

## Article

# Paving the Way for CCK2R-Targeted Peptide Receptor Radionuclide Therapy with [<sup>177</sup>Lu]Lu-DOTA-MGS5 in Patients with Small Cell Lung Cancer

Taraneh Sadat Zavvar <sup>1</sup>, Giulia Santo <sup>1</sup>, Leonhard Gruber <sup>2</sup>, Ariane Kronthaler <sup>1</sup>, Judith Hagenbuchner <sup>3</sup>, Ira Skvortsova <sup>4,5</sup>, Inken Piro <sup>6</sup>, Katja Steiger <sup>6</sup>, Vladan Martinovic <sup>7</sup>, Danijela Minasch <sup>8</sup>, Judith Löffler-Ragg <sup>7</sup>, Gianpaolo di Santo <sup>1</sup>, Irene J. Virgolini <sup>1</sup> and Elisabeth von Guggenberg <sup>1,\*</sup>

<sup>1</sup> Department of Nuclear Medicine, Medical University of Innsbruck, 6020 Innsbruck, Austria

<sup>2</sup> Department of Radiology, Medical University of Innsbruck, 6020 Innsbruck, Austria

<sup>3</sup> 3D Bioprinting Core Facility, Department of Child and Adolescence Health, Pediatrics I, Medical University of Innsbruck, 6020 Innsbruck, Austria

<sup>4</sup> EXTRO-Lab, Department of Therapeutic Radiology and Oncology, Medical University of Innsbruck, 6020 Innsbruck, Austria

<sup>5</sup> Tyrolean Cancer Research Institute (TKFI), 6020 Innsbruck, Austria

<sup>6</sup> Institute of Pathology, TUM School of Medicine and Health, Technical University of Munich, 81675 Munich, Germany

<sup>7</sup> Department of Pulmonology, Hospital Natters, 6161 Natters, Austria

<sup>8</sup> Department of Radiation Oncology, Medical University of Innsbruck, 6020 Innsbruck, Austria

\* Correspondence: elisabeth.von-guggenberg@i-med.ac.at

## Abstract

**Background/Objectives:** Peptide receptor radionuclide therapy (PRRT) is an established treatment for neuroendocrine tumors (NETs), enabling targeted radiation delivery via radiolabeled peptides. Small cell lung cancer (SCLC) remains a major therapeutic challenge due to its aggressive nature and poor prognosis. Despite advances, relapse rates are high and effective therapies are limited. We previously demonstrated the diagnostic potential of the cholecystokinin-2 receptor (CCK2R)-targeting minigastrin analog [<sup>68</sup>Ga]Ga-DOTA-MGS5 in PET/CT imaging of different NETs. Building on this, we developed and evaluated [<sup>177</sup>Lu]Lu-DOTA-MGS5 as a therapeutic PRRT agent. **Methods:** Preclinical studies investigating the receptor-mediated cellular internalization and intracellular distribution over time in A431 cells with and without CCK2R expression were performed using the fluorescent tracer ATTO-488-MGS5. Short- and long-term cytotoxic effects of [<sup>177</sup>Lu]Lu-DOTA-MGS5 were evaluated on the same cell line using trypan blue exclusion and clonogenic survival assays. CCK2R expression was assessed by immunohistochemistry in 42 SCLC tissue specimens. In addition, the first PRRT with [<sup>177</sup>Lu]Lu-DOTA-MGS5 was conducted in a patient with extensive disease SCLC (ED-SCLC) after confirming CCK2R-positive uptake in [<sup>68</sup>Ga]Ga-DOTA-MGS5 PET/CT. **Results:** Rapid binding and internalization into A431-CCK2R cells, with progressive accumulation in intracellular compartments, was observed for ATTO-488-MGS5. Short-term irradiation effects of [<sup>177</sup>Lu]Lu-DOTA-MGS5 were comparable for 4 h and 24 h incubation and were between the effects obtained with 2 and 4 Gy of external beam radiotherapy (EBRT). Clonogenic survival of A431-CCK2R cells incubated with increasing activity of [<sup>177</sup>Lu]Lu-DOTA-MGS5 decreased in a dose-dependent manner. Immunohistochemistry on SCLC specimens confirmed moderate to high CCK2R expression in 16 out of 42 SCLC samples. In the first patient with SCLC treated with four cycles of [<sup>177</sup>Lu]Lu-DOTA-MGS5 with a total activity of 17.2 GBq, an improvement in clinical symptoms was observed. **Conclusions:** The preclinical and clinical results confirm the feasibility of [<sup>177</sup>Lu]Lu-DOTA-MGS5 PRRT in patients with SCLC and support further



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clinical studies investigating the therapeutic value and clinical applicability of this new CCK2R-targeted theranostic approach in larger patient cohorts.

**Keywords:** peptide receptor radionuclide therapy; cholecystokinin-2 receptor; minigastrin; SPECT/CT imaging; first-in-human

## 1. Introduction

Peptide receptor radionuclide therapy (PRRT) has made significant progress as a highly effective treatment modality for various cancers, in particular neuroendocrine tumors (NETs). PRRT employs radiolabeled peptides that bind to specific receptors, enabling targeted delivery of cytotoxic radiation directly to cancer cells, thus minimizing damage to surrounding healthy tissues [1].

With the approval of [<sup>177</sup>Lu]Lu-DOTA-TATE (Lutathera®) targeting somatostatin receptors (SSTRs) by the European Medicines Agency (EMA) in 2017 and the FDA in 2018, following the positive outcomes of the NETTER-1 phase III trial, a broader clinical application of PRRT has become possible. The study demonstrated significantly improved progression-free survival in patients with advanced midgut NETs treated with [<sup>177</sup>Lu]Lu-DOTA-TATE [2]. In various studies, significant improvements in patient outcomes with PRRT in terms of overall response rates and quality of life were shown [3–5].

Lung neuroendocrine neoplasms (NENs) represent a heterogeneous group of tumors characterized by neuroendocrine morphology and immunophenotype. According to the 2022 WHO classification, they are divided into well-differentiated NETs—including typical carcinoid (low-grade) and atypical carcinoid (intermediate-grade) NETs, as well as NETs with elevated mitotic counts and/or proliferation indices—and poorly differentiated neuroendocrine carcinomas (NECs), which comprise small cell (lung) carcinoma and large cell neuroendocrine carcinoma [6].

Notably, in contrast to gastroenteropancreatic neuroendocrine tumors (GEP-NETs), SSTR expression in pulmonary NETs shows greater heterogeneity. Recent studies report that only 24–44% of patients with metastatic pulmonary NET demonstrate uniformly positive SSTR expression on positron emission tomography (PET) imaging [7–9]. The expression of SSTRs was analyzed by immunohistochemistry in SCLC tissue samples, revealing that 38% of the specimens were positive for the SSTR subtype 2 [10]. Consequently, there is a clear need to investigate alternative radiopharmaceuticals beyond those targeting SSTRs, in order to provide effective diagnostic and therapeutic options for patients lacking sufficient SSTR expression.

Small cell lung cancer (SCLC), accounting for 13% to 15% of all lung cancer cases globally, represents a significant clinical challenge. Extensive disease is associated with a particularly poor prognosis, with the five-year survival rate commonly reported to be less than 10% [11–13]. The treatment strategies for SCLC have evolved, with the current standard of care primarily involving a combination of platinum-based chemotherapy—either cisplatin or carboplatin—paired with etoposide. This regimen is recommended by several guidelines, for both limited disease (LD-SCLC) and extensive disease (ED-SCLC) [13–15]. Recent updates have incorporated the use of immune checkpoint inhibitors such as atezolizumab or durvalumab in conjunction with standard chemotherapy, particularly for patients with ED-SCLC, reflecting a shift towards more comprehensive therapeutic approaches [15,16]. The management of SCLC has been shown to benefit from additional interventions such as prophylactic cranial irradiation (PCI), especially in responding patients, as it can signifi-

cantly reduce the risk of symptomatic brain metastases [17]. Lurbinectedin and topotecan can be considered as subsequent systemic therapy options [13,14].

Despite advancements in cancer treatment, there remains a significant need to improve therapeutic options and explore novel treatment modalities for patients with SCLC, as current therapies often provide limited efficacy and are associated with high relapse rates. Furthermore, emerging strategies focusing on personalized approaches may pave the way for more effective and individualized treatment paradigms, potentially improving prognosis in this challenging disease [18,19]. Therefore, the exploration of novel agents, including radiopharmaceuticals and combination therapies, is essential for future research and clinical application in the management of SCLC [20,21].

Previously, we have demonstrated the ability of the new radiolabeled minigastrin analogue [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 targeting the cholecystokinin-2 receptor (CCK2R) to accurately detect malignant lesions by means of PET/CT imaging, supporting its potential as a valuable diagnostic tool in patients with advanced medullary thyroid carcinoma (MTC) as well as SCLC [22,23]. Within a phase I/IIA clinical trial, the safety and dosimetry, as well as the diagnostic performance of [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5, was evidenced in patients with advanced MTC and a patient with bronchopulmonary NET [24].

