Therapeutic efficacy of heterogeneously distributed radiolabelled peptides: Influence of radionuclide choice

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ABSTRACT

Purpose: To model dose-response relationships for in vivo experiments with radiolabelled peptides enabling maximum therapeutic efficacy while limiting toxicity to kidney and bone marrow.

Methods: A multiregional murine kidney phantom, with a kinetic model for cortex and outer medulla distribution, were used to predict renal toxicity. Maximum tolerated activities to avoid nephrotoxicity (at 40 Gy Biological Effective Dose BED) and hematologic toxicity (at 2 Gy) were compared. The therapeutic efficacy of 90Y, 177Lu, 177Lu and 213Bi was assessed at their respective maximum tolerated activities based on cellular-level dosimetry accounting for activity and tumor heterogeneity. These results were compared with average tumor-dosimetry-based predictions.

Results: The kidney was found to be the dose-limiting organ for all radionuclides, limiting the administered activity to 44 MBq 177Lu, 34 MBq 161Tb, 19 MBq 90Y and 13 MBq 213Bi, respectively. The average S-values for the initial heterogeneous activity distribution in the tumor volume are not significantly different from the homogeneous ones. The in vivo tumor cell survivals predicted by assuming uniform dose-rate distributions are not significantly different from those for heterogeneous dose-rate predictions. The lowest in vivo survival was found for 213Bi (2%) followed by 161Tb (30%), 177Lu (37%) and 90Y (60%). The minimal effective dose rate for cell kill is 13-14 mGy/h for β-emitters and 2.2 mGy/h for the α-particle emitter 213Bi below these values proliferation takes over.

Conclusions: Radionuclides emitting α-particles have the highest potential for improving therapeutic efficacy in tumors and metastases with uniform receptor expression, after careful evaluation of their burden to the healthy organs.

Introduction

Peptide receptor radionuclide therapy (PRRT) employs receptor-mediated binding to deliver a cytotoxic dose to neuroendocrine tumors (NETs). Somatostatin analogues (i.e., [177Lu]Lu-DOTA-[Tyr3]octreotate or [177Lu-DOTATATE and [90Y] Y-DOTA-[Tyr3]octreotide or 90Y-DOTATOC) were proven highly efficacious in treatment of patients bearing inoperable or metastatic NETs overexpressing the somatostatin receptor type-2 (SSTR2) [1,2]. However, despite their success in improving patient’s quality of life while mitigating adverse events, patients invariably relapse on average 2-3 years after starting treatment. In this respect, the use of dosimetry-guided treatment planning and alternative radionuclides may lead to improvement of progression free survival.

Optimization of clinical PRRT by dosimetry-guided treatment planning relies on the evaluation of absorbed dose-effect relationships in preclinical experiments in order to assess treatment efficacy and toxicity. Regarding treatment efficacy, tumor size and targeted receptor expression may guide the radionuclide choice in terms of physical characteristics. For instance, simulations show that a low energy β-emitting radionuclide, such as 177Lu (E mean = 133 keV) yields a high probability of cure in the 1-3-mm tumor size range. Instead, the higher
energy β-emitter ($E_{\text{mean}} = 933$ keV) $^{90}$Y shows optimal tumor control in the 28–42-mm range [3]. These results were confirmed in rats with subcutaneously implanted somatostatin receptor-positive tumors [4,5]. Moreover, on one hand, the use of these medium-to-long range β particle-emitting radionuclides might overcome heterogeneous activity distributions by cross-irradiation; on the other hand, the relatively large loss of radiation in surrounding tissue and low linear energy transfer (LET) characterizing β-particles might reduce treatment efficacy. In this respect, a promising alternative to $^{177}$Lu is $^{161}$Tb, because of its additional release of low energy (up to 50 keV per decay) conversion and Auger electrons compared to $^{177}$Lu. Octreotate internalizes into the cells and possibly accumulates into the Golgi apparatus, making only the conversion electron energy high enough to reach the cell nucleus [6]. Indeed, theoretical dose calculations demonstrated that $^{161}$Tb may outperform $^{177}$Lu in treating small metastatic lesions [7–9]. Furthermore, studies with cells and mice bearing small tumor xenografts confirmed this finding [10]. Finally, a more potent option, suitable for treatment of small tumor clusters and metastases, are higher LET particles, such as the α-emitter $^{213}$Bi, as shown in mice [11] and, more recently, in clinical trials [12].

Once a clear dose-response relationship between tumor volume reduction and tumor absorbed dose is found, the challenge is to deliver the highest dose to the tumor while sparing healthy tissues. Therefore, the establishment of absorbed dose-effect relationships for healthy organs, as well, is a fundamental step to limit toxicity while maximizing treatment efficacy. In PRRT for NETs the main dose-limiting tissues are the liver, as well, is a fundamental step to limit toxicity while maximizing the establishment of absorbed dose-effect relationships for healthy or.

