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ORIGINAL ARTICLE

Anti-L1CAM radioimmunotherapy is more effective with the radiolanthanide terbium-161 compared to lutetium-177 in an ovarian cancer model

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Abstract
Purpose The L1 cell adhesion molecule (L1CAM) is considered a valuable target for therapeutic intervention in different types of cancer. Recent studies have shown that anti-L1CAM radioimmunotherapy (RIT) with ^{67}Cu- and ^{177}Lu-labelled internalising monoclonal antibody (mAb) chCE7 was effective in the treatment of human ovarian cancer xenografts. In this study, we directly compared the therapeutic efficacy of anti-L1CAM RIT against human ovarian cancer under equitoxic conditions with the radiolanthanide ^{177}Lu and the potential alternative ^{161}Tb in an ovarian cancer therapy model.
Methods Tb was produced by neutron bombardment of enriched ^{160}Gd targets. ^{161}Tb and ^{177}Lu were used for radiolabelling of DOTA-conjugated antibodies. The in vivo behaviour of the radioimmunoconjugates (RICs) was assessed in IGROV1 tumour-bearing nude mice using biodistribution experiments and SPECT/CT imaging. After ascertaining the maximal tolerated doses (MTD) the therapeutic impact of 50 % MTD of ^{177}Lu- and ^{161}Tb-DOTA-chCE7 was evaluated in groups of ten mice by monitoring the tumour size of subcutaneous IGROV1 tumours.
Results The average number of DOTA ligands per antibody was 2.5 and maximum specific activities of 600 MBq/mg were achieved under identical radiolabelling conditions. RICs were stable in human plasma for at least 48 h. ^{177}Lu- and ^{161}Tb-DOTA-chCE7 showed high tumour uptake (37.8–39.0 %IA/g, 144 h p.i.) with low levels in off-target organs. SPECT/CT images confirmed the biodistribution data. ^{161}Tb-labelled chCE7 revealed a higher radiotoxicity in nude mice (MTD: 10 MBq) than the ^{177}Lu-labelled counterpart (MTD: 12 MBq). In a comparative therapy study with equitoxic doses, tumour growth inhibition was better by 82.6 % for the ^{161}Tb-DOTA-chCE7 than the ^{177}Lu-DOTA-chCE7 RIT.
Conclusions Our study is the first to show that anti-L1CAM ^{161}Tb RIT is more effective compared to ^{177}Lu RIT in ovarian cancer xenografts. These results suggest that ^{161}Tb is a promising candidate for future clinical applications in combination with internalising antibodies.

Keywords ^{161}Tb · ^{177}Lu · Radioimmunotherapy · Ovarian carcinoma · L1CAM · mAb chCE7

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Introduction

Ovarian cancer is a gynaecological malignancy with high mortality, because it is often diagnosed late. Approximately 75 % of the patients will have already developed metastases at

the time of diagnoses, and debulking surgery is still the crucial step in ovarian cancer therapy [1]. Some success has been achieved in the treatment of ovarian cancer during the last few decades, but the largest percentage of patients will have a relapse with a median progression-free survival of 18 months [2]. New therapeutic approaches with antibodies and/or selective kinase inhibitors (targeted therapy) have not made any significant improvement for patients until now [3, 4]. Overall mortality due to ovarian cancer remains unchanged, and new therapeutic strategies to control the disease are very desirable.

The L1 cell adhesion molecule (L1CAM) is a highly glycosylated type I transmembrane protein that is overexpressed in various tumours and is considered a promising target for novel therapies (reviewed in: [5–9]). The aberrant expression of L1CAM is associated with tumour cell invasion and motility [7], and with poor prognosis and a high risk for progression in ovarian, uterine, and colorectal cancers [10–12]. The restricted expression of L1CAM in normal tissue [13] has led to the use of anti-L1CAM monoclonal antibodies in targeted ovarian cancer therapies [14–17]. To increase the efficacy of antibody-based L1CAM therapy, we introduced ^{67}Cu [16] and ^{177}Lu [15] for radioimmunotherapy (RIT) in ovarian cancer therapy models. The choice of the therapeutic low-ß$^-$-energy emitting nuclides for RIT matches the size of small residual disease found after tumour resection in ovarian cancer patients. The used monoclonal antibody chCE7 is a chimeric IgG1 molecule that binds with high affinity to human L1CAM. We could demonstrate that chCE7 binds near the RGD sequence in the sixth Ig-like domain of human L1CAM and can prevent the binding to integrins [18]. The antigen-antibody complex internalised by endocytosis [19] and metallic radionuclides like ^{67}Cu or ^{177}Lu were trapped intracellularly. Limiting factors for a more widespread use of ^{67}Cu in preclinical and clinical evaluations is probably due to the limited availability of ^{67}Cu and due to the production-related limited specific activity that can be reached.