In addition, we have reported the successful radiopharmaceutical development and preclinical evaluation of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5, establishing a solid foundation for its further advancement as a therapeutic agent [25]. In this previous work, a standardized radiopharmaceutical preparation with confirmed stability was established and the experimental data on non-clinical pharmacology and toxicology was described. The performed studies confirmed the high CCK2R affinity and improved targeting properties of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. Furthermore, the first dose estimates and toxicological studies in support of the clinical translation were provided and the initial peptide dose for clinical application was set.

The data collected in the present work will be part of the Investigational Medicinal Product Dossier (IMPD) to support a phase I clinical trial application with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 in patients with SCLC. In this new study, we report on the CCK2R expression in tumor samples from patients with SCLC evaluated by immunohistochemistry. We further investigated the receptor interaction at the cellular level using the peptide analog MGS5 conjugated to a fluorescence dye. The therapeutic effects of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 were also assessed by comparing CCK2R-expressing cells incubated with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 with the same cell line irradiated by external beam radiotherapy (EBRT). In addition, we report on the first application of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 in a patient with ED-SCLC. The results provide a preliminary basis for future clinical trials in this patient group.

## 2. Materials and Methods

### 2.1. Cell Cultures

The A431 human epidermoid carcinoma cell line (American Type Culture Collection, Manassas, VA, USA) stably transfected with the plasmid pCR3.1 containing the complete human CCK2R coding sequence (A431-CCK2R) and the same cell line transfected with the empty vector alone (A431-mock) were originally generated by Dr. Luigi Aloj and used with his permission [26]. A431 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (*v/v*) fetal bovine serum and 5 mL of a 100× penicillin–streptomycin–glutamine mixture. Cells were cultured in a humidified atmosphere containing 5% CO<sub>2</sub> at 37 °C. Cells were passaged three times per week with 10× 2.5% trypsin–EDTA solution at a 1:2–1:3 ratio. All media and supplements were purchased from Sigma-Aldrich (Darmstadt, Germany) or Invitrogen Corporation (Lofer,

Austria). Strict aseptic culture conditions were adhered to, preventing fungal, bacterial or mycoplasma contamination.

## 2.2. Fluorescence Microscopy

ATTO-488 NHS-ester (ATTO-TEC, Siegen, Germany) was conjugated to the N-terminus of the MGS5 peptide sequence via amide bond formation. The reaction was carried out in acetonitrile under basic conditions (10  $\mu$ L of DIPEA, pH~9) for 1 h. The conjugation reaction resulted in an approximately 30% yield. After coupling, the ATTO-488-MGS5 product was purified by preparative HPLC to isolate the labeled fraction. ATTO-488-MGS5 was obtained with a purity >95%, lyophilized and stored at  $-20^{\circ}\text{C}$  for up to 2 months. The identity was confirmed by mass analysis using a Q Exactive HF mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

The cell uptake of the fluorescently labeled ligand was analyzed on A431-CCK2R and A431-mock cells. Cells were seeded on labteks or glass plates coated with 0.1 mg/mL collagen (Corning, New York, NY, USA) at a density of 30,000 or 500,000 cells, 36 to 48 h prior to assay. In order to differentiate the nucleus, nuclei were stained using 1  $\mu$ g/mL Hoechst 33342 (Merck, Vienna, Austria) 30 min before imaging. Then cells were incubated with 100 nM of ATTO-488-MGS5, and cellular microscopy was performed using an Axiovert 200 M microscope (Zeiss, Oberkochen, Germany), using a setting of  $\lambda_{\text{exc}} = 405$  nm for Hoechst nuclear staining and  $\lambda_{\text{exc}} = 488$  nm for the fluorescent ligand. Image analyses were performed with Axiovision Software (version 4.8, Zeiss, Oberkochen, Germany). Receptor-specific uptake in A431-CCK2R cells was confirmed by additional blocking studies by incubating the cells with a 100-fold excess of DOTA-MGS5 (10  $\mu$ M) 10 min prior to ATTO-488-MGS5 exposure and using A431-mock cells without receptor expression as a negative control.

Quantitative evaluation of ATTO-488-MGS5 uptake was performed by densitometric analysis of time-lapse fluorescence microscopy images. For each time course, 5–6 well-isolated A431-CCK2R cells were selected, resulting in 16 cells from three independent experiments. For each cell and time point, two regions of interest (ROIs) were defined: (i) the whole-cell area and (ii) the intracellular compartment excluding the plasma membrane. Mean fluorescence intensity was measured for both ROIs, and the membrane-associated signal was calculated as follows:

$$I_{\text{membrane}} = I_{\text{whole cell}} - I_{\text{intracellular}}$$

All membrane and intracellular fluorescence values were normalized to the 5 min time point after tracer addition, and fold-change over time was used to describe the uptake kinetics.

## 2.3. Cell Viability Assay

To investigate the cytotoxic effect of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 on A431-CCK2R cells in comparison with EBRT, cells were counted using a TC20 automated cell counter (Bio-Rad Laboratories, Hercules, CA, USA), seeded at a density of  $1 \times 10^5$  cells per well in 6-well plates and incubated at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . After 24 h, cells were irradiated with varying doses (2, 4 and 8 Gy) using a linear accelerator (Elekta Precise, Elekta Oncology Systems, Crawley, England, UK) or by incubation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 (~250 kBq, 2 nM/well) for 4 and 24 h followed by replacement with fresh medium. Incubation periods of 4 h and 24 h were selected based on the previously established internalization kinetics and the stability data obtained for [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 [25].

At the time point of 72 h after treatment, cell viability was assessed using a Beckman Coulter Vi-CELL AS cell viability analyzer (Beckman Coulter, Fullerton, CA, USA). The

supernatant from each well was collected in 15 mL Falcon tubes. Cells were then washed twice with 1 mL of serum-free medium, and the wash fractions were combined with the respective supernatants. Trypsin (300  $\mu$ L) was added to each well, and plates were incubated until detachment. Following detachment, 1 mL of medium was added to each well, and cells were rinsed and transferred to their respective Falcon tubes. This washing step was repeated twice, reaching a total volume of 3 mL.

Cells were pelleted by centrifugation in a benchtop centrifuge (Megafuge™ 8; Thermo Fisher Scientific, Waltham, MA, USA) at 2000 rpm for 8 min at 4 °C, and the supernatant was discarded. Pellets were resuspended in 1 mL of cold serum-free medium. Samples were loaded into the Vi-CELL AS analyzer according to the manufacturer's instructions. The number of viable cells in each treatment group was normalized to the mean number of viable cells in the non-irradiated control group and expressed as a percentage of the control.

#### 2.4. Clonogenic Survival Assay

To investigate the clonogenic survival after incubation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5, A431-CCK2R cells were counted in the automated cell counter and serial dilutions were performed to achieve a final concentration of 1000 cells/mL (1 cell/ $\mu$ L). Based on the treatment with increasing activities of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 (250 kBq–2 MBq, ~100 nM in each well), cell numbers seeded in 6-well plates were adapted and incubated at 37 °C with 5%  $\text{CO}_2$ . After 24 h of incubation to allow for cell attachment, cells were treated with the increasing activities of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 for 24 h at 37 °C. After this treatment, the medium was aspirated and the cells were gently washed twice with phosphate-buffered saline (PBS) and then incubated at 37 °C for 12 days to allow colony formation. At the end of the incubation period, the culture medium was removed, and the wells were gently rinsed twice with PBS.

Colonies were fixed by adding 1 mL of a 3:1 methanol–glacial acetic acid solution to each well and incubated for 20 min at room temperature in a chemical hood. The solution was removed and the fixed cells gently rinsed twice with 1 mL of PBS.

Staining was performed using 1 mL of Giemsa stain (modified solution; Sigma-Aldrich, St. Louis, MO, USA, CAS No. 32884) diluted 3.5:10 in Milli-Q water. Plates were incubated with the staining solution for 2 h at room temperature. After staining, wells were rinsed twice with Milli-Q water, and plates were inverted on absorbent paper to air-dry.

Colonies were counted manually, with each visible purple-stained cluster considered as one colony. The number of colonies in each treatment group was normalized to the plating efficiency of a control group not subjected to incubation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 and expressed as percentage of clonogenic survival. Each data point represents the mean  $\pm$  standard deviation (SD) of four independent experiments, each performed in triplicate.

#### 2.5. Immunohistochemical Staining

The expression of CCK2R in SCLC was assessed by immunohistochemistry on tissue specimens available at the tissue archive of the Institute of Pathology of the Technical University of Munich, Germany. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Technical University of Munich (2024-130-S-DFG-SB) on 9 April 2024. Informed consent for participation was not required by local legislation (Bayerisches Krankenhausgesetz—Art. 27 Datenschutz). A total of 42 tissue specimens, each from a distinct patient (25 men and 17 women) with histologically confirmed SCLC, were analyzed. The samples comprised tissue samples taken from 41 primary tumors and one lymph node metastasis. Slides were deparaffinized and rehydrated using a decreasing concentration of alcohol, followed by heat-induced

epitope retrieval at 100 °C for 40 min in Bond™ H1 solution. Endogenous peroxidase activity was blocked, and sections were incubated with a mouse monoclonal CCK2R (E-3) antibody raised against amino acids 1–85 mapping at the N-terminus of CCK2R of human origin (sc-166690; Santa Cruz Biotechnology, Dallas, TX, USA) at a dilution of 1:75 for 15 min at room temperature.