The co-emitted Auger/conversion electrons of $^{161}$Tb raised the concern of a potentially higher probability of renal toxicity than $^{177}$Lu-DOTATATE due to a higher dose to the cortex [16], even though a very recent first-in-human application of $^{161}$Tb-DOTATOC showed that the treatment was well tolerated and no related adverse events were reported [17].

Moderate toxicity was instead observed in a clinical trial of $^{213}$Bi-DOTATOC administered with a renal protection solution in patients' refractory to $^{177}$Lu-DOTATATE and $^{90}$Y-DOTATOC treatment, while acute haematotoxicity was even less pronounced than with the preceding β therapies [12].

The aim of this work is to evaluate the relative therapeutic potential of $^{90}$Y, $^{177}$Lu, $^{161}$Tb and $^{213}$Bi for treatment of solid tumors, adopting our previously developed dosimetric model using the SSTR2 levels to account for tumor (cancer/healthy cells) and activity heterogeneity at cellular scale [18]. The comparison was carried out preventing kidney and bone marrow toxicity, thereby modeling the spatial and temporal distribution of radioactivity in kidney sub-regions and accounting for dose rate and fractionation of treatment delivery. The same biodistribution profiles were used for all radio labelled analogues [18].

**Material and Methods**

**Limiting organ dosimetry: Red bone marrow**

In order to determine the administered activity corresponding to a maximum tolerated dose (MTD) in the bone marrow of 2 Gy, simulation of self- and cross- S-values were combined with cumulated activities extrapolated from $^{177}$Lu-DOTATATE data [19].

**Self- and cross-absorbed dose rate S-values for bone marrow dosimetry**

The Moby program [20] was used to generate a voxel phantom of 128×128×432 elements, with cubic voxels of 234 μm. The original phantom was re-scaled to represent a mouse with body weight of about 25 g and its lungs were simulated in full exhaled condition. The mouse image was then expanded with voxels of zero value (i.e., empty) in order to allow for the addition of a spherical subcutaneous tumor xenograft on one of the flanks (Fig. 1) using Python [21]. The tumor did not invade the tissue beneath the skin, resulting in a half sphere-like geometry. The phantom consists of 30 tissues, including the tumor, serving as both source and target in the absorbed dose rate S-value calculations. The compositions of each organ was taken from ICRP 23 [22]. The skeleton was subdivided in ribs, spine, skull, humeri, femurs, tibiae and others (i.e., radii, ulnae, fibulae, patellae and reminder) and assumed to be made of soft tissue (1 g/cm$^3$ vs. 1.2 g/cm$^3$) in order to evaluate the bone marrow S-values by rescaling for the marrow cellularity of each bone [23]. The material of voxels outside the phantom was air. Density and volume of organs is detailed in Table 1. The activity was assumed to be homogeneously distributed in each source region.

The Gate MC toolkit version 9.0 [24] was used to perform simulations and score the average absorbed dose for target tissues with the DoseByRegion actor (locally deposited energy per dose voxel mass).

The radioactive source for the β-emitting radionuclides was sampled using the predefined ion source definition (ENSDF database), which includes all the spectral components of $^{177}$Lu, $^{90}$Y and $^{161}$Tb. The 4 radionuclides involved in the decay chain of $^{213}$Bi were instead sampled by means of user defined spectra, accounting for the emission of all particle types, described in 11 separate macro-sources. The definition of the spectral components of $^{213}$Bi were taken from ICRP 107 and the probability of an event coming from each macro-source and the energy emitted per decay is reported in Table 2, together with the relative branching ratio, taken into account in the source sampling. Recoil energy associated with $^{213}$Bi and $^{213}$Po was ignored.

The Livermore physics list, including a low-energy electromagnetic
model based on publicly available evaluated data tables from the Livermore data library and with a production cut-off of 20 μm. The radionuclides were located uniformly either in the cortex or in the outer medulla region and the absorbed fractions of energy for the emitted radiation of 213Bi decay chain. The branching ratios (BR) of each radionuclide is reported in the last row. The decay data for these radionuclides were taken from ICRP 107. A cut off value of 1 keV was used in the energy transport calculations for electrons.

For 213Bi calculations the S-values reported at nephron level by above. For the bone marrow calculations, a blood based approach with a red-marrow-to-blood ratio (RMBLR) = 1 [25] was adopted assuming muscle as a surrogate for the whole body distribution (i.e., TIAClμdr ≅ TIACblod ≅ TIACmbr). The TIACs of the bone marrow contained in the 7 segmented bones was then calculated accounting for the cellularity [23].

Assuming an absorbed-dose threshold of 2 Gy to hypothetically reduce the probability of severe marrow depression [26], the maximum safe administered activity was computed as described below. Both self- and cross- absorbed doses to each bone marrow region were accounted for when dealing with β-emitting radionuclides, while the cross-dose was neglected for the 213Bi scenario. A relative biological effectiveness (RBE) of 2 [27] was used to determine the RBE-weighted absorbed dose for α-particles to the bone marrow.