In this study, we directly compared the electron (ß$^-$) emitters ^{177}Lu and ^{161}Tb for RIT. Both radionuclides are similar with respect to half-life (6.7 and 6.9 days, respectively), ß$^-$-energy (mean 134 keV vs. 154 keV), and chemical properties, and emit low-energy photons suited for gamma camera imaging. The main difference between these two lanthanides is the number of emitted conversion and Auger electrons (referred to as Auger electrons). ^{161}Tb emits 16 times more Auger electrons per decay (3–50 keV) than ^{177}Lu (Table 1).

Auger electrons produce short-range damage that may have an advantage for the eradication of small tumour nodules. In this study, we compared biodistributions and therapeutic anticancer efficacies of ^{177}Lu- and ^{161}Tb-DOTA-chCE7 immunoconjugates in human ovarian tumour-bearing nude mice under equitoxic conditions.

Materials and methods

Cell lines and antibodies

IGROV1 human ovarian carcinoma cells were obtained from Istituto Nazionale per lo Studio e la Cura dei Tumori (Milano, Italy). Cells were analysed by STR profiling (DSMZ, Braunschweig, Germany). Cell culture conditions and antibodies are described in Supplementary Information (SI).

L1CAM expression on IGROV1 cells

L1CAM expression on IGROV1 cells was analysed by flow cytometry (SI).

Conjugation of antibodies to DOTA and radiolabelling

Conjugation of antibodies to DOTA was performed as previously described [15]. The molar excess of p-SCN-Bn-DOTA (Macrocyclics, Dallas, TX, USA) was adapted individually for the different antibodies to achieve a similar number of DOTA-ligands coupled to the antibodies. The reaction mixture was adjusted to pH 9–10 using a saturated Na_3PO_4 solution and was incubated overnight (16 h) at 4 °C. Excess ligands were removed by gel filtration chromatography on a NAP-5 column (GE Healthcare, Glattbrugg, Switzerland) which was eluted with 0.25 M ammonium acetate (pH 5.5) for labelling. The number of chelators coupled per mAb molecule was determined by mass spectrometry [15]. Immunoconjugates were stored at −80 °C until use.

Carrier-free ^{161}Tb was produced in-house by irradiation of highly enriched gadolinium-160 targets at the spallation-induced neutron source at Paul Scherrer Institute (PSI, Villigen, Switzerland) or at the high-flux nuclear reactor at the Institut Laue-Langevin (Grenoble, France) and separated by cation exchange chromatography as described before [20]. ^{177}Lu was obtained from ITG (Garching, Munich, Germany). Both radionuclides were used for radiolabelling according to published procedures [21]. Briefly, 200–1,000 μg of the immunoconjugates in a total volume of 500 or 900 μL of 0.25 M ammonium acetate buffer (pH 5.5) was reacted with 100–600 MBq of ^{177}Lu or ^{161}Tb solution at 37 °C for 1 h. After incubation, EDTA was added to a final concentration of 5 mmol/L and the mixture was incubated for 5 min to complex unchelated lutetium or terbium. Purification of the labelled antibodies was achieved by fast protein liquid chromatography (FPLC) size exclusion chromatography on a Superose 12 column (GE Healthcare, Glattbrugg, Switzerland) in phosphate buffered saline (PBS) with a flow rate of 0.5 mL/min. Fractions of 500 μL were collected and the major peak fractions were pooled.

Table 1 Comparison of the decay properties of ^{177}Lu and ^{161}Tb

Isotope	^{177}Lu	^{161}Tb
Nuclide availability	Good	Limited
ß$^{-}$-energy av/decay (intensity)	134 keV (100 %)	154 keV (100 %)
Conversion and Auger electrons (intensity)	3–50 keV (14 %)	3–50 keV (224 %)
Eγ/E$_X$ (intensity)	208 keV (10 %)	75 keV (10 %)
	113 keV (6 %)	45–53 keV (39 %)
Half life	6.7 days	6.9 days

Data from: National Nuclear Data Centre Brookhaven National Laboratories

Quality control of radiolabelled preparations

The immunoreactive fraction of labelled antibody conjugates was measured by cell-binding assays and data were analysed according to the Lindmo method [22]. Stability of the labelled antibodies after incubation in human plasma at 37 °C was analysed by FPLC size exclusion chromatography on a TSKgel G3000Wxl column (Tosoh Bioscience, Stuttgart, Germany) with sodium phosphate buffer (0.3 M NaCl, 0.05 M Na_2HPO_4, pH 6.2) as mobile phase at a flow rate of 1 mL/min.