The Bond Polymer Refine Detection Kit (DS9800; Leica Biosystems, Wetzlar, Germany) without the post-primary reagent was used for antibody detection, followed by DAB (3,3'-diaminobenzidine, BS04-500; Medac Diagnostica, Wedel, Germany) staining using brown chromogen (DS9800, Bond Polymer Refine Detection; Leica Biosystems, Wetzlar, Germany), hematoxylin counterstaining, dehydration, clearing and mounting using Pertex mounting medium (00801; Histolab, Goeteborg, Sweden). Slides were digitally scanned with a resolution of 0.252 µm per pixel (equivalent to a 40× objective) using a Leica Aperio Imager AT2 (Leica Biosystems, Wetzlar, Germany). Images were analyzed using Aperio ImageScope software (version 12.4.6.5003). The same procedure was applied to the tissue specimen of the patient with ED-SCLC treated with [<sup>177</sup>Lu]Lu-DOTA-MGS5. Antibody specificity was evaluated using specimens from human stomach tissue, known to physiologically express CCK2R, and normal lung tissue lacking CCK2R expression (Supplementary Figure S2). The absence of non-specific binding was confirmed using another mouse monoclonal antibody (407317; Sigma-Aldrich/Merck, Darmstadt, Germany).

All samples were evaluated for CCK2R immunoreactivity in terms of a positive signal (brown DAB precipitate). Staining intensity (I, score: 0–3) and frequency (P, %) were assessed for each tumor sample. A final immunoreactivity score was defined as  $IRS = I \times (P/100)$ —with IRSs being in a final range between 0 and 3. All IRS scoring was performed by the same experienced pathologist.

## 2.6. Clinical Case Presentation

A patient diagnosed with SCLC (initial stage: T4 N3 M1b with primary tumor of the left central lung and possible metastasis to the left adrenal gland) completed first-line systemic therapy with carboplatin/etoposide/atezolizumab (schedule: day 1–day 3, every three weeks) followed by maintenance therapy with atezolizumab and prophylactic cranial irradiation (fractions of 2 Gy each, reaching a total dose of 30 Gy; cognitive preservation with memantine). Subsequently, the entire primary tumor region, including the selectively involved lymph nodes on the left central side, was irradiated (fractions of 2 Gy, resulting in a total dose of 60 Gy). After completing EBRT approximately six months after diagnosis, the disease remained stable for the following four months. Ten months after diagnosis, a new metastasis to the right adrenal gland occurred, and re-induction therapy with carboplatin/etoposide/atezolizumab was administered (a total of four cycles; schedule: day 1–day 3, every three weeks). In the follow-up CT scan, progressive disease was identified again, and third-line therapy with cyclophosphamide/doxorubicin/vincristine was initiated (a total of four cycles; schedule: day 1–day 2, every three weeks). Further progression was seen at the next follow-up CT scan, and the therapy was switched to fourth-line treatment with weekly topotecan (day 1, day 8 and day 15). After 2 cycles, the disease again progressed. This time, there was progression of the primary tumor, liver metastases and new lymph node metastases. Thus, alternative image-guided treatment strategies were evaluated. PET/CT imaging with [<sup>68</sup>Ga]Ga-DOTA-MGS5 targeting the CCK2R, performed seventeen months after initial diagnosis, showed intense uptake in tumor lesions, while only faint somatostatin receptor-related uptake was observed in PET imaging with [<sup>68</sup>Ga]Ga-DOTA-TOC [23]. Thus, the patient was considered eligible for PRRT with [<sup>177</sup>Lu]Lu-DOTA-MGS5. PRRT was performed under the responsibility of the treating physician and after tumor board approval. According to the Austrian Medicines Act

(Arzneimittelgesetz §8), named patient use (“Heilversuch”) was not subject to authorization by or notification to the authorities. Written informed consent was obtained from the patient. All procedures were in accordance with the principles of the 1964 Declaration of Helsinki and its subsequent amendments. The retrospective analysis of the data of the patient was exempted from consultation with the Ethics Committee by the Medical University of Innsbruck.

## 2.7. [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 PRRT

### 2.7.1. Automated Radiolabeling Process

An activity of 1500 MBq for dosimetric evaluation and four activities of 4000 MBq [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 for therapeutic use were prepared using an automated synthesis module (Modular-Lab PharmTracer<sup>®</sup>; Eckert & Ziegler Eurotrope GmbH, Berlin, Germany) following a previously published method [25]. Each batch was subjected to a full quality control evaluation before release for patient administration. The specifications and analytical methods for the quality control of the final product (as shown in Supplementary Table S1) were established based on experience with in-house production of different radiotherapeutics and Ph. Eur. monographs available for other radiopharmaceuticals, and the stability of the final product was evaluated previously [25,27,28]. The determination of the peptide content was based on a calibration curve and with a limit of detection of 1  $\mu\text{g/mL}$  and a limit of quantification of 5  $\mu\text{g/mL}$ .

### 2.7.2. Treatment Regimen

Adequate hydration was ensured through intravenous infusion of 1000 mL of 0.9% saline solution at a rate of 300 mL/h, starting 30 min before and continuing for 2 h after treatment administration. The patient received prophylactic antiemetic therapy with 4 mg ondansetron, as well as 4 mg dexamethasone and 40 mg pantoprazole, given 20 min before the treatment. Prophylactic antiemetic therapy was repeated 12 h after therapy.

[ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 was administered intravenously via a dedicated peristaltic infusion pump system over a period of 15–20 min. The patient was discharged at the third day following therapy. The treatment was repeated approximately every four weeks.

### 2.7.3. Image Acquisition Protocol

Whole-body SPECT/CT after each therapy cycle was performed using a dual-head SPECT/CT system (NM/CT 870 DR; GE Healthcare, Chicago, IL, USA) approximately 24 h after injection of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. The acquisition consisted of 60 projections over 360° with an angular step of 6° and a dwell time of 20 s per projection. A medium-energy general-purpose collimator was used. The primary energy window was set to 187.2–228.8 keV and the scatter window to 169.1–186.9 keV. SPECT images were reconstructed using the manufacturer’s proprietary algorithms (OSEM with resolution recovery), using 2 iterations and 10 subsets and no post-reconstruction filtering. The reconstructed matrix size was 128  $\times$  128, with a pixel size of 4.42 mm. Photon scatter correction was performed using the dual energy window method, and attenuation correction was performed using a low-dose CT scan acquired at 120 kVp, 30 mAs effective, with CareDose4D activated. Whole-body planar imaging was performed in anterior and posterior views using the same system. The scan was conducted in supine position, feet-first orientation. The table traversed 1.14 m at a speed of 10 cm/min. The matrix size was 1024  $\times$  256, with a pixel spacing of 2.40 mm. Imaging after the fourth therapy cycle had to be performed on a different camera system and with a different protocol (for details, see the Supplementary Materials).

For dosimetry, both planar and SPECT/CT imaging were performed at nominal time points of 30 min and 4, 24, 72 and 96 h post-injection (p.i.). The acquisition protocol for the

first three time points followed the procedure described above. For the 72 and 96 h scans, the dwell time per projection for SPECT acquisition was increased to 24 s, and the planar scan speed was reduced to 8 cm/min to compensate for the reduced count statistics at later time points.

