Simultaneous Auger e- and β⁻-Particle Therapy of Metastasized NET Using ^{161}Tb-DOTATOC

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ABSTRACT

Peptide receptor-targeted radionuclide therapy (PRRT) is among the most successful treatment options for patients suffering from metastasized neuroendocrine tumors (NET). A critical point in the management of the disease, however, is the frequent relapse after 2-3 years, due to single cancer cells and micro-metastases that escaped the therapy.[1] The aim of this project was to develop a novel therapy concept for NET patients, based on the use of ^{161}Tb-labeled somatostatin analogues (DOTATOC and DOTA-LM3). ^{161}Tb is a therapeutic radionuclide with similar decay properties (β^-, γ) to those of the clinically-established ^{177}Lu. In addition, it emits conversion and Auger electrons which are particularly effective for the treatment of single cancer cells.[2,3,4] ^{161}Tb was produced by neutron irradiation of ^{160}Gd targets and subsequent chemical separation. The efficacy of ^{161}Tb-labeled somatostatin analogues to kill tumor cells was investigated in comparison to the clinically-established ^{177}Lu-labeled counterparts. In view of a clinical study, the production of ^{161}Tb-DOTATOC was established on an automated system, according to Good Manufacturing Practice (GMP). Finally, a first-in-man application of ^{161}Tb-DOTATOC in Bad Berka, Germany, demonstrated the feasibility of using ^{161}Tb for clinical scintigraphy and SPECT.

^{161}Tb PRODUCTION

^{161}Tb was produced via the ^{160}Gd(n,γ)^{161}Gd→^{161}Tb nuclear reaction which led, after radiochemical purification, to carrier-free ^{161}Tb in quantities >10 GBq. The quality of the produced ^{161}Tb was excellent, as indicated by the radiolabeling capacity of ≥99% at a molar activity of 100 MBq/nmol using DOTATOC.[5]

Nuclear data[6]

Decay mode	β^- (593 keV), Auger e^-
Half-life	6.96 d [7]

Figure 1. Production route for ^{161}Tb by irradiation at neutron source at the Institute Laue Langevin (ILL), France, or Necsa, South Africa.

Radiochemical Purification

Figure 2. Concept of ^{161}Tb chemical separation from enriched Gd target material.

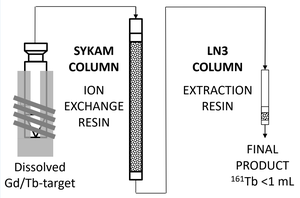
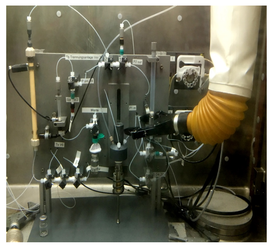


Figure 3. ^{161}Tb separation panel.



Specification	^{177}LuCl$_3$[10]	^{161}TbCl$_3$
pH	1–2	1–2
Sterility	Not required if the final radiopharmaceutical is sterilized	Not required if the final radiopharmaceutical is sterilized
Bacterial endotoxins (LAL test)	<175 IU/$V_{injectable\ dose}$	<175 IU/$V_{injectable\ dose}$
Radionuclidic purity (γ spectrometry)	^{175}Yb ≤ 0.01 %	^{160}Tb ≤ 0.007 %
Radiochemical purity (TLC)	≥ 99.0 % as ^{177}LuCl$_3$	≥ 99.0 % as ^{161}TbCl$_3$

PRECLINICAL EVALUATION

The two somatostatin receptor (SSTR)-targeting peptides,[8,9] DOTATOC (SSTR agonist) and DOTA-LM3 (SSTR antagonist) were labeled with either ^{161}Tb or ^{177}Lu and evaluated in preclinical experiments.

In Vitro Therapy Study

In vitro cell viability (MTT) and cell survival (clonogenic) assays were performed to investigate the difference between ^{161}Tb- and ^{177}Lu-labeled peptides on SSTR-positive AR42J rat pancreatic tumor cells.