Biodistribution and SPECT/CT imaging

Animal studies were conducted in compliance with the Swiss laws on animal protection. All experiments were approved by the cantonal committee on animal experiments and permitted by the responsible cantonal authorities (permission numbers 75528 and 75535). Housing and animal husbandry was conducted according to local law on animal protection. Female CD1-foxn1^{nu} mice, 4–5 weeks old (Charles River, Sulzfeld, Germany) were inoculated subcutaneously (right shoulder) with 7×10^6 IGROV1 cells and biodistribution studies and SPECT/CT (NanoSPECT/CT, Bioscan Europe; Paris, France) imaging were performed 14 days later. For biodistribution studies 1–3 MBq of ^{177}Lu- or ^{161}Tb-DOTA-chCE7 (30 μg) was injected into a tail vein of tumour-bearing nude mice and mice were euthanised at the indicated time points. Tumours and major organs were collected, weighed, and counted for radioactivity together with an aliquot of the injected solution in a gamma counter. Each group with IGROV1 xenografts consisted of five mice. Results are expressed as percentage of injected activity per gram (%IA/g). In vivo imaging was done 72 h post i.v. injection of 6 MBq ^{177}Lu-DOTA-chCE7 or ^{161}Tb-DOTA-chCE7 (38 μg) or of 6 MBq isotope matched ^{177}Lu-DOTA control IgG or ^{161}Tb-DOTA control IgG (38 μg). SPECT data were acquired by Nucline software (version 1.02, Bioscan, Washington DC, USA). SPECT data were reconstructed iteratively with HiSPECT software (version 1.4.3049, Scivis, Göttingen, Germany). SPECT and CT data were automatically co-registered as both modalities shared the same axis of rotation. The fused datasets were analysed with the InVivoScope postprocessing software (version 1.44, Bioscan, Washington DC, USA).

Dose escalation study

Groups of four non-tumour-bearing nude mice (female CD1-foxn1^{nu}, Charles River, Sulzfeld, Germany) were treated intravenously with escalating doses of ^{177}Lu-DOTA-chCE7 (8, 10, 12, and 14 MBq; each 65 μg mAb) or ^{161}Tb-DOTA-chCE7 (6, 8, 10, and 12 MBq; each 65 μg mAb). After injection, mice were weighed every other day and monitored daily for humane endpoint. After 2 weeks, we changed the inspection intervals to every third day until the end of the experiment (47 days). The maximum tolerated dose (MTD) is defined as the first dose level below the dose leading to >20 % decrease in total body weight in at least one of the mice, or an early reaching of a defined endpoint of at least one mouse [23]. Peripheral blood mononuclear cell (PBMC) viability was analysed 13 days post i.v. injection of RICs in whole peripheral blood using the Guava ViaCount assay (Guava Technologies, Cardiff, UK) measured on a guava easyCyte flow cytometer (Millipore, Zug, Switzerland) according to the instructions given by the supplier.

Radioimmunotherapy experiments

Based on the results of the dose escalation studies, RIT studies were performed in female nude mice (eight per group) with subcutaneous tumours (IGROV1 human ovarian carcinoma, mean volume 133±50 mm^3, 8 days after cell inoculation) and a 50 % MTD of ^{177}Lu-DOTA-chCE7 or ^{161}Tb-DOTA-chCE7 (30 μg). Equivalently labelled, unspecific matched control IgGs and PBS were used as controls. All injections were done i.v. via the lateral tail vein. Tumour growth and the weight of the mice were evaluated every 2–3 days. Humane endpoint criteria were defined as weight loss of more than 20 % of the initial body weight or a tumour volume of more than 1,000 mm^3 or ulceration of the tumours. The tumour volume (V) was calculated using the following equation: $V = (L \times W^2)/2$, where W is the width of the tumour (small diameter), and L the length (larger diameter), both in millimetres. The relative tumour volume (RTV) of each individual tumour was

calculated as Vx/Vo (Vx = tumour volume at a given time, Vo = tumour volume at the start of therapy).

Statistical analysis

Excel software (Microsoft Office 2003) was used for statistical analyses as described in Supplementary Information.

Results

Conjugation of antibodies to DOTA and labelling of the conjugates with ^{177}Lu and ^{161}Tb

The average number of ligands per mAb chCE7 was 2.5 determined by mass spectrometry. For the control antibodies we obtained a similar number of ligands per molecule. For both radionuclides, ^{177}Lu and ^{161}Tb, maximum specific activities of 600 MBq/mg antibody have been achieved under identical reaction conditions.