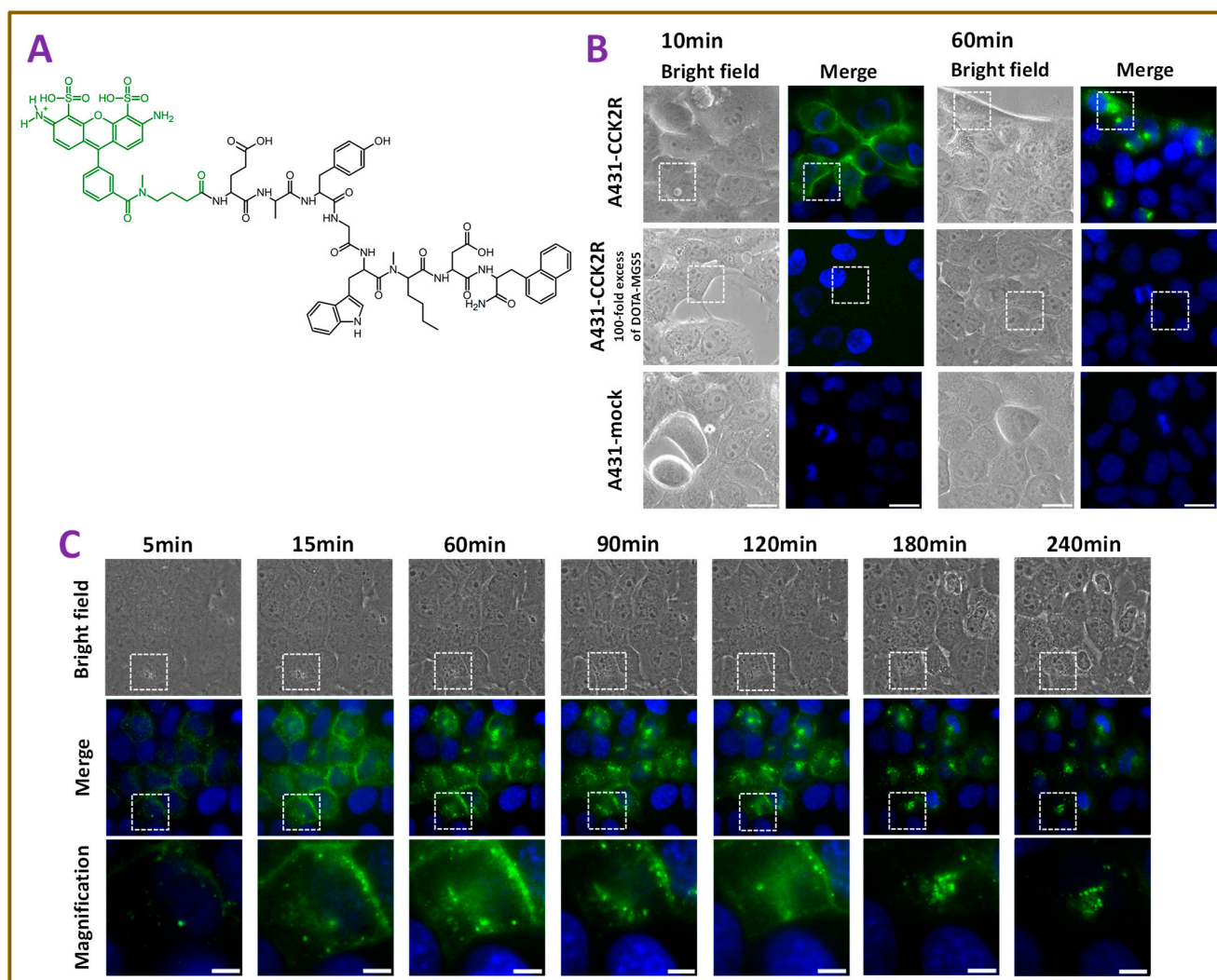
#### 2.7.4. Quantification and SPECT-SUV

The gamma camera sensitivity was calibrated according to the GE Healthcare-recommended protocol (DICOM Conformance Statement: NM General Purpose 600/800 Series, Rev. 15, 2018). A Petri dish containing a known activity of lutetium-177 was used as the calibration source, yielding a calibration factor of 5.2 cps/MBq. To correct for partial volume effects (PVEs), recovery coefficients (RCs) were determined using an IEC-NEMA phantom with fillable spheres of varying diameters, and a Jaszczak phantom containing a 60 mm sphere (Data Spectrum Corporation, Hillsborough, NC, USA). For each sphere, RCs were calculated, and a logarithmic regression model was fitted to generate a recovery curve. Two selected well-delineable lesions were segmented on 24 h p.i. SPECT images using a 41% isocontour threshold in Affinity 3.0 (Hermes Medical Solutions, Stockholm, Sweden). Subsequently, SUV<sub>max</sub> values normalized to body weight were computed and corrected for PVEs using the corresponding RCs.

### 3. Results

#### 3.1. Fluorescence-Based Receptor-Specific Cell Uptake

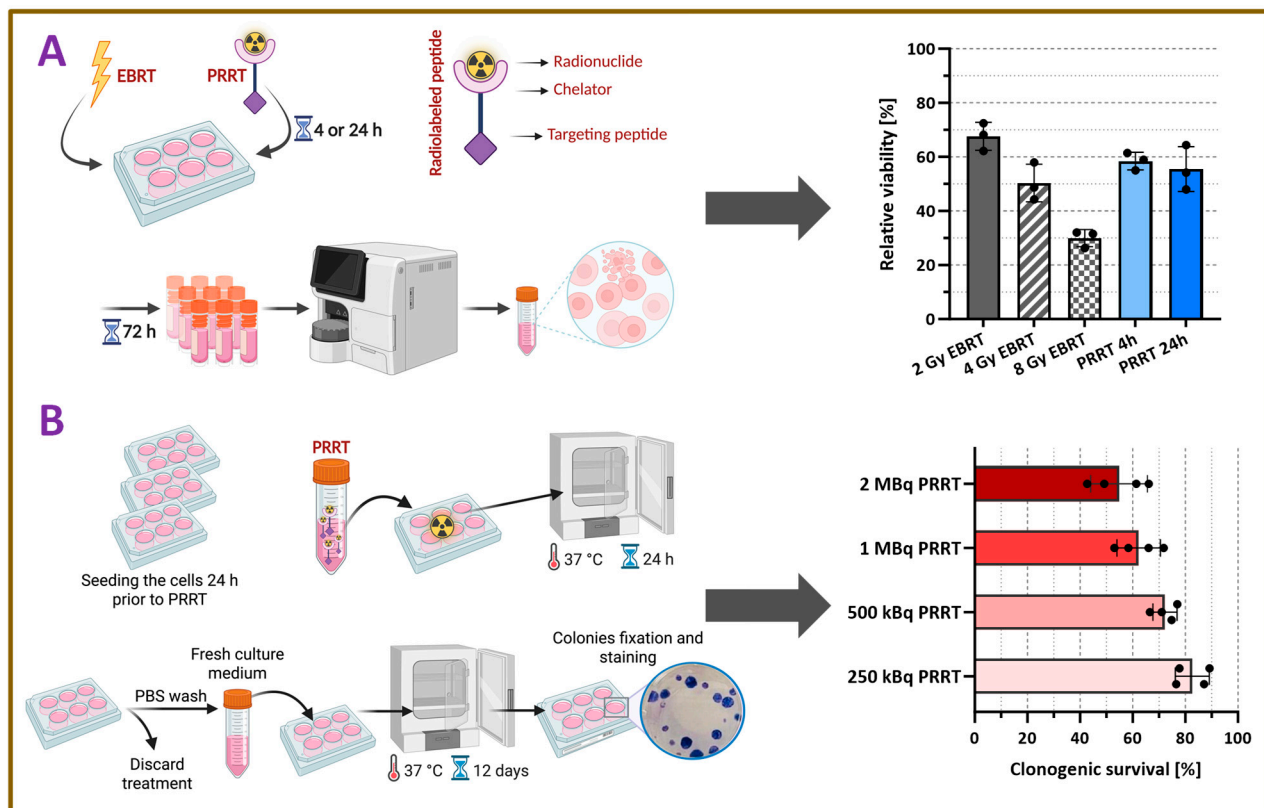
The structural formula of ATTO-488-MGS5 (MW: 1635 g/mol) is shown in Figure 1A. Cellular microscopy tracking of the fluorescent ligand in A431-CCK2R cells demonstrated rapid internalization of ATTO-488-MGS5. No uptake was visible in A431-mock cells imaged at 10 min and 60 min after incubation with ATTO-488-MGS5. Blocking experiments for the same time points showed also that pre-incubation with a 100-fold excess of DOTA-MGS5 prior to exposure to the fluorescently labeled ligand prevented the uptake of ATTO-488-MGS5, further confirming that the uptake occurs specifically through CCK2R (Figure 1B). Time-lapse fluorescence microscopy of the same field of view in A431-CCK2R cells revealed the dynamic internalization of ATTO-488-MGS5. Upon addition of the fluorescent ligand, rapid binding to the cell surface was observed. Within minutes, the fluorescently labeled ligand was internalized, leading to progressive accumulation of ATTO-488-MGS5 in intracellular compartments over time, with a significant intracellular fluorescent signal persisting even 4 h after incubation (Figure 1C). Quantitative analysis of fluorescence intensities across three independent experiments ( $n = 3$ ) confirmed that the membrane-associated signal increased rapidly and peaked within the first 60–90 min, while the intracellular fluorescence rose more gradually, exceeding the membrane-associated signal starting at 20 min, peaking at around 90–120 min and persisting for several hours. The corresponding uptake curves are provided in Supplementary Figure S1.