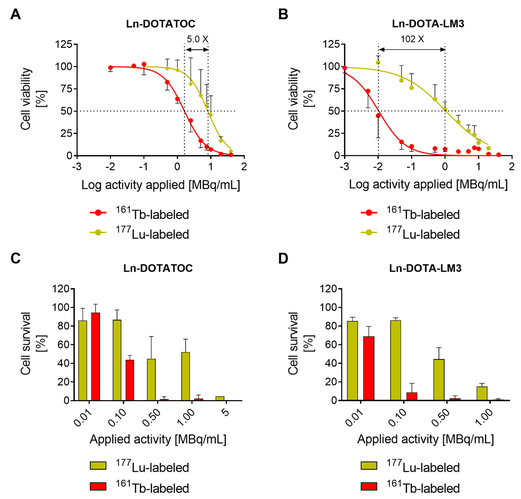


Figure 4. *In vitro* results showed more effective reduction in AR42J-tumor cell viability (**A/B**) and survival (**C/D**) after treatment with ^{161}Tb-labeled DOTATOC (**A/C**) and ^{161}Tb-labeled DOTA-LM3 (**B/D**) compared to the respective ^{177}Lu-labeled counterpart. (Ln = ^{161}Tb or ^{177}Lu).

Dual-Isotope SPECT/CT Imaging

AR42J-tumor-bearing mice were simultaneously injected with equal activities of ^{161}Tb- and ^{177}Lu-DOTATOC or ^{161}Tb- and ^{177}Lu-DOTA-LM3 to demonstrate equal biodistribution of the ^{161}Tb- and ^{177}Lu-labeled counterparts.

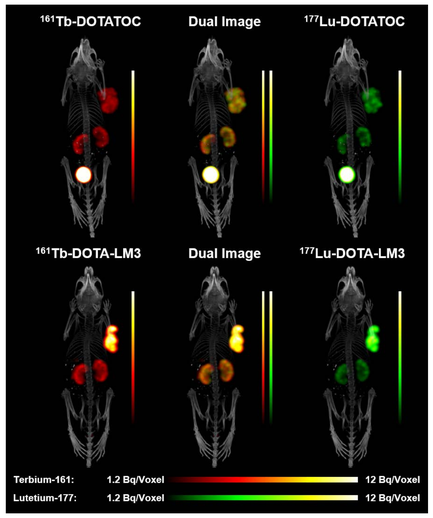


Figure 5. Dual-isotope (^{161}Tb/^{177}Lu) SPECT/CT images demonstrating equal *in vivo* distribution of ^{161}Tb- and ^{177}Lu-labeled peptides in the same animal. Mice injected with radiolabeled DOTA-LM3 showed higher tumor uptake and tumor-to-kidney ratios than mice injected with radiolabeled DOTATOC.

Preclinical Therapy Study

The superior effect of ^{161}Tb was confirmed in a therapy with tumor-bearing mice that received either two injections (Day 0 and Day 7) of 10 MBq (0.2 nmol) ^{161}Tb-DOTATOC or ^{177}Lu-DOTATOC or two 10 MBq injections of ^{161}Tb-DOTA-LM3 or ^{177}Lu-DOTA-LM3.

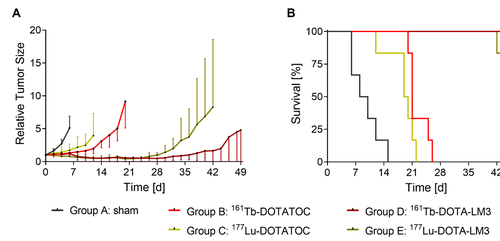
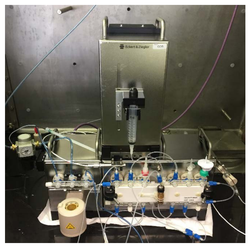