Aggregates, small fragments, and free radionuclides were separated from the radiolabelled antibody fraction by FPLC size exclusion chromatography. The immunoreactivity of the antibodies analysed by cell binding assays ranged from 78 to 84 %. Both radioimmunoconjugates were stable in human plasma at 37 °C for at least 48 h. No sign of degradation, release of radioactivity or aggregation was observed (SI).

Comparative biodistribution of ^{177}Lu-DOTA-chCE7 and ^{161}Tb-DOTA-chCE7

In order to compare the biological behaviour of the DOTA conjugated chCE7 antibodies in ^{177}Lu- and ^{161}Tb-labelled form biodistribution experiments were performed on nude mice with human IGROV1 ovarian carcinoma xenografts. The L1CAM expression on the cell surface of IGROV cells was confirmed by FACS analysis (SI). Thirty micrograms of RICs were injected i.v. and accumulation of radioactivity in all major organs and tumours was measured (Table 2). Uptake of radioactivity in tumours was high after 72 h (31.5–33.4 %IA/g) and increased to 37.8–39.0 %IA/g after 144 h. Both RICs showed almost identical behaviour in vivo.

SPECT/CT imaging with ^{177}Lu- and ^{161}Tb-DOTA-chCE7 allowed an excellent visualization of implanted L1CAM-expressing ovarian carcinomas (Fig. 1). Subcutaneous IGROV1 tumours (7×10^6 tumour cells, inoculated 14 days in advance) were visualised with high resolution 72 h after injection of 6 MBq (38 μg) of ^{177}Lu- and ^{161}Tb-labelled RICs. The images showed low blood activities and low activities in liver and other organs which leads to the low background. The labelled unspecific isotope-matched antibodies did not accumulate at the tumour side (Fig. 1b, d).

Table 2 Biodistribution of ^{177}Lu- and ^{161}Tb-labelled antibody chCE7 in nude mice with subcutaneous ovarian cancer (IGROV1)

^{177}Lu-DOTA-chCE7		
Organ (%IA/g)	72 h	144 h*
Tumour	33.4±7.7	37.8±1.5
Blood	10.4±2.5	8.7±0.9
Liver	6.8±1.4	6.6±1.5
Spleen	7.0±1.7	7.5±2.2
Kidney	3.4±0.8	2.8±0.2
Heart	4.6±1.2	3.7±0.3
Stomach	0.6±0.1	0.6±0.1
Intestine	0.8±0.1	0.9±0.2
Muscle	1.5±0.5	1.7±0.5
Bone	1.6±0.3	1.7±0.4
^{161}Tb-DOTA-chCE7		
Organ (%IA/g)	72 h	144 h
Tumour	31.5±8.5	39.0±13.1
Blood	8.2±3.8	7.0±4.6
Liver	6.7±1.2	6.6±1.4
Spleen	7.7±2.9	8.9±5.0
Kidney	3.1±0.6	2.6±0.9
Heart	3.7±1.5	3.1±1.6
Stomach	0.4±0.1	0.3±0.1
Intestine	1.1±0.1	0.9±0.1
Muscle	0.9±0.3	0.7±0.3
Bone	2.0±0.4	1.5±0.3

Groups of five (*four) animals were injected i.v. with 1.0 MBq (30 μg) of ^{177}Lu-DOTA-chCE7 or 1.5 MBq (30 μg) of ^{161}Tb-DOTA-chCE7. Data are presented as %IA/g ± SD

Determination of the maximum tolerated dose

A direct comparison of the therapeutic effects of ^{177}Lu- and ^{161}Tb-labelled mAb chCE7 can be based on experimentally determined maximum tolerated dose (MTD). The dose escalation study was done in groups of four non-tumour bearing nude mice treated with 8, 10, 12, or 14 MBq ^{177}Lu-DOTA-chCE7 (Fig. 2a) and 6, 8, 10, or 12 MBq ^{161}Tb-DOTA-chCE7 (Fig. 2b). The amount of the injected RICs was adjusted to 65 μg. Within the first 10 days, one mouse died unexpectedly in the 12 MBq ^{177}Lu-DOTA-chCE7 group. This death was not regarded as related to the application of the RIC (no signs of distress, no change in behaviour, and no radiation syndrome), and therefore, it was not taken into the evaluation.