**Figure 1.** Fluorescence imaging of A431-CCK2R and A431-mock cells. (A) Structural formula of ATTO-488-MGS5. (B) Uptake of ATTO-488-MGS5 in A431-CCK2R cells after 10 min and 60 min of incubation (upper row). The middle row shows A431-CCK2R cells pre-incubated with DOTA-MGS5 (10  $\mu$ M) for 10 min prior to ATTO-488-MGS5 exposure to demonstrate receptor-specific blocking. The lower row shows A431-mock cells incubated with ATTO-488-MGS5 with no uptake. (C) Time-dependent uptake of ATTO-488-MGS5 in A431-CCK2R from 5 min to 240 min. Cell nuclei are stained with Hoechst (blue), and ATTO-488-MGS5 (100 nM) is shown in green. Image size: 90  $\times$  90  $\mu$ m, magnification: 25  $\times$  25  $\mu$ m (white dashed frame), scale bar: 5  $\mu$ m (white line).

### 3.2. Short- and Long-Term Cytotoxic Effects of Radiation

The short-term irradiation effect was determined by analyzing the cell viability using the trypan blue exclusion method (Figure 2A). The viability of A431-CCK2R cells irradiated with EBRT was dose-dependent, with relative viability decreasing from  $67.6 \pm 5.2\%$  at 2 Gy to  $30.0 \pm 3.2\%$  at 8 Gy. Statistically significant differences were observed between all EBRT dose levels (2 Gy vs. 4 Gy:  $p = 0.0265$ ; 4 Gy vs. 8 Gy:  $p = 0.0098$ ; 2 Gy vs. 8 Gy:  $p < 0.0001$ ). The response of A431-CCK2R cells to [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 ( $\sim 250$  kBq, 2 nM/well) was similar for the 4 h and 24 h incubation periods and comparable to EBRT with 2–4 Gy, with relative viabilities of  $58.5 \pm 3.3\%$  and  $55.5 \pm 8.3\%$  at 4 h and 24 h incubation, respectively ( $p > 0.05$  for all). The effect of both PRRT conditions was, however, significantly lower than the effect observed in the 8 Gy EBRT group ( $p < 0.01$ ).

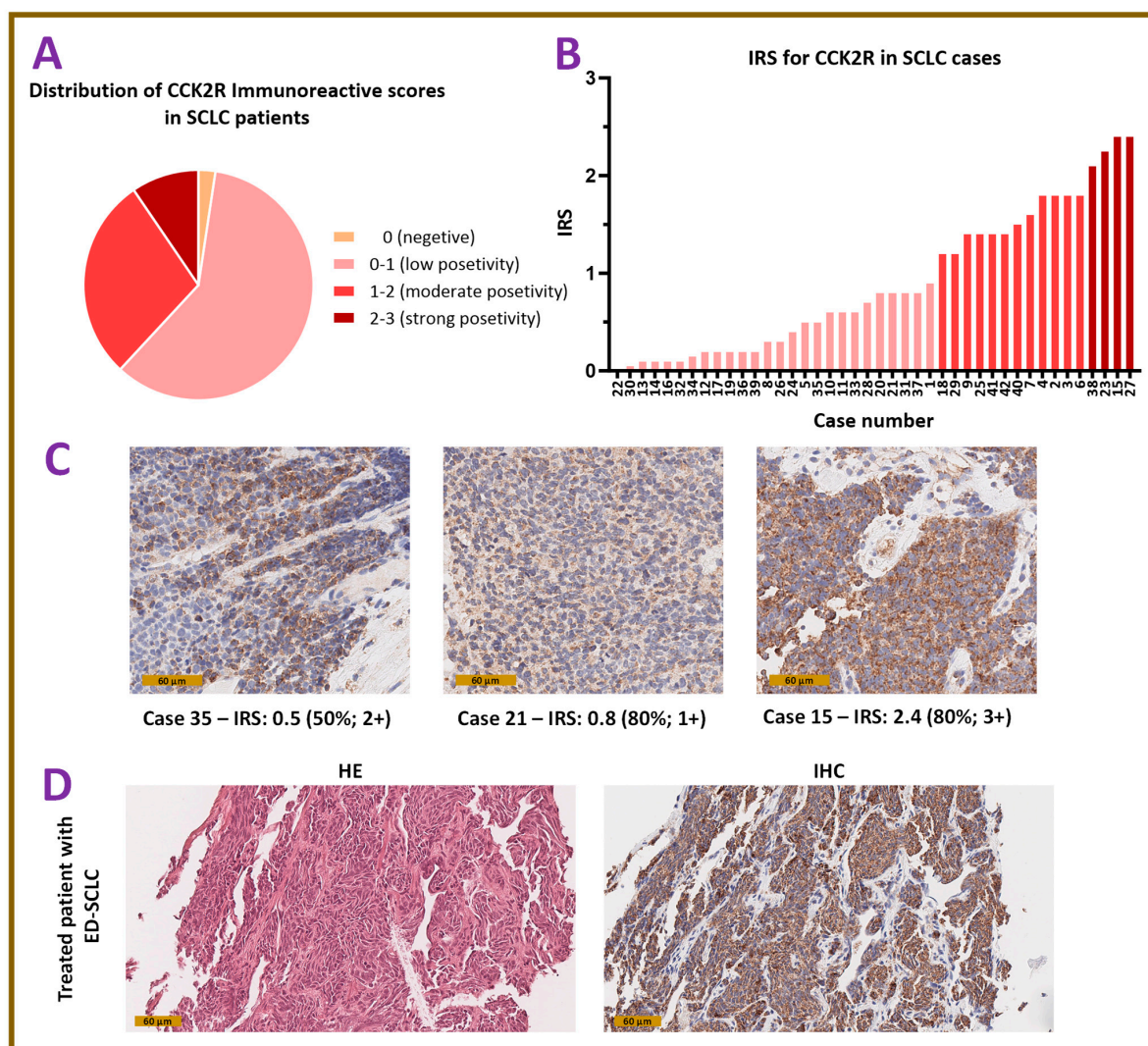


**Figure 2.** In vitro radiation effect on A431-CCK2R cells. (A) Schematic illustration of the short-term cell viability assay using EBRT or PRRT and percentage of relative viability 72 h after exposure to different doses of EBRT and varying incubation periods with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 ( $n = 3$ ). (B) Schematic illustration of clonogenic assay and percentage of clonogenic survival 12 days after treatment with different activities of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 ( $n = 4$ ).

In order to investigate the long-term effect of PRRT on A431-CCK2R cells, clonogenic assays were performed with different concentrations of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. Clonogenic survival of A431-CCK2R cells decreased in a dose-dependent manner following treatment with 250 kBq to 2 MBq [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. The percentage of cell survival was reduced to  $82.7 \pm 6.5\%$  with 250 kBq/well and decreased to  $54.9 \pm 10.9\%$  with 2 MBq/well (Figure 2B).

### 3.3. Immunohistochemical Findings

Immunohistochemical evaluation of tissue specimens from patients with SCLC revealed moderate to high CCK2R expression in more than one third of the specimens evaluated (38.1%, 16 patients out of 42). Immunoreactivity was mainly observed to be cytoplasmic with a granular staining pattern compatible with endosomal localization. Of the evaluated tissue specimens, one was negative with an IRS score of zero, 25 patients had an IRS score between 0 and 1, 12 patients had moderate CCK2R expression with an IRS score of 1 to 2, and 4 patients were strongly positive with an IRS score of 2 to 3, with the maximum IRS of 2.4 corresponding to 80% of tumor cells with strong positivity (Figure 3A–C). Immunohistochemical evaluation was also performed for the first patient treated with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. As shown in Figure 3D, the biopsy sample from the bronchial mucosa of the left upper lobe of the lung from this patient was strongly positive for CCK2R expression with an IRS score of 2.1. Negative and positive control staining can be found in Supplementary Figure S2.



**Figure 3.** CCK2R immunoreactivity as analyzed on tissue specimens of patients with SCLC: (A) distribution of CCK2R immunoreactivity scores (IRSs); (B) case-by-case visualization sorted by IRSs; (C) exemplary images of IHC staining for scores 0.5–2.4; (D) representative images of HE and IHC staining of biopsy samples from the small cell lung cancer (SCLC) lesion in the left upper lobe of the patient treated with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. Images are displayed at 400 $\times$  magnification; brown staining indicating CCK2R expression.

### 3.4. Pharmaceutical Formulation

A lower administered activity of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 with a radioactivity concentration of 105.2 MBq/mL and a total activity of 1505 MBq was produced for the patient-specific dosimetry study. Subsequently, four different batches of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 with a radioactivity concentration of  $458.2 \pm 10.0$  MBq/mL and a final product activity of  $6507.3 \pm 193.4$  MBq were prepared for PRRT. Quality control of each individual batch confirmed a radiochemical purity (RCP) of  $97.9 \pm 0.2\%$  (range: 97.7–98.2%) as analyzed by radio-HPLC. In addition, instant thin layer chromatography (iTLC) confirmed the presence of minor impurities with values of  $0.12 \pm 0.08\%$  for free lutetium-177 and  $0.09 \pm 0.05\%$  for colloidal lutetium-177. The quality control results for all therapeutic batches prepared for patient administration are shown in Supplementary Table S1. All batches produced complied with the defined specifications for the various parameters tested (appearance, pH, radioactivity concentration, radionuclide identity, identity, radiochemical purity, peptide

content, apparent specific activity, ethanol content, bacterial endotoxins and sterility) and met the required acceptance criteria for release.