### Limiting organ dosimetry: Kidney

A biological effective dose (BED) based treatment planning was performed to determine the MTD for nephrotoxicity. A regional kidney dosimetry model was used to determine the outer medulla and cortex S-value for β-emitting radionuclides. The uptake in outer medulla and cortex was modelled to determine the biodistribution profiles at regional level. Within the cortex most (95 %) of the activity was assumed to be located in the proximal tubes [13].

### Regional kidney dosimetry model

A multiregional mathematical kidney model of 0.15 ml (as in MOBY) was developed in order to perform separate detailed Monte Carlo simulations accounting for the inhomogeneous distribution of the activity and the relatively long range of β-emitting radionuclides. The kidney was divided into several regions: cortex, outer stripe (outer medulla), inner stripe (outer medulla), inner medulla, pelvis and papilla. These regions were modelled as prolate ellipsoids, except for the renal pelvis (cone) in MCNP6 (Fig. 2, Table 4). Volumes and densities are detailed in Table 4. Materials were defined as in ICRU report 46.

The radionuclides were located uniformly either in the cortex or in the outer medulla region and the absorbed fractions of energy for the emitted radiation of 177Lu, 161Tb and 186Y were simulated. From the absorbed fractions, the absorbed dose rate S-values to the cortex were derived.

The decay data for these radionuclides were taken from ICRP 107. A cut off value of 1 keV was used in the energy transport calculations for electrons.

For 213Bi calculations the S-values reported at nephron level by

### Table 1
Organ volumes and tissue densities of the murine model MOBY. Percentage of total marrow cellularity [23] for each bone is reported in parenthesis. The bones are solid structures made of soft tissue.

| Tissue            | Volume (mm³) | Density (g/cm³) |
|-------------------|--------------|----------------|
| Heart wall        | 68.11        | 1.05           |
| Body              | 17124.55     | 1.05           |
| Liver             | 1649.89      | 1.06           |
| Langs             | 414.01       | 0.3            |
| Stomach wall      | 64.56        | 1.04           |
| Stomach content   | 401.97       | 1.04           |
| Pancreas          | 322.90       | 1.04           |
| Kidneys           | 302.63       | 1.05           |
| Spleen            | 94.93        | 1.06           |
| Small intestine   | 939.69       | 1.03           |
| Large intestine   | 293.74       | 1.03           |
| Bladder           | 59.25        | 1.04           |
| Vascular defense  | 22.01        | 1.06           |
| Testis            | 296.32       | 1.04           |
| Ribs              | 205.99       | 8 ( % )        |
| Spine             | 413.11 (52 %)| 1              |
| Skull             | 477.82 (11 %)| 1              |
| Brain             | 449.76       | 1.04           |
| Thyroid           | 12.24        | 1.04           |
| Large intestine air| 276.64   | 0.00129        |
| Small intestine air| 706.21    | 0.00129        |
| Humeri            | 33.08 (5 %)  |                |
| Femurs            | 96.80 (10 %)| 1              |
| Tibiae            | 89.87 (4 %)  | 1              |
| Other bones       | 300.62 (10 %)| 1              |
| Gall bladder      | 13.07        | 1.04           |
| Heart content     | 181.60       | 1.03           |
| Airways           | 76.34        | 1.03           |
| Tumor             | 118.51       |                |

### Table 2
Particles and energy (E) per decay (Bq s) emitted by the 213Bi decay chain. The branching ratios (BR) of each radionuclide is reported in the last row.

| Particle type | 213Bi (yield) | (MeV) | 213Po (yield) | (MeV) | 209Tl (yield) | (MeV) | 209pb (yield) | (MeV) |
|--------------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|
| γ + x        | 9.63E-01      | 1.28E-01 | 7.13E-05      | 3.75E-05 | 6.62          | 2.14E-00 | 0             |      |
| β            | 9.79E-01      | 4.25E-01 | 0             | 1      | 6.55E-01      | 1      | 1.97E-01      |      |
| e⁻           | 6.26E-01      | 1.92E-02 | 2.12E-05      | 1.23E-06 | 3.46          | 3.22E-02 | 0             |      |
| α            | 2.09E-02      | 1.22E-01 | 1.00E-00      | 8.38E-00 | 0             | 0      | 0             |      |
| BR           | 1             | 9.79E-01 |               | 2.09E-02 | 1             |        |               |      |

### Table 3
Time integrated activity coefficient (TIAC) per gram of tissue in organs with physiologic uptake and in tumor, following a single (1-exp) or a double exponential (2-exp) clearance pattern.

| TIAC (h/g) | Spleen | Pancreas | Kidney | Liver | Stomach | Duodenum + intestine | Muscle | Tumor |
|-----------|--------|----------|--------|-------|---------|----------------------|--------|-------|
| Decay     | 1-exp  | 2-exp    | 2-exp  | 2-exp | 2-exp   | 1-exp                | 1-exp  | 1-exp |
| 177Lu     | 0.11   | 0.34     | 1.31   | 0.08  | 0.65    | 0.17                 | 0.05   | 3.68  |
| 161Tb     | 0.10   | 0.34     | 1.28   | 0.08  | 0.63    | 0.16                 | 0.04   | 3.43  |
| 186Y      | 0.05   | 0.28     | 1.03   | 0.05  | 0.44    | 0.09                 | 0.03   | 2.02  |
| 213Bi     | 2.04E-03 | 0.03    | 0.06   | 3.11E-03 | 0.02   | 4.65E-03             | 1.70E-03 | 0.06  |
Hobbs et al. [28] were adopted.