Twelve to 14 days after administration of the highest dose of radioactivity (14 MBq of ^{177}Lu-labelled and 12 MBq of ^{161}Tb-labelled immunoconjugate) we observed a significant decrease in body weight for almost all mice in these treatment groups (Fig. 2a, b). The mice looked pale; their agility dropped, and mice showed signs of distress and acute radiation syndrome. A representative picture of such a mouse is shown in (SI). At the same time, the number of red blood cells

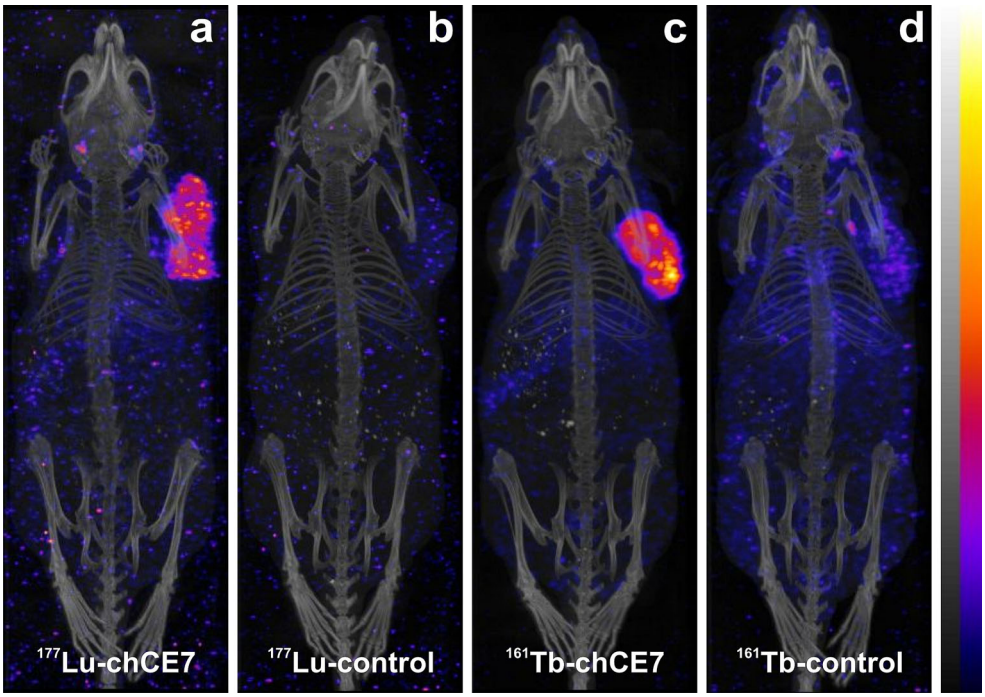

Fig. 1 Whole-body SPECT/CT imaging of tumour-bearing nude mice. The images were taken 72 h after i.v. injection of six MBq radioimmunoconjugates (38 μg). **a** ^{177}Lu-DOTA-chCE7, **b** ^{177}Lu-DOTA-matched-control IgG, **c** ^{161}Tb-DOTA-chCE7, **d** ^{161}Tb-DOTA-matched-control IgG. The mice are had one IGROV1 tumour on the right shoulder

and the viability of the peripheral white blood cells dropped (Table 3). The MTD for the ^{177}Lu-labelled compound was found to be higher (12 MBq) compared to the ^{161}Tb-labelled compound (10 MBq).

Comparative therapy study of ^{177}Lu-DOTA-chCE7 and ^{161}Tb-DOTA-chCE7

An experiment comparing the therapeutic efficacy of anti-L1CAM RIT using ^{177}Lu-DOTA-chCE7 and ^{161}Tb-DOTA-chCE7 was performed in nude mice with subcutaneous tumour xenografts (IGROV1 human ovarian carcinoma, mean volume 133±50 mm^3, 8 days after cell inoculation). Radiolabelled antibodies were injected i.v. A 50 % MTD dose of ^{161}Tb-DOTA-chCE7 (5 MBq) induced prominent tumour growth retardation compared with mice that received corresponding control treatments (Fig. 3). The effect of 50 % MTD of ^{177}Lu-DOTA-chCE7 (6 MBq) was less prominent. After a short delay phase, the mean relative tumour volume (RTV) in all control groups increased roughly linearly and increased three times compared to therapy started after a tumour growth delay (TGD) of 40 days (in the case of ^{161}Tb-DOTA-unspecific matched control IgG), 47 days (in the case of ^{177}Lu-DOTA-unspecific matched control IgG) and 54 days (in the case of PBS). In both anti-L1CAM targeted therapy groups, a mean three-fold increase of the start RTV was not reached, because the first mouse in these groups attained the endpoint before. Therefore, we calculated the TGD in these groups with a RTV increase of 2.3 for ^{177}Lu-DOTA-chCE7 (TGD 69 days) and a RTV increase of 2.5 for ^{161}Tb-DOTA-chCE7 (TGD 118 days). This fact underestimates the tumour growth delay for both therapies.