### 3.5. First Patient Experience of PRRT with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5

Due to disease progression observed at the radiologic follow-up examination during fourth-line treatment with topotecan (Figure 4a–f) and following confirmation of intense tracer uptake on [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 PET/CT, the patient underwent a preliminary dosimetric evaluation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 to calculate the absorbed dose to organs at risk and the tumor. After the intravenous administration of 1.5 GBq [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 (corresponding to a peptide dose of less than 100  $\mu\text{g}$ ), scintigraphic images were acquired at 30 min, 4 h, 24 h, 72 h and 96 h p.i. (Supplementary Figure S3A). As previously described, the patient-specific dosimetry evaluation revealed absorbed doses of 0.28 Gy/GBq to the kidneys, 0.022 Gy/GBq to the bone marrow and 0.42 Gy/GBq to the stomach. Additionally, the mean absorbed dose to the tumor was  $12.5 \pm 10.2$  Gy/GBq (range: 1.2–28 Gy/GBq) across five well-delineated lesions [25]. The time–activity curves for kidneys, stomach wall and two selected lesions were fitted and extrapolated using non-linear regression. As shown in Supplementary Figure S3B, tracer clearance exhibited organ- and lesion-specific kinetics. The kidneys demonstrated a rapid initial decline in activity, consistent with fast clearance, while the CCK2R-positive stomach wall showed a slower and more prolonged washout. The thoracic wall lesion and gastrointestinal tract lesion displayed similar kinetic profiles, characterized by a gradual mono-exponential decrease in activity over time.

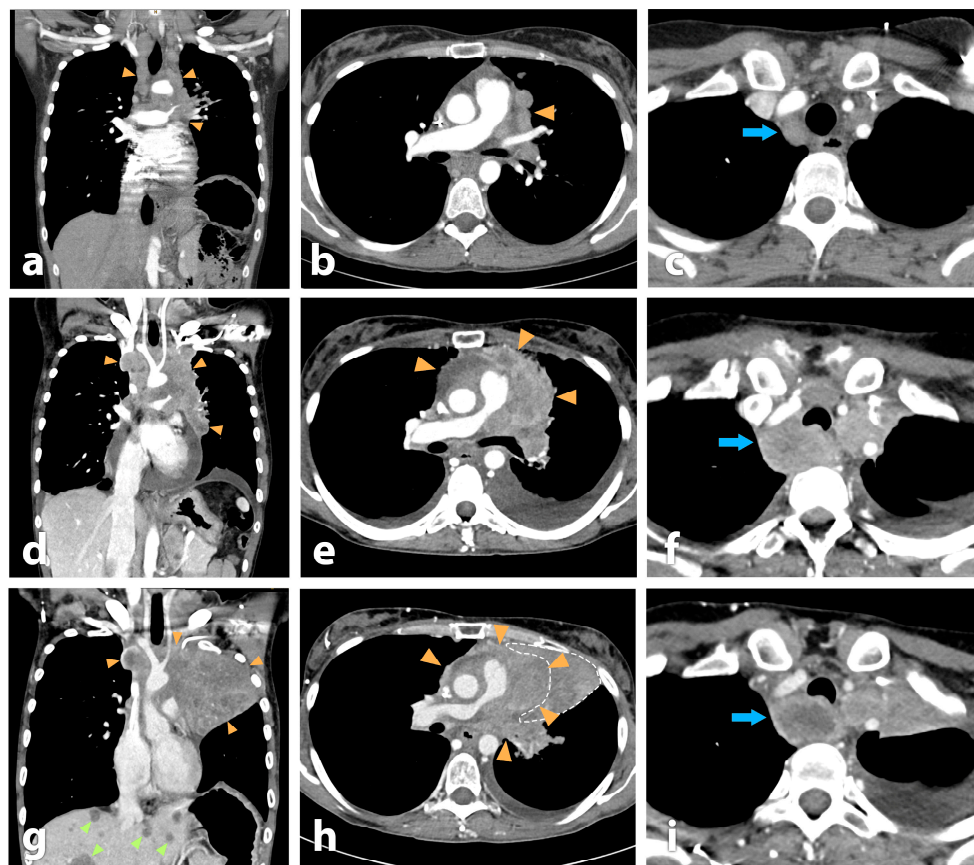
After confirming the treatment's feasibility due to the low dose load to organs at risk, the patient proceeded with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 therapy. Prior to the start of treatment, the patient was alert, in good general condition and reported no pain. Nausea was noted on an empty stomach. The patient's weight was 66 kg, with a BMI of 21.8 kg/m<sup>2</sup>. Blood values, including white blood cell count, hemoglobin and thrombocytes, as well as kidney parameters (creatinine: 0.66 mg/dL, eGFR > 60 mL/min/1.73 m<sup>2</sup>) and liver function tests (GOT: 31 U/L, GPT: 15 U/L, GGT: 72 U/L), were all within the acceptable range to support PRRT. Tumor markers indicated a high neuron-specific enolase (NSE) level of 166.0 ng/mL, while chromogranin A was within the normal range.

About two years after the initial diagnosis, the patient underwent four monthly cycles of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 therapy, with a cumulative administered activity of 17.2 GBq (mean activity per cycle: 4.3 GBq, corresponding to a peptide dose of less than 100  $\mu\text{g}$  per cycle). The therapy was well-tolerated, with no acute or delayed adverse effects. Additionally, no hematological, renal or hepatic toxicities were detected in laboratory tests performed during the treatment period. NSE levels showed a slight reduction after the first three administrations but increased by the fourth administration, alongside a rise in chromogranin A levels (Supplementary Figure S4).

The patient's performance, as measured by the Karnofsky Index, remained at 100% throughout treatment. Following the first administration, the patient experienced mild nausea. After the second administration, clinical symptoms showed improvement, although nausea and mild appetite loss persisted for a few days post-therapy, accompanied by a weight loss of approximately 2 kg. By the fourth administration, nausea and appetite loss were less pronounced, although the patient reported a mild onset of dyspnea.

Serial coronal and axial fused SPECT/CT images acquired after each treatment cycle with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 demonstrated overall stable uptake (Figure 5). In the two well-delineated lesions evaluated, only a minor decrease in SUV<sub>max</sub> was found (Supplementary Figure S5). Blood values, including white blood cell count, hemoglobin and thrombocytes, as well as kidney parameters (creatinine: 0.64 mg/dL, Egfr > 60 mL/min/1.73 m<sup>2</sup>) and liver function tests (GOT: 44 U/L, GPT: 14 U/L, GGT: 74 U/L), remained within an acceptable

range until the last cycle of PRRT. SPECT/CT imaging performed after the fourth cycle revealed new disseminated liver metastases, which were [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5-negative, indicating disease progression and dedifferentiation (Supplementary Figure S6). Imaging after the last therapy cycle had to be performed on a different camera system and with a different protocol which did not allow for a direct comparison of uptake values (for details, see the Supplementary Materials).

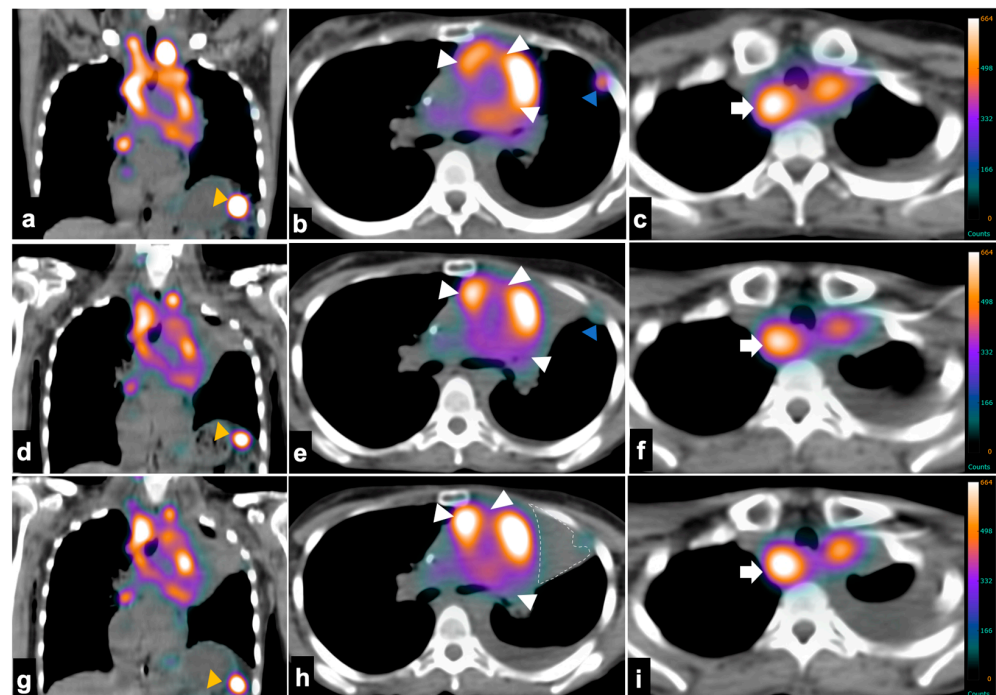


**Figure 4.** Coronal and axial computed tomography (window/level = 400/40 Hounsfield units) of the chest 4 months prior to (a–c), immediately prior to (d–f) and 3 months after starting PRRT (g–i) with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. Over the time course progressive tumor extension (orange arrowheads) into the mediastinum was observed, leading to increasing compression of mediastinal structures, illustrated by a progressive narrowing of the right pulmonary artery (b,e,h). Notice also areas of increasing tumor necrosis during growth, as demonstrated by a retrotracheal mass (blue arrows; (c,f,i)) and progressive infiltration of the left main bronchus leading to paracardial atelectasis (dashed outline). At the last check-up, disseminated liver metastases were noticed (green arrowheads (g)).