**Biodistribution modeling and biological effective dose (BED) calculations**

The Linear-Quadratic (LQ) model is an empirical relation between the cell survival $S$ and absorbed dose $D$ following a linear and quadratic exponential function: $S = \exp \left(-\frac{\alpha D}{\beta} - \beta D^2\right)$. Both the cell survival and the renal damage dose response curves for $\alpha$-particle radiation follow only a linear exponential relation, reflecting that the repair of cellular damage is reduced favoring single event cell kill, expressed in the linear radiation sensitivity parameter $\alpha$ [1]. In general the Biological Effective Dose $BED$ is defined for a dose given in $N$ fractions as:

$$BED = D \left(1 + \frac{GD/N}{\alpha/\beta}\right),$$

with $G$ the Lea-Catcheside factor, expressing the dose rate and DNA-damage repair function over time.

The kidney absorbed-dose limit of 23 Gy determined for external beam radiotherapy (EBRT) [29] was adjusted to the PRRT-equivalent via the BED formalism derived with the LQ model [30]. First, assuming $\alpha/\beta = 2.6$ Gy and an absorbed dose per fraction of 2 Gy for EBRT, the equivalent BED was found. The BED was converted into the maximum absorbed dose in the renal cortex (Eq. (1)) per therapy cycle of PRRT, assuming 4 therapy cycles for all radionuclides. The $G$-factor was evaluated for 2 source regions (i.e., outer medulla and cortex) following a bi-exponential time-activity curve, according to the formula by Baechler et al. [31].

For this purpose, a differential clearance between cortex and outer medulla was assumed based on renal clearance data of female and male mice by Melis et al. [32]. The ratio of activity in the cortex relative to the outer medulla was modelled with an exponential build-up curve followed by a plateau after 96 h and the sum of cortex and outer medulla activity was set equal to the whole-kidney activity (Table 3). A summary of the bi-exponential fitting parameters for the biological renal clearance is reported in Table 5. Fig. 3 shows a plot of the fractional activity in the whole kidney, outer medulla, and cortex versus time, used as input together with the regional $S$-values to evaluate the $G$-factor.

The renal pelvis is modeled as a conical surface between two ellipsoidal surfaces.

### Table 4
Dimensions and geometrical characteristics of the mouse kidney model defined in MCNP, the numbers in column ID refer to the kidney regions shown in Fig. 2.

| ID | Region | Density (g/cm$^3$) | Volume (cm$^3$) | External surface | Internal surface |
|----|--------|-------------------|----------------|-----------------|-----------------|
|    |        | R (cm) | H (cm) | R (cm) | H (cm) |
| 10 | Cortex | 1.04  | 7.28E-02 | 0.268 | 0.500 | 0.214 | 0.400 |
| 20 | Outer stripe medulla | 1.04  | 2.66E-02 | 0.214 | 0.400 | 0.178 | 0.360 |
| 30 | Inner stripe medulla | 1.03  | 2.21E-02 | 0.178 | 0.360 | 0.150 | 0.270 |
| 40 | Inner medulla | 1.03  | 2.06E-02 | 0.150 | 0.270 | 0.085 | 0.150 |
| 50 | Papilla & Pelvis | 1.04  | 4.48E-03 | 0.085 | 0.150 | 0 | 0 |
| 60 | Papilla | 1.04  | 2.26E-04 | 0.080 | 0.268 | 0.134 | 0.201 |
| 70 | Pelvis | 1.04  | 1.58E-03 | 0.080 | 0.268 | 0.134 | 0.201 |

Ellipsoidal surfaces of equation:\n
$$\frac{x^2}{R} + \frac{y^2}{R} + \frac{z^2}{H} = 1$$

contour kidney regions.

The renal pelvis is modeled as a conical surface between two ellipsoidal surfaces.