The average RTV was reduced significantly in the targeted ^{177}Lu- and ^{161}Tb-DOTA-chCE7 therapy groups compared to all controls (p-values 0.004 to <0.00001). The average RTV dropped significantly ($p<0.05$) in the ^{161}Tb-RIT group compared to the ^{177}Lu-RIT group. A tumour growth inhibition (TGI) of 67.5–85.7 % was obtained for ^{177}Lu anti-L1CAM RIT and was higher than 94 % for the ^{161}Tb-RIT in comparison to control treatments. The TGI of ^{161}Tb-RIT was better by 82.6 % compared to ^{177}Lu-RIT.

Discussion

In this study, we investigated the efficacy of anti-L1CAM radioimmunotherapy using mAb chCE7 labelled with ^{177}Lu or ^{161}Tb. For both radiolanthanides, we used the same labelling procedures and obtained stable radiolabelled DOTA conjugated antibodies with high specific activities. Biodistribution studies showed that both radioconjugates distributed similarly in tumour-bearing nude mice. The immunoreactivity of both radioconjugates was equal. Terbium and lutetium have similar ionic radii (Tb: 0.923 Å; Lu: 0. 861 Å) for six-coordinate complexes [24, 25], and both lanthanides build extremely stable complexes with DOTA ligands [26], which is reflected in the low uptake of radioactivity in bones. It has been described that nude mice exhibit often-different endogenous IgG concentrations that can influence blood pool, spleen and liver uptake of injected mAbs [27, 28]. To