The follow-up contrast-enhanced CT performed at the fourth cycle of PRRT demonstrated continued disease progression, with primary tumor progress leading to increasing compression of mediastinal structures, illustrated by a progressive narrowing of the right pulmonary artery, increasing central necrosis of the retrotracheal mass and progressive infiltration of the left main bronchus with paracardial atelectasis (Figure 4). New disseminated liver metastases were also noted together with further progression of lymph nodal metastasis (Figure 4g–i).

PRRT was discontinued, and one month later fifth-line therapy with lurbinectedin was initiated. A rise in liver function parameters (GOT: 261 U/L, GPT: 65 U/L, GGT: 749 U/L) in line with progressive disseminated liver metastases was noted, and due to a marked decline in the patient's general condition following the first cycle with lurbinectedin, treatment was discontinued and the patient transitioned to symptom-related management. With

ongoing tumor progression, the patient passed away due to respiratory insufficiency and multiorgan failure one month later.



**Figure 5.** Coronal and axial fused single-photon emission tomography/computed tomography (SPECT/CT) after the first (a–c), second (d–f) and third (g–i) cycles of treatment with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. Imaging showed substantial stable disease over time (white arrowheads) with a slight response in small tumor lesions such as those in the gastrointestinal tract ((a,d,g)—yellow arrowheads) and in the left thoracic wall ((b,e,h)—blue arrowheads)—the same ones used for dosimetry studies. The retrotracheal mass (white arrow; (c,f,i)) appears to demonstrate constant uptake during the course of treatment.

#### 4. Discussion

In previous work, we have presented the preclinical development of the CCK2R-targeting minigastrin analog DOTA-MGS5 radiolabeled with gallium-68 and lutetium-177 [25,27,29]. Besides the full preclinical characterization of DOTA-MGS5 radiolabeled with different radiometals, the initial clinical translation of PET/CT imaging with [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 was achieved [22,23]. The aim of the present study was to provide further evidence on the applicability of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 for CCK2R-targeted PRRT in patients with SCLC in support of a first clinical trial application.

Using radioligand-based assays, we characterized and evaluated the receptor affinity and cellular uptake kinetics of DOTA-MGS5 comprehensively [25,27,29]. However, these assays do not allow the visualization of the receptor-mediated internalization processes. To investigate the uptake process at the cellular level, we synthesized the fluorescently labeled analog ATTO-488-MGS5, enabling real-time imaging of tracer–receptor interactions and intracellular trafficking. In dynamic fluorescent microscopy studies on A431-CCK2R cells incubated with Atto-488-MGS5, a rapid translocation from the cell surface to intracellular compartments leading to prolonged intracellular sequestration was confirmed, providing the basis for efficient receptor-specific tumor targeting.

Furthermore, cell-based assays were performed to investigate the therapeutic effect of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. In the cell viability studies performed using A431-CCK2R cells, the therapeutic effect of 250 kBq of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 was between the effects of EBRT with 2 and 4 Gy, which was used as a control treatment. The observed early

cytotoxic response to PRRT in this assay is consistent with known radiation-induced effects, such as cellular stress, cell cycle arrest and cell death. However, it has to be noted that the response of the cells to PRRT with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 delivering continuous low-dose-rate irradiation might differ from EBRT with short-term high-dose-rate exposure. The radiobiological differences between PRRT and EBRT primarily relate to the absorbed dose rate and the spatial characteristics of energy deposition. EBRT delivers homogeneous irradiation at a high absorbed dose rate, resulting in relatively uniform dose delivery to the cells. In contrast, PRRT is characterized by a low absorbed dose rate with protracted, heterogeneous irradiation, where energy deposition depends on the receptor-mediated uptake of the radiolabeled compound, the half-life of the radioisotope and the particle path length of the emitted radiation. Consequently, activity distribution is highly heterogeneous, leading to regions receiving substantial radiation exposure while neighboring territories may partially escape irradiation. These differences are known to influence the induction of DNA damage, repair kinetics and cellular survival, and should therefore be considered when comparing the biological effects of PRRT and EBRT *in vitro* [30,31]. For this reason, clonogenic assays were performed in addition to investigate the clonogenic survival after incubation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. The observed dose-dependent effects on clonogenic survival suggest a higher level of irreparable DNA damage with increasing activities of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 leading to long-term reproductive death. This observation is in line with lethal DNA damage through continuous low-dose irradiation, ultimately impairing the ability of cells to undergo multiple rounds of cell division [31]. A limitation of these experiments is the exclusive use of the engineered A431-CCK2R cell model for *in vitro* experiments. Currently, no human SCLC cell line with natural, endogenous overexpression of CCK2R is available, and consequently most published studies on CCK2R-targeting radiopharmaceuticals rely either on A431-CCK2R cells, which stably express the human receptor, or on the AR42J rat pancreatic tumor cell line with physiological CCK2R expression. In ongoing experiments, we are performing comparative studies with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 in A431-CCK2R and AR42J cells, including also A431-mock cells and blocking with excess peptide in AR42J cells to evaluate the receptor-specificity of the treatment. To further elucidate the cellular effects of PRRT, we intend to conduct additional *in vitro* assays, such as the  $\gamma\text{H2AX}$  assay, which will allow us to better characterize DNA double-strand break formation and repair dynamics, in addition to our current findings [32,33].

In lieu of a human SCLC cell line for preclinical testing, we have investigated the applicability of this new personalized therapeutic approach in patients with SCLC by performing immunohistochemistry analysis of the CCK2R receptor expression in tumor samples from patients with SCLC. The analysis of 42 tumor specimens confirmed moderate to high CCK2R expression in more than one third of the specimens evaluated, supporting that [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 can be used to target CCK2R in this patient group for selecting patients eligible for PRRT with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5. As shown previously for SSTRs, there is a moderate to strong correlation between SSTR expression and  $\text{SUV}_{\text{max}}$  of [ $^{68}\text{Ga}$ ]Ga-DOTA-TATE or [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC PET scans in different tumor entities [9,34,35]. No similar studies have been performed for CCK2R targeting so far. When investigating CCK2R expression in NETs by receptor autoradiography, Reubi et al. [36] reported a high incidence of CCK2R expression in MTC (92%, 22 out of 24 samples), SCLC (57%, 8 out of 14 samples), astrocytomas (65%, 11 out of 17 samples) and stromal ovarian cancer (100%, 3 out of 3 samples). In further studies, also, insulinomas and vipomas, as well as some bronchial and ileal carcinoids, were suggested as potential CCK2R targets, especially in patients with low or no SSTR expression. In addition, gastrointestinal stromal tumors (mean density of 8641 dpm/mg) and leiomyosarcomas (mean density of 7283 dpm/mg) express CCK2R at a high density [36–38]. Based on our immunohistochemical analysis in 38% of

the SCLC tissue specimens analyzed, high overexpression of CCK2R could be confirmed, meeting the essential condition for the effectiveness of any receptor-based radionuclide therapy. In a new clinical trial which has recently been started in our center (EUCT n. 2024-514584-25-00), we are currently investigating the correlation of CCK2R expression and uptake values of [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 PET/CT imaging in patients with SCLC.