### Table 5
Bi-exponential fitting parameters for outer medulla and cortex region of female and male mouse models, including whole kidney parameters.

| Parameters | Whole kidney | Male mouse model | Female mouse model |
|------------|--------------|------------------|--------------------|
|            |              | Cortex | Outer medulla | Cortex | Outer medulla |
| Initial fraction | 1.00 | 0.44  | 0.56  | 0.23  | 0.77  |
| Plateau | 0.04 | 0.00  | 0.00  | 0.00  | 0.00  |
| %Fast | 76.10 | 71.16 | 82.17 | 89.36 | 79.83 |
| $T_{1/2}$ – FAST (h) | 8 | 9.35  | 7.45  | 19.88 | 6.48  |
| $T_{1/2}$ – SLOW (h) | 47.90 | 61.45 | 47.21 | 155.00 | 51.23 |
For the $^{213}$Bi-DOTATATE scenario, only $\alpha$-particles were assumed to cause nephrotoxicity. The time-integrated activity was evaluated at nephron level, assuming most of the radioactivity (95%) to be retained in the proximal tubules with respect to the glomeruli cells [13]. The BED formula, in this case, was corrected with an RBE [30] of 2 and the $G$-factor was approximated to 1. Hence, the maximum absorbed dose was evaluated as follows:

$$D_{\text{Cortex}} = \frac{\alpha/\beta}{2G/N} \left( \sqrt{1 + 4G/N \text{BED}} - 1 \right)$$

The maximum injected activity was then evaluated from the absorbed dose determined by either Eq. (1) or Eq. (2), knowing TIACs and $S$-values for the corresponding compartments.

It should be noticed that the same radiosensitivity parameters ($\alpha/\beta$) were used for all radionuclides, except for $^{213}$Bi.

### Therapeutic efficacy: Tumor dosimetry and in vivo survival correlation

The $\text{SSTR}_2$ expression of NCI-H69 xenografts assessed by immunofluorescent stainings in previous experiments [19] was used to assess the heterogeneity in the absorbed dose distribution over the tumor volume and the average absorbed dose $S$-value, as reported previously solely for $^{177}$Lu-DOTATATE [18]. Briefly, square tissue sections with $3.2 \times 3.2 \text{ mm}$ side and resolution of $0.625 \text{ m\mu/pixel}$ from 4 independent mice before injection were used to reconstruct 16 voxelized computational models (heterogeneous tumor cell distribution) and the corresponding 16 voxelized sources (heterogeneous radionuclide distribution). The input data for the Monte Carlo (MC) simulations is represented by $507 \times 507 \times 289$ voxels of $5.7 \times 5.7 \times 10 \text{ m\mu size}$.

For comparison, the $S$-value and absorbed dose rate distribution calculations, assuming an equivalent uniform spherical phantom (representing the tumor) were also performed.

The Gate MC toolkit version 9.0 was used to perform simulations and score 3-dimensional absorbed dose maps (resolution: $5.7 \times 5.7 \times 10 \text{ m\mu}$) within the defined geometry. The average dose was also calculated for tumorous and healthy cells with the DoseByRegion actor (deposited energy per dose voxel mass). The radioactive source definition and the physics list adopted for these calculations were the same as reported for the bone marrow $S$-value calculations. However, in order to display the absorbed dose distribution at voxel level, the production cut-off was lowered to 1 $\text{ m\mu}$ for $^{177}$Lu, $^{161}$Tb and $^{90}$Y, whilst a step size of 1 $\text{ m\mu}$ was added for the $^{213}$Bi-case.

The in vivo survival was determined either accounting for the heterogeneous absorbed dose rate distribution or by means of a single averaged $S$-value (i.e., average approach) over the tumor volume as described previously [18] for both uniform (spherical) and heterogeneous scenario.

The radiobiological parameters used to describe the in vivo tumor cell survival according to the LQ model for the $\beta$-emitting radionuclides ($^{177}$Lu, $^{90}$Y, $^{161}$Tb) are: $\alpha = 0.14 \text{ Gy}^{-1}$, $\alpha/\beta = 100 \text{ Gy}$ or $10 \text{ Gy}$, $T_\text{p} = 60 \text{ h}$ and $T_\text{D} = 14.5 \text{ d}$, where $T_\text{p}$ and $T_\text{D}$ are used to indicate repair and tumor repopulation half-life, respectively.

A linear dose-response was assumed for $^{213}$Bi-DOTATATE exposure with an RBE of 3.4 [11].

### Results

#### Bone marrow dosimetry

The absorbed dose per administered activity for the bone marrow is reported in Fig. 4, distinguishing among the segmented bone marrow regions. The major contribution to the self- bone marrow dose is delivered by the bone marrow region with the highest cellularity (i.e., spine), regardless of the radionuclide used. Most of the cross-dose to the bone marrow for $^{177}$Lu and $^{161}$Tb is delivered by the total body (Fig. 4A and B), whilst the cross-dose for $^{90}$Y is more diverse including a significant contribution from tumor to lower limbs (i.e., tibiae and femurs), from stomach and liver to ribs and finally, from kidneys and stomach to spine (Fig. 4C). The electrons and photons contribution to the bone marrow absorbed dose is negligible ($\approx 2\%$) compared to the $\alpha$-particle component.

Finally, limiting the absorbed dose to the bone marrow to 2 Gy, the maximum administered activities are 231 MBq, 181 MBq, 22 MBq and 250 MBq for $^{177}$Lu, $^{161}$Tb, $^{90}$Y and $^{213}$Bi, respectively.

#### Kidney dosimetry

The simulated absorbed energy fractions and $S$-values for outer medulla and cortex activity are summarized in Table 6 and Table 7, respectively.

