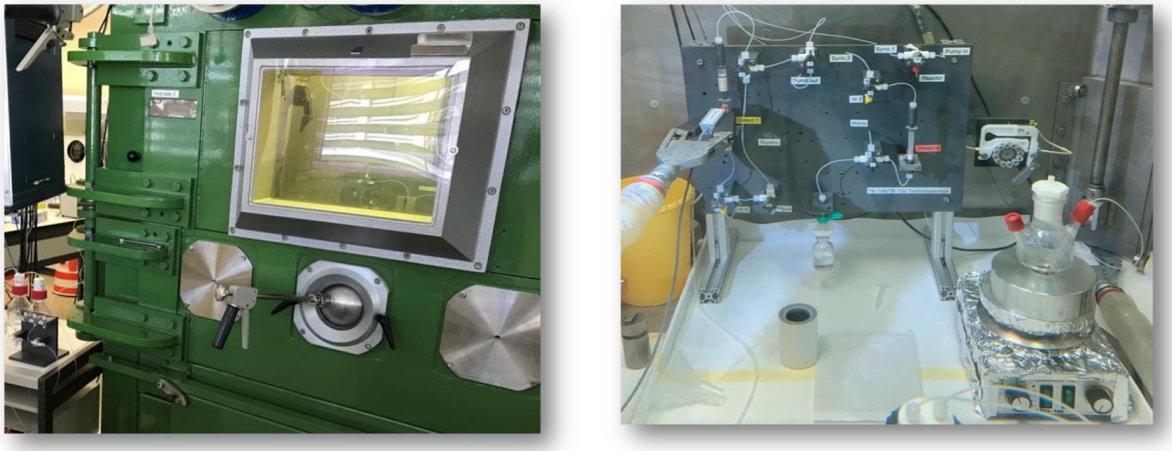

Figure 3 The hot cell at PSI where the foils are dissolved and separated, along with impurities, from the desired radionuclide.

Summary of results and discussion from 2021 and 2022

Two one-week campaigns to produce ^{149}Tb took place in November 2021 and March 2022, respectively. Collections were performed overnight using Zn-coated gold foils. The foils, containing the desired product and its A=149 isobars were transported to PSI for processing. The chemical separations were performed using an adjusted/updated method. Usable products (yields around 100 MBq) were produced using a two-column separation system in a hot cell, allowing the labelling of ^{149}Tb to somatostatin analogues and its use for preclinical studies.

Excellent results in terms of chemical separation and preclinical studies were obtained in these campaigns; however, these results require corroboration along with therapy studies using other somatostatin-based ligands. Up to 110 MBq ^{149}Tb was separated from the collection material, with a radiochemical separation efficiency of >90% and a final radionuclidic purity >99.9%. The product was labelled to DOTA-derivatized peptides at up to 20 MBq/nmol molar activity: this was the first time that this quality was achieved. *In vitro* cell viability assays using AR42J tumour cells were performed using ^{149}Tb-DOTALM3 and ^{149}Tb-DOTATATE (Figure 4), along with *in vivo* PET/CT imaging of AR42J tumour-bearing mice with these ligands (Figure 5).

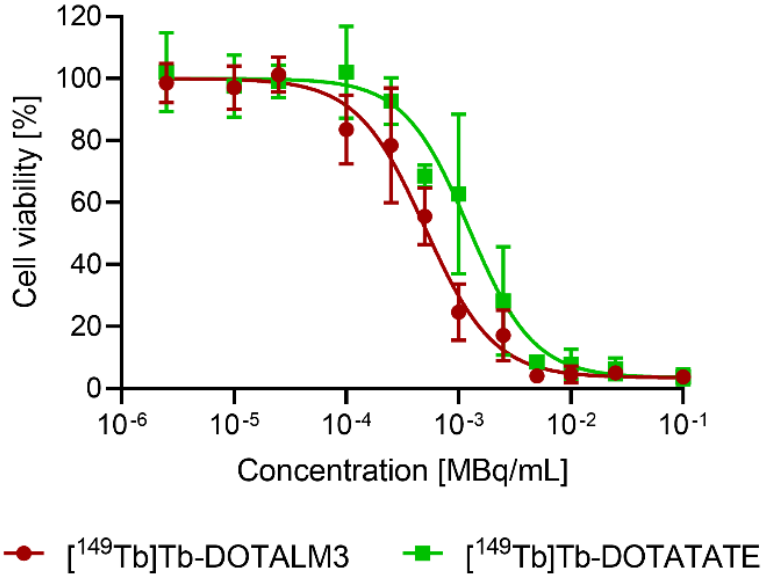

Figure 4 *In vitro* cell viability assays using AR42J tumour cells.