Figure 6. Tumor growth curves shown until the first mouse of the group was euthanized (**A**) and survival (**B**) of AR42J-tumor-bearing mice treated with sham (Group A), ^{161}Tb-DOTATOC (Group B), ^{177}Lu-DOTATOC (Group C), ^{161}Tb-DOTA-LM3 (Group D) or ^{177}Lu-DOTA-LM3 (Group E). The treatment was applied at Day 0 and at Day 7 (10 MBq; 0.2 nmol). Median Survival of Group A = 9 d; Group B = 21 d; Group C = 19.5 d; Group D = n.d. (all of the mice survived until end of study); Group E = 48.5 d (3 mice survived until end of study).

Synthesis of ^{161}Tb-DOTATOC

A protocol for the synthesis of ^{161}Tb-DOTATOC, compliant with cGMP, was established using an automated cassette module system (Eckert&Ziegler).



Product specifications	
Final volume	20.0 mL ± 2.0 mL
Activity (SLED)	1 ± 0.1 GBq – 7.4 ± 0.74 GBq
DOTATOC	max 200 µg / 20 mL
Molar activity	53 ± 5.3 MBq/nmol
Shelf-life	24 h

Production yield > 95 %

FIRST-IN-MAN APPLICATION OF ^{161}Tb-DOTATOC

Radiolabeling of DOTATOC with ^{161}Tb was performed at the Klinikum Bad Berka in Germany (Prof. R. Baum). A 70-year-old male patient with a metastatic, functional neuroendocrine neoplasm of the pancreatic tail was injected with ^{161}Tb-DOTATOC. Scintigraphy and SPECT were recorded. The image quality was comparable to the ^{177}Lu-labeled counterpart.

Whole-Body Scintigraphies

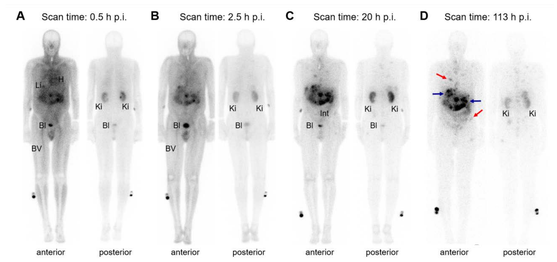


Figure 7. Whole-body images obtained at 0.5 h p.i. (**A**), 2.5 h p.i. (**B**), 20 h p.i. (**C**) and 113 h p.i. (**D**) of ^{161}Tb-DOTATOC. Early blood-pool activity was noticed in the heart (H) and blood vessels (BV). Physiological uptake was seen in the soft tissues, liver (Li), kidneys (Ki), and the bladder (Bl). Pathological uptake was demonstrated in bilobar liver (blue arrows) and multifocal osseous metastases (red arrows).

SPECT/CT Imaging

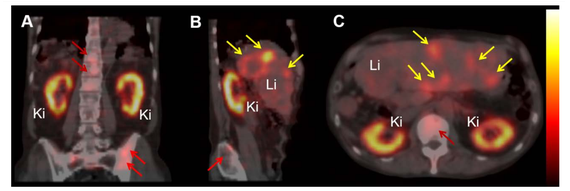


Figure 8. SPECT/CT images obtained at 19 h after injection of ^{161}Tb-DOTATOC. (**A**) Coronal section, (**B**) sagittal section and (**C**) transverse section. Uptake of ^{161}Tb-DOTATOC was seen in bilobar hepatic metastases (yellow arrows), as well as multiple osteoblastic skeletal metastases in the vertebral column (dark red arrows) and the pelvis (red arrows).

CONCLUSIONS

^{161}Tb was successfully developed and investigated from bench to bedside within this accelerator grant supported by the NETRF. In view of a clinical study, the GMP production method of ^{161}Tb-DOTATOC was set up. A clinical Phase I study is planned in future, in order to investigate the specific features of ^{161}Tb for the treatment of disseminated disease in patients with NETs.

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