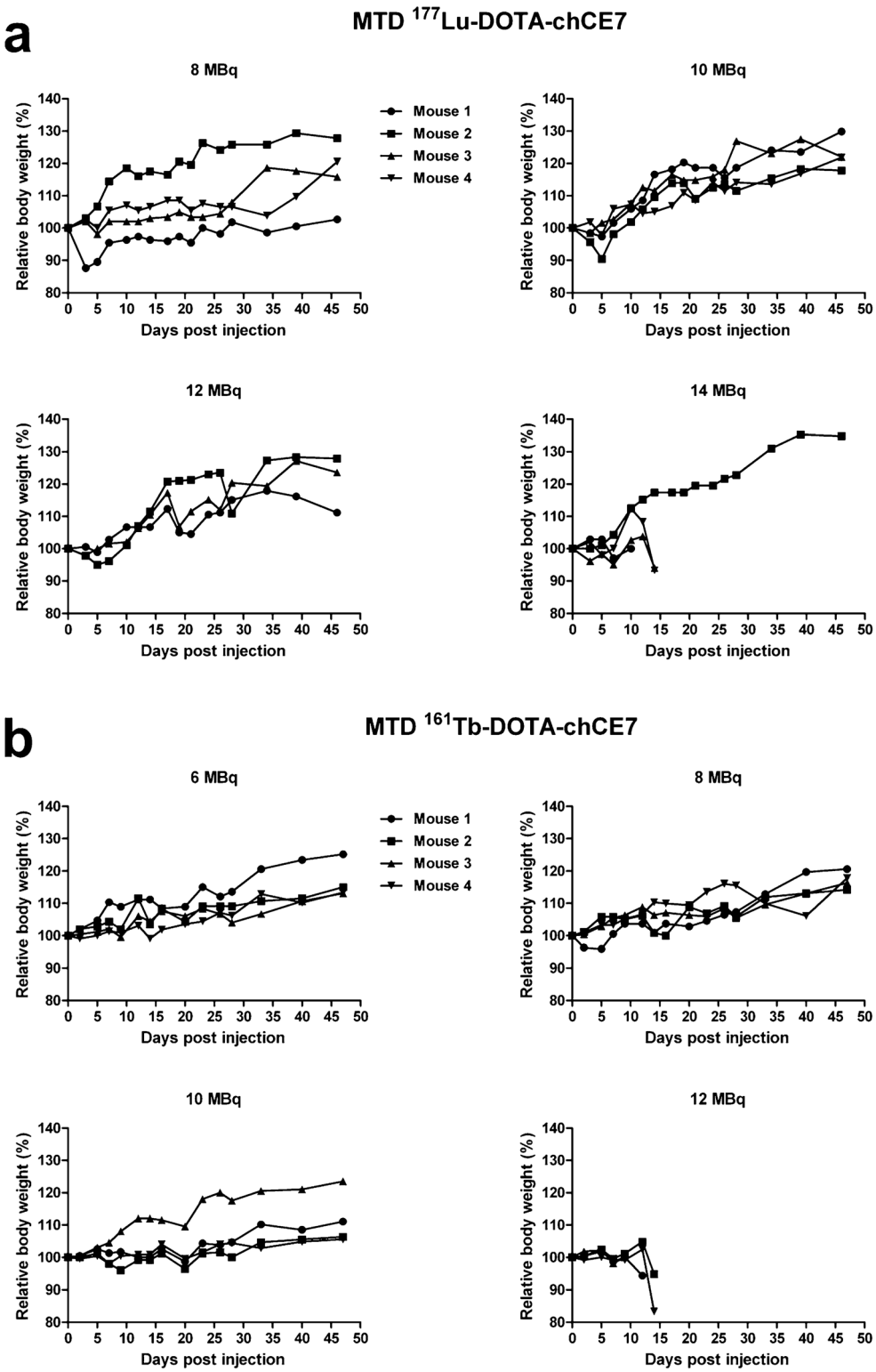

Fig. 2 Dose escalation studies with **a** ^{177}Lu-DOTA-chCE7 or **b** ^{161}Tb-DOTA-chCE7 in nude mice. Mice were injected i.v. with increasing doses of ^{177}Lu- or ^{161}Tb-labelled antibody chCE7 (n=4 per dose). The amounts of injected antibodies were constant at 65 μg for each dose. Body-weight and health of mice were monitored for 47 days. The maximum tolerated dose was 12 MBq for ^{177}Lu-DOTA-chCE7 and 10 MBq for ^{161}Tb-DOTA-chCE7

compensate for most of these effects we injected 30 μg IgG per mouse in the biodistribution experiments. We cannot exclude that such effects influenced the biodistribution of ^{177}Lu-DOTA-chCE7 in one mouse at 144 h post injection. The mouse showed unusually low activity in tumour and organs (SI). We assume that this low activity was due to a small amount of administered antibody. This mouse was not considered for statistical analysis. The clear SPECT/CT images confirmed the biodistributions we obtained for both radioconjugates. Therefore, both radiolanthanides are suitable

Table 3 Peripheral blood mononuclear cell (PBCM) viability in tumour-free nude mice after treatment with increasing doses of radiolabelled antibodies (n=4/dose)

	Viable [% of total]	Apoptotic [% of total]	Dead [% of total]	Total [%]
Control	99.0	<1.0	<1.0	100
8 MBq ^{177}Lu-DOTA-chCE7	95.3	4.0	0.7	100
10 MBq ^{177}Lu-DOTA-chCE7	83.1	14.0	2.9	100
12 MBq ^{177}Lu-DOTA-chCE7	83.8	14.6	1.6	100
14 MBq ^{177}Lu-DOTA-chCE7[a]	48.7	43.5	7.8	100
6 MBq ^{161}Tb-DOTA-chCE7	97.7	2.0	0.3	100
8 MBq ^{161}Tb-DOTA-chCE7	94.2	5.6	0.2	100
10 MBq ^{161}Tb-DOTA-chCE7	96.7	3.3	0.0	100
12 MBq ^{161}Tb-DOTA-chCE7[a]	40.2	39.4	20.4	100

Blood samples were analysed 13 days post i.v. injection of RICs

[a] Less than 160 nucleated cells were counted (normally 1,000 nucleated cells were counted)

in the same way for imaging in small animals. The low gamma ray energies of ^{161}Tb may limit the clinical application for diagnostic purposes.