Assuming the cortex region as target, only a small fraction of the $\beta$-particles emitted from $^{90}$Y localized in the cortex reaches the target (23%) if compared to $^{177}$Lu (80%) and $^{161}$Tb (99%) (Table 6). On the contrary, when the radionuclides are assumed to be located in the outer medulla, similar energy fractions are absorbed in the cortex.

The calculated factors leading to translate a BED of 41 Gy to PRRT-equivalent absorbed dose and the corresponding maximum injected activity are reported in Table 8.

The maximum tolerated absorbed dose to renal cortex (i.e., glomeruli cells) for $^{213}$Bi-DOTATATE is 8 Gy, corresponding to 13.74 MBq and 23.45 MBq of injected activity for male and female mice, respectively. The female mouse biodistribution results in the highest dose per injected activity because of the higher residence time estimated in the outer medulla for this mouse model.

#### Tumor dosimetry

Significant differences are found in the absorbed dose rate...
distributions caused by each radionuclide depending on the range of its particles and physical half-life. The dose rate map for the same tissue section (one of the 4 available tissue sections) theoretically treated with longer range radionuclides ($^{177}$Lu and $^{90}$Y) and the corresponding cumulative dose volume histograms (cDVH, a graphical summary of the absorbed dose distribution by indicating the volumes with absorbed dose exceeding equi-spaced dose intervals in a histogram) are reported in Fig. 5 A. The distributions of shorter-range radionuclides ($^{161}$Tb and $^{213}$Bi) are shown in Fig. 5 B. The equivalent spherical distributions are reported for comparison in both figures (Fig. 5 A and B). The distributions corresponding to the other 3 tissue sections stained for the SSTR2 are reported in the Supplemental Material (Supplemental Material).

The absorbed dose rate maps of $^{177}$Lu and $^{161}$Tb are very similar; however, the cDVH shows a longer tail corresponding to a greater presence of localized absorbed dose spots for $^{161}$Tb with respect to $^{177}$Lu. The absorbed dose rate map of $^{90}$Y is not significantly different from that of the spherical homogenous case scenario; however, a significant portion of the absorbed dose is delivered outside the tumor volume. The radionuclide with the most heterogeneous absorbed dose distribution is $^{213}$Bi, which shows a similar distribution pattern as $^{161}$Tb, with absorbed dose rate spots 2 times higher than the respective average values.

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Table 6

Absorbed energy fractions in several target regions as defined in Table 4. Each radionuclide is emitted either from the cortex or from the outer medulla. The radiation types emitted by each radionuclide are defined by the subscripts. AE and IE indicate Auger electrons and internal conversion electrons, respectively.

| Target region | Source region: cortex | Source region: outer medulla |
|---------------|-----------------------|-----------------------------|
| $^{90}$Y      | $^{177}$Lu | $^{177}$Lu(AE+IE) | $^{161}$Tb | $^{161}$Tb(AE) | $^{161}$Tb(IE) |
| $^{213}$Bi    | $^{177}$Lu | $^{177}$Lu(AE+IE) | $^{161}$Tb | $^{161}$Tb(AE) | $^{161}$Tb(IE) |
| 10            | 23.49%     | 79.22%             | 94.32%     | 77.13%     | 99.88%         | 98.87%     |
| 20            | 7.02%      | 7.19%              | 2.17%      | 7.79%      | 0.05%          | 0.43%      |
| 30            | 4.37%      | 0.67%              | 0.00%      | 0.83%      | 0.00%          | 0.00%      |
| 40            | 3.44%      | 0.04%              | 0.00%      | 0.07%      | 0.00%          | 0.00%      |
| 50            | 0.67%      | 0.00%              | 0.00%      | 0.00%      | 0.00%          | 0.00%      |
| 60            | 0.03%      | 0.00%              | 0.00%      | 0.00%      | 0.00%          | 0.00%      |
| 70            | 0.27%      | 0.16%              | 0.05%      | 0.18%      | 0.00%          | 0.01%      |
| Total kidney  | 39.29%     | 87.27%             | 96.55%     | 85.99%     | 99.93%         | 99.32%     |
significantly different from the corresponding uniform one \((S_{\text{hom}} - S_{\text{het}} < 0.04)\), regardless of the type of radionuclide. The average absorbed doses to the tumor corresponding to an administered activity of 44 MBq of \(^{177}\text{Lu}\), 34 MBq of \(^{161}\text{Tb}\), 19 MBq of \(^{90}\text{Y}\) and 13.74 MBq of \(^{213}\text{Bi}\) are 12.9 Gy, 14.8 Gy, 5.9 Gy and 4.2 Gy (corresponding to 14 Gy \(_{\text{RBE}=3.4}\)) respectively. It should be noticed that the calculated absorbed doses are limited by the toxicity to healthy organs and by the physical half-life characterizing each radionuclide, i.e., the cumulated activity of shorter lived radionuclides, such as \(^{90}\text{Y}\) and \(^{213}\text{Bi}\), is smaller than longer lived ones, as well.