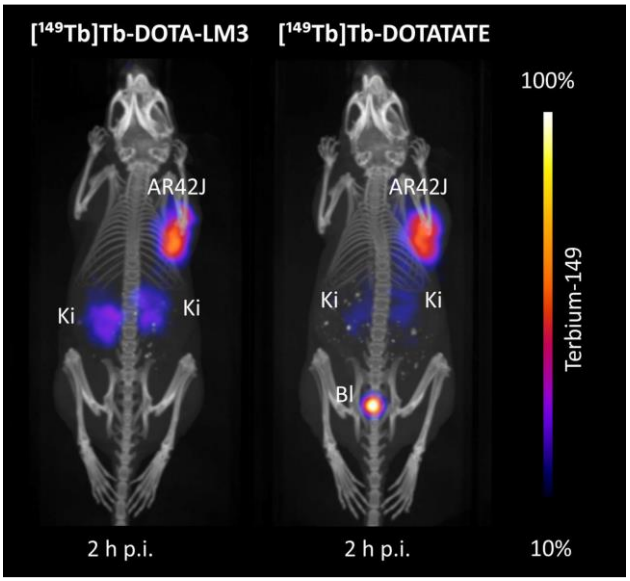

Figure 5 *In vivo* PET/CT imaging, 2 h p.i., of AR42J tumour-bearing mice using ^{149}Tb-DOTALM3 (left) and ^{149}Tb-DOTATATE (right).

Therapy studies were performed with these radioligands to investigate the efficacy to delay the tumour growth. These data are currently being processed and analysed.

Further collections are necessary for the following investigations:

- We need to investigate labelling at higher molar activity to determine the quality of ^{149}Tb.

- We need to investigate the stability of the ^{149}Tb-labeled somatostatin analogues in vitro, as the alpha-particle emission may damage the peptide; a suitable formulation with adequate scavengers has to be developed.
- Further replicates of the *in vitro* viability assay are required to corroborate the already acquired data.
- We need to perform additional *in vitro* experiments such as the colony-forming assay to demonstrate the effect based on a different experimental setting.
- We would like to investigate radiobiological effects such as the extent of DNA double-strand breaks in tumour cells treated with ^{149}Tb-peptides.
- We need to complete our in vivo studies with a particular focus on the investigation of the tolerability of this therapy concept on radiosensitive normal tissue (kidneys and bone marrow).

The previous studies were performed using a previously-irradiated target, which had been prepared in 2018. Although the yields from this target were acceptable, larger yields would be possible from a fresh unit. Now that concerns about the collection chamber and removal of the irradiated foil have been allayed, being able to collect activities up to 500MBq would allow for more systematic studies in a shorter time than currently the case.

It should be noted that at the time of writing, MEDICIS is not yet ready to schedule production runs for high activity collections required for preclinical *in vivo* studies using ^{149}Tb. Such biological experiments require long lead times to prepare the mice and grow tumours under controlled conditions. If insufficient activity is collected, the corresponding experiments would fail completely. We propose to continue the well-established collections of ^{149}Tb from ISOLDE until adequate ^{149}Tb activities can be collected at MEDICIS.

Requested shifts:

(i) Envisaged measurements and requested isotopes
10 shifts for the collection of ^{149}Tb

(ii) Number of shifts (based on newest yields) required for each isotope
The given yields were observed in the last beam time, averaged over several hours of collection.

Isotope	Cumulative yield (/uC)	target – ion source	Shifts (8h)
^{149}Tb	6E8	Ta foil + W surface ionizer and Dy RILIS	10

Total shifts: 10

References:

[1] C. Müller, et al. J Nucl Med 2012,53:1951.

[2] Müller et al., Pharmaceuticals 7, 353 (2014).

[3] Umbricht et al., Scientific Reports 9, 17800 (2019).

[4] Müller et al., EJNMMI Radiopharmacy and Chemistry 1,5 (2016).

[5] Müller et al., EJNMMI Research 6, 35 (2016).

[6] Baum et al., Dalton Transactions 46, 14638 (2017).

[7] G.-J. Beyer et al. Eur J Nucl Med Mol Imaging 2004;31:547.

Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

Please describe here below the main parts of your experimental set-up:

Part of the experiment	Design and manufacturing
Shielded ENSAR2 collection chamber	☒ To be used without any modification ☐ To be modified

HAZARDS GENERATED BY THE EXPERIMENT

Additional hazard from flexible or transported equipment to the CERN site:

Domain	Hazards/Hazardous Activities		Description
Mechanical Safety	Pressure	☐	[pressure] [bar], [volume][l]
	Vacuum	☐	
	Machine tools	☐	
	Mechanical energy (moving parts)	☐	
	Hot/Cold surfaces	☐	
Cryogenic Safety	Cryogenic fluid	☐	[fluid] [m^3]
Electrical Safety	Electrical equipment and installations	☐	[voltage] [V], [current] [A]
	High Voltage equipment	☐	[voltage] [V]
Chemical Safety	CMR (carcinogens, mutagens and toxic to reproduction)	☐	[fluid], [quantity]
	Toxic/Irritant	☐	[fluid], [quantity]
	Corrosive	☐	[fluid], [quantity]

	Oxidizing	☐	[fluid], [quantity]
	Flammable/Potentially explosive atmospheres	☐	[fluid], [quantity]
	Dangerous for the environment	☐	[fluid], [quantity]
Non-ionizing radiation Safety	Laser	☐	[laser], [class]
	UV light	☐	
	Magnetic field	☐	[magnetic field] [T]
Workplace	Excessive noise	☐	
	Working outside normal working hours	☐	
	Working at height (climbing platforms, etc.)	☐	
	Outdoor activities	☐	
Fire Safety	Ignition sources	☐	
	Combustible Materials	☐	
	Hot Work (e.g. welding, grinding)	☐	
Other hazards			