For direct comparison of the therapeutic efficacy of the ^{177}Lu- or ^{161}Tb-labelled chCE7 antibodies, we determined the MTD for both radioimmunoconjugates. Tumour-free mice were chosen for the dose escalation study to simulate the worst case, i.e. no tumour accumulation of the antibody. Consequently, the RICs follow their biological half-life in blood and distribute without accumulation at a tumour site in the body. For the dose escalation study we used 65 μg of labelled antibody per mouse (resulting from the highest dose that was given) to exclude the influence of endogenous IgG concentrations and to hold the conditions for the whole dose range constant. The higher radiotoxicity of mAb ^{161}Tb-DOTA-chCE7 (MTD: 10 MBq) compared to ^{177}Lu-DOTA-chCE7 (MTD: 12 MBq) is certainly caused in part by the higher ß$^-$ energy emitted by ^{161}Tb. This is in agreement with observations of Brouwers et al. [29] who found a low MTD of 5.6 MBq for a ^{90}Y-labelled internalising chimeric antibody in nude mice. Yttrium-90 has mean ß$^-$ energy of 900 keV. It is most likely that the additional Auger and conversion electrons emitted by ^{161}Tb influenced the radiotoxicity too. The short-range electrons exert their greatest toxicity effects only after internalisation or on the cell membrane of the target cell [30–33]. Inside the cell, Auger electrons are able to deposit high energy around the decay site which leads to ionisation and excitations, chemical transmutations, local effects of charged species, and nuclear recoil [30, 34]. The biological effects induced by Auger electrons are, for the most part, indirectly caused [30]. The significance of intracellular position near the cell nucleus has been discussed [30, 34–36]. In this context, the group of Reilly reported that nuclear localizing sequences coupled to an ^{111}In-labelled antibody promoted specific nuclear uptake in tumour cells and enhanced the radiotoxicity of the antibody [37, 38]. On the other hand, Guo et al. [39] showed that an increased nuclear uptake of the Auger emitter ^{64}Cu in a radioimmunotherapy regime of HCT116 tumour-bearing nude mice had no advantage. In this comparative RIT study between ^{177}Lu- and ^{161}Tb-labelled monoclonal antibodies using 50 % MTD we could clearly demonstrate that the ^{161}Tb-labelled mAb chCE7 performed much better than the ^{177}Lu-labelled counterpart. The differences were significant and tumour growth inhibition was 82.6 % higher for ^{161}Tb-DOTA-chCE7 vs. ^{177}Lu-DOTA-chCE7. We assume that the better therapeutic efficacy of ^{161}Tb-RIT is the result of both the higher ß$^-$ energy and the 16-fold higher emission rate of conversion and Auger electrons. More than half of these electrons (3–50 keV) emitted by ^{161}Tb have an energy higher than 16 keV. This could explain why a nuclear localization is not necessary for the great effect of ^{161}Tb. It is known that Auger electrons induce bystander effects, which can harm neighbouring cells [40]. This may

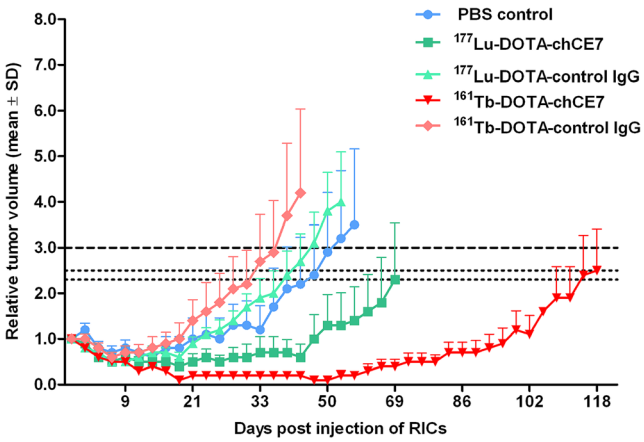

Fig. 3 Therapeutic efficacy of ^{177}Lu- and ^{161}Tb-DOTA-chCE7 RIT in nude mice bearing subcutaneous IGROV1 tumours. Tumour-bearing nude mice were treated i.v. with 50 % MTD of ^{177}Lu- or ^{161}Tb-labelled anti-L1CAM mAb chCE7 or labelled control antibodies or PBS. Data represent the mean relative tumour volumes ± SD. Tumour growth curves were stopped when the first tumour in a treatment group reached 1,000 mm^3. The *dashed line* represents a three-fold increase of the RTV. The *dotted lines* represent a) a 2.3-fold increase of the RTV (for the ^{177}Lu-DOTA chCE7 treatment) or b) a 2.5-fold increase of the RTV (for the ^{161}Tb-DOTA-chCE7 treatment)

also have influenced the therapeutic efficacy of the RIT. At present, dosimetric estimations are not able to reflect all radiobiological responses induced by conversion and Auger electrons [35].

Conclusions

Our study is, to our knowledge, the first to show that ^{161}Tb-RIT is more effective in an ovarian cancer model under equitoxic conditions in comparison to ^{177}Lu-RIT. For both radiolanthanides the same well established chemistry can be used and biodistributions of the RICs were similar. The ^{161}Tb-labelled antibody showed a slightly higher radiotoxicity in a dose escalation study compared to the ^{177}Lu-labelled mAb. Overall, ^{161}Tb is potentially the better candidate for RIT with internalisation antibodies. Next, we will investigate if we can optimise the RIT with ^{161}Tb in combination with radiosensitising agents, and we will analyse the exact molecular mechanisms of the radiobiological effects induced by the Auger electrons.

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Conflict of interest None

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