The \textit{in vivo} survival results, using the uniform (spherical) \(S\)-values for the dose calculations, are reported in Fig. 6A, as no significant difference has been found between the uniform and heterogeneous dose distribution assumption (Supplemental Material), as indicated for illustration purposes for one radionuclide \((^{213}\text{Bi})\) in Fig. 6B.

The minimal effective dose rate for cell kill, below which proliferation takes over, are reported in Table 9, together with the lowest survival rate.

**Discussion**

In this study, the potential of PRRT planning for various therapeutic radionuclides to control tumor growth has been assessed accounting for dose-limiting kidney and bone marrow toxicity. The results reported in this study, for a tumor diameter of about 3 mm, are of particular relevance for adventian or consolidation therapy in which tumor targets are threefold reduction in renal uptake could be justified only by the radionuclide during PRRT is influenced by the radionuclide used.

In more detail, since \(^{161}\text{Tb}\) and \(^{177}\text{Lu}\) are both lanthanides similar pharmacokinetics can be expected for both tumor and healthy organs. An absorbed dose threshold for the kidney of 24 Gy has been assumed (based on external beam therapy data), analyzing morphological changes in kidney structure of nude mice treated with \(^{177}\text{Lu}\)-DOTATATE [38]. This value is in good agreement with the maximum absorbed dose evaluated in our work for the \(\beta\)-emitting radionuclides (29 Gy – 31 Gy). Interestingly, the MTD in patients falls also in a similar range (23 – 28 Gy) [39]. It should be noted, however, that similar average absorbed doses to the cortex region do not cause similar pattern of damage because of the inherent physical difference (i.e., half-life and particle range) characterizing the analyzed radionuclides. As evident from the dose rate maps calculated for the resected tumor sections, the shorter the range of the emitted particle and the half-life of the mother nuclide, the more pronounced would be the damage in localized area with high radioactivity as the cortex region. For this reason, the maximum administered activity resulted to be lower for \(^{161}\text{Tb}\) (34 MBq) if compared to \(^{177}\text{Lu}\) (44 MBq).

On the other hand, demonstration of the existence of an absorbed dose-response relationship for bone marrow toxicity from PRRT has proven elusive. For this reason, the 2 Gy safety limit usually assumed for \(^{131}\text{I}\) therapy, was chosen as limiting value to prevent myelotoxicity in this work. Limit, however, does not prevent induction of late hematologic toxicity such as induction of leukemia, which is another well-known, though fortunately rare late adverse event [40].

The tumor uptake of \(^{177}\text{Lu}\)-DOTATATE (5.4 ± 1.2 \%IA/g) used in this study is comparable with the data of Chan et. al on H69 tumor-bearing mice treated with \(^{213}\text{Bi}\)-DOTATATE (7.5 ± 2.2 \%IA/g), as well [41]. However, \(^{177}\text{Lu}\)-DOTATATE is not a good surrogate to determine kidney (44.0 ± 9.6 \%IA/g vs 5.4 ± 0.87 \%IA/g) and muscle uptake (2.1 ± 0.8 \%IA/g vs 0.16 ± 0.17 \%IA/g) of \(^{213}\text{Bi}\)-DOTATATE. Indeed, the difference in complexation chemistry of \(^{213}\text{Bi}\) in the DOTA-chelator compared to \(^{177}\text{Lu}\) and the binding of free \(^{213}\text{Bi}\) to metallothionein in the kidneys are plausible causes of higher kidney uptake (12 ± 3.7 min/g compared to 3.5 min/g derived from \(^{177}\text{Lu}\)-DOTATATE). This threefold reduction in renal uptake could be justified only by the administration of a renal protection solution immediately prior to the treatment [42], which was not accounted for in this work. Nonetheless, our calculations resulted in a maximum safe activity of 13.74 MBq and are thus in line with earlier results of 13.0 ± 1.6 MBq (without renal protection) by Chan et al. [43]. This might be related to the different dosimetric approach used for the calculations. Adopting the nephron

**Table 7**

| Target region | Absorbed dose rate per unit activity (mGy MBq⁻¹ s⁻¹) | Source region: cortex | Source region: outer medulla |
|---------------|-----------------------------------------------|------------------------|-----------------------------|
| \(^{90}\text{Y}\) | \(^{177}\text{Lu}\) | \(^{161}\text{Tb}\) | \(^{90}\text{Y}\) | \(^{177}\text{Lu}\) | \(^{161}\text{Tb}\) |
| 10            | 0.47  | 2.53E-01 | 3.47E-01 | 3.54E-01 | 5.34E-01 | 6.47E-01 |
|               | 20    | 0.35  | 6.50E-02 | 7.88E-02 | 5.56E-02 | 7.80E-02 |
|               | 30    | 0.29  | 6.26E-02 | 8.92E-02 | 5.72E-02 | 1.19E-01 |
|               | 40    | 0.24  | 4.27E-02 | 7.83E-02 | 4.40E-02 | 1.17E-01 |
|               | 50    | 0.22  | 3.01E-02 | 1.79E-02 | 3.79E-02 | 1.84E-02 |
|               | 60    | 0.21  | 3.72E-02 | 4.82E-02 | 3.73E-02 | 2.77E-02 |
| 70            | 0.24  | 2.11E-02 | 2.64E-02 | 3.62E-02 | 3.70E-02 | 4.57E-02 |