We have recently reported the first evidence of [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5-positive lesion uptake in a patient with ED-SCLC [23]. The patient underwent dosimetric evaluation with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 [25], revealing high tumor doses in the range of 1.2–28 Gy/GBq combined with favorable pharmacokinetics leading to a low radiation burden of 0.28 Gy/GBq to the kidneys and 0.42 Gy/GBq to the stomach due to physiological uptake in this organ. Based on a tumor board decision, a first therapeutic attempt with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 was undertaken in this patient. A treatment regimen with an administered activity of 4 GBq at intervals of one month was applied to this patient with extensive tumor progression. The patient was treated with a total of four cycles, reaching a total administered activity of 17.2 GBq. This approach was chosen in the attempt to provide palliative treatment for the patient, improving the patient's quality of life while minimizing potential side effects. Besides mild nausea and mild appetite loss for a few days post-therapy, the treatment was well tolerated by the patient, who reported a sustained state of psychological and physical well-being. Moreover, the patient maintained full autonomy in daily activities and showed subjective improvement in dyspnea and asthenia, mostly within the first three cycles of treatment. Post-therapy SPECT/CT scans after each treatment cycle showed visually stable uptake across the first three cycles. Also, the SPECT/CT scan after the fourth cycle performed with a different camera system showed a similar uptake pattern. However, despite clinical stability during PRRT with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5, the patient showed progressive tumor extension in the diagnostic CT scan performed at the fourth cycle. Due to newly appearing disseminated liver metastases without [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5-uptake as well as further progression of the primary tumor, PRRT was discontinued. Together with the rise in tumor marker levels (i.e., NSE and chromogranin A) the tumor progression evidenced in the diagnostic CT and dedifferentiation noted on SPECT/CT imaging suggested resistance to treatment. Of note, the patient received treatment with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 after progression under various chemotherapy regimens and at a very advanced stage of the disease. Based on the dosimetric evaluation performed [25], a mean absorbed dose of 215 Gy (range: 21–482 Gy) could be delivered to the tumor lesions during PRRT with [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 in this patient. The calculated tumor-absorbed dose is in line with the tumor-absorbed dose of treatment with [ $^{177}\text{Lu}$ ]Lu-DOTA-TATE [39]. Even though no clear stabilization of the disease was achieved, the improvement in quality of life during the four months of PRRT was of particular importance for the patient. The absorbed doses of healthy tissue remained at acceptable levels of 5 Gy for the kidneys, 0.4 Gy for the bone marrow and 7 for the stomach. Fifth-line therapy with lurbinectedin initiated one month after discontinuation of [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 PRRT was also discontinued, and the patient passed away one month later due to the extensive tumor burden and worsening of the patient's general condition.

By means of the CCK2R-targeting theranostic pair [ $^{68}\text{Ga}$ ]Ga-DOTA-MGS5 and [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5, we could offer an attractive and highly specific alternative treatment option to the patient. Such a theranostic approach, combining imaging and therapy, allows for personalized treatment planning and monitoring [40]. The use of CCK2R-targeted PRRT resulted in a relief of the clinical symptoms and an improvement in the patient's quality of life.

So far,  $^{177}\text{Lu}$ -labeled peptides targeting CCK2R have been used for PRRT in patients with advanced MTC. In this patient group, the  $^{177}\text{Lu}$ -labeled CCK2R-targeting agonist

DOTA-(DGLu)<sub>6</sub>-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH<sub>2</sub> ([<sup>177</sup>Lu]Lu-PP-F11N) showed a favorable biodistribution with the main accumulation in the stomach and kidneys combined with a median absorbed tumor dose of 0.88 Gy/GBq, which was considered sufficient for a therapeutic approach [41]. In a dose escalation study, six patients with advanced MTC were treated with up to three to four cycles of [<sup>177</sup>Lu]Lu-PP-F11N (3 × 6 GBq or 4 × 8 GBq) at intervals of 8–10 weeks without signs of dose-limiting toxicity [41,42]. In another study, two patients with NETs showing sub-optimal uptake in SSTR-targeting [<sup>68</sup>Ga]Ga-DOTA-TATE PET/CT imaging were treated with the <sup>177</sup>Lu-labeled CCK2R-targeting agonist DOTA-(DGLu)<sub>6</sub>-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH<sub>2</sub> ([<sup>177</sup>Lu]Lu-CP04), demonstrating the potential theranostic application in patients with low-SSTR-expressing NETs [43]. In the first patient treated with [<sup>177</sup>Lu]Lu-DOTA-MGS5 a tumor dose of 12.5 ± 10.2 Gy/GBq (range: 1.2–28 Gy/GBq) was observed, confirming the high potential of this new radiopharmaceutical in this patient group. The high tumor burden in the patient, consistent with a very advanced and progressed stage of the disease at the time of treatment, may have limited the therapeutic efficacy. With increasing dedifferentiation, NETs become more aggressive and do not express the receptor targets for therapy [44]. This was possibly also the case in this first patient treated with [<sup>177</sup>Lu]Lu-DOTA-MGS5. In a future clinical trial (EUCT n. 2024-518039-12-00), we will investigate [<sup>177</sup>Lu]Lu-DOTA-MGS5 PRRT in a larger patient cohort to identify patients who could benefit from this new treatment at an earlier stage of the disease.

## 5. Conclusions

In this study, we demonstrated the receptor-mediated cellular internalization and strong intracellular retention of MGS5 in CCK2R-expressing cells. In addition, short- and long-term radiation effects of [<sup>177</sup>Lu]Lu-DOTA-MGS5 were demonstrated using different cell-based assays. The moderate to high CCK2R expression in SCLC supports the potential of a theranostic approach using DOTA-MGS5, with [<sup>68</sup>Ga]Ga-DOTA-MGS5 employed in PET/CT imaging for patient selection. This study provides the first proof of feasibility of CCK2R-directed PRRT with [<sup>177</sup>Lu]Lu-DOTA-MGS5 in a patient with SCLC. However, despite the high uptake of [<sup>177</sup>Lu]Lu-DOTA-MGS5 in most of the lesions, only an improvement in quality of life was achieved in this advanced-stage patient. The therapeutic efficacy and clinical applicability of this CCK2R-targeted approach need to be verified in a larger patient cohort.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/pharmaceutics18010138/s1>, Figure S1: Quantitative analysis of membrane-associated and intracellular fluorescence over time; Figure S2: CCK2R immunoreactivity: (A) normal lung tissue lacking CCK2R expression (magnification: ×100), (B) human stomach tissue known to physiologically express CCK2R (magnification: ×300), (C) anti-integrin β<sub>6</sub> Mouse mAb staining of sample from patient 15 showing no tumor cell staining, indicating absence of nonspecific mouse IgG background (magnification: ×400); Table S1: Acceptance criteria and quality control results of four individual therapeutic batches of [<sup>177</sup>Lu]Lu-DOTA-MGS5; Figure S3: Dosimetric evaluation with 1.5 GBq [<sup>177</sup>Lu]Lu-DOTA-MGS5: (A) representative serial whole-body planar images of the patient with ED-SCLC, (B) time–activity curves extrapolated for two lesions, as well as for the stomach and kidney, were derived from the serial SPECT/CT scans; Figure S4: Trend of tumor markers during PRRT with [<sup>177</sup>Lu]Lu-DOTA-MGS5 (reported as ng/mL); Figure S5: (A) Fused coronal view of a whole-body SPECT/CT scan acquired 24 h p.i. of [<sup>177</sup>Lu]Lu-DOTA-MGS5. The thoracic wall lesion is masked in blue, and the gastrointestinal tract lesion is masked in yellow. (B) A comparison of lesion uptake at 24 h p.i., indicated by SUV<sub>max</sub>, is shown for both well-delineated lesions during dosimetry and the first three treatment cycles; Figure S6: Coronal and axial fused SPECT/CT images (a–c) after the fourth cycle of treatment with [<sup>177</sup>Lu]Lu-DOTA-MGS5; next to

stable uptake in CCK2R-positive disease new disseminated liver metastases without [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 uptake was noticed in the CT scan (black arrowheads; d).

**Author Contributions:** E.v.G. and T.S.Z. contributed to the study conception and design and prepared the radiopharmaceutical preparations for clinical use. T.S.Z. performed the majority of the in vitro experimental work. J.H. conceptualized and interpreted the fluorescence microscopy studies. I.S. conceptualized and reviewed the cell toxicity studies. I.P. and K.S. performed the immunohistochemistry evaluations and interpreted the results. A.K. analyzed and interpreted the patient-specific dosimetry calculations. G.d.S., I.J.V., G.S., L.G., D.M., V.M. and J.L.-R. were involved in patient management and interpretation of clinical results. T.S.Z. drafted the manuscript and prepared the figures. E.v.G., L.G., V.M. and I.J.V. edited and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The retrospective immunohistochemical analysis of the CCK2R expression in SCLC tissue specimens was approved by the Medical Ethics Committee of the Technical University of Munich (2024-130-S-DFG-SB) on 9 April 2024. The retrospective analysis of the presented data of the SCLC patient was exempted by the Medical University of Innsbruck from consultation with the Ethics Committee.

**Informed Consent Statement:** Informed consent for the immunohistochemistry study was not required as per local legislation (Bayerisches Krankenhausgesetz, Art. 27 Datenschutz). Written informed consent was obtained from the patient prior to [ $^{177}\text{Lu}$ ]Lu-DOTA-MGS5 PRRT in accordance with the Declaration of Helsinki.

**Data Availability Statement:** Data presented in this study is contained within the article. Further inquiries can be directed to the corresponding author.

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**Conflicts of Interest:** E.v.G. is listed as an inventor on the European patent application (no. 17174973) and is a consultant for Evergreen Theragnostics, Inc. in product development. Evergreen Theragnostics has financially supported the immunohistochemistry analyses at TUM. No other conflict of interest relevant to this article was reported.

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