**Table 8**

Comparison of G-factor, maximum tolerated dose to the cortex (Max D), TIACs in cortex (TIACₘₜ) and outer medulla (TIACₒₘₜ), dose to the cortex per injected activity (D/IA) and maximum tolerated administered activity (Max A) for each radionuclide, using the female or male mouse model. The most restrictive result is reported in bold.

| Radionuclide | G-factor Female | Max D (Gy) Female | Max D (Gy) Male | TIACₘₜ (s) Female | TIACₒₘₜ (s) Female | D/IA (Gy/MBq) Female | Max A (MBq) Female | G-factor Male | Max D (Gy) Male | Max D (Gy) Male | TIACₘₜ (s) Male | TIACₒₘₜ (s) Male | D/IA (Gy/MBq) Male | Max A (MBq) Male |
|--------------|-----------------|------------------|-----------------|------------------|------------------|---------------------|-------------------|---------------|-----------------|-----------------|-----------------|------------------|-------------------|-----------------|
| \(^{177}\text{Lu}\) | 0.103 | 31.11 | 21.35 | 2122.28 | 2129.42 | 1995.63 | 2092.57 | 1286.71 | 1563.21 |
| \(^{161}\text{Tb}\) | 0.104 | 31.03 | 31.26 | 3189.34 | 2227.93 | 3137.10 | 2197.69 | 2393.83 | 1729.35 |
| \(^{90}\text{Y}\) | 0.149 | 28.81 | 29.02 | 44.94 | 47.65 | 34.77 | 36.12 | 19.77 | 21.67 |
model of Hobbs et al. [28] and assuming 95% of the radioactivity retained in the proximal tubules results in a sparing effect to the glomeruli dose in comparison to whole organ dosimetry calculations, assuming homogenous uptake in the mouse cortex. Interestingly, comparing the regional dosimetry approach accounting for the differential kinetics of outer medulla and cortex with the results evaluated at nephron level, no significant difference was found in the MTD to the cortex. While the regional S-values account for both $\beta$- and $\alpha$-radiations, the S-values adopted at nephron level neglects the $\beta$-radiation contribution. Given that most of the radioactivity is retained in the proximal tubules, one could argue that longer range radiation might have a greater impact than shorter range radiation, whose dose deposition would be confined to the proximal tubules. Hence, the higher cross-dose from the proximal tubules to the radiation-sensitive glomeruli may even make the average dose concept not suitable for understanding radiation effects for $\beta$-emitter radionuclides. Moreover, a synergistic effect might be expected when dealing with a mixed radiation field.

For this reason, a more complex description of the absorbed dose distribution at functional level, including different radiosensitivities of the sub-units in the cortex would be beneficial. Another reason to further characterize the kidney structure is the unusual response of kidney to PPRT with respect to EBRT. Early tubular damage is known to lead to nephritis (glomeruli damage) because of the glomeruli obstruction caused by the induction of fibrosis after radiation-induced inflammation [44]. In PRRT this morphological change is not seen and tubular damage (hydronephrosis) is predominant with respect to nephritis. Only a direct comparative study would unambiguously characterize RBE, radiosensitivity and pharmacokinetic profiles of $^{213}$Bi, $^{90}$Y and $^{177}$Lu-labeled peptides in the kidneys and its sub-units. Such analysis is beyond the aim of this study, focused primarily on tumor efficacy comparison of different radionuclides.

The bone marrow was not identified as the limiting organ in the current analysis, in agreement with many studies in mice showing myelotoxicity as generally mild and transient for PRRT with $\beta$-emitters [45,46].

Using the cumulated activity in the blood as surrogate for the bone marrow assumes no specific binding in the marrow itself. The blood-to-bone-marrow activity ratio was measured to be unity in patients after PRRT [24], conforming the prior assumption. Unfortunately, none of the bone marrow dosimetry models for PRRT show a clear correlation between bone marrow dose and toxicity data. Our findings agree with data of patients treated with $^{177}$Lu-DOTATATE, for which the contribution of
the cross-dose from source organs and tumors to the bone marrow dose is significant [25]. This implies that the variability of body masses and tumor burdens should be taken into account carefully.

The unrealistically high maximum safe activity found for myelotoxicity when using $^{213}$Bi-DOTATATE is certainly caused by the significantly lower TIAC when extrapolating data from $^{177}$Lu-DOTATATE. Assuming a TIAC of 0.033 h/g instead of 0.0017 h/g, obtained by fitting the data of Chan et al. [41], would lead to a maximum tolerated activity of 6 MBq and 11 MBq with an RBE of 2 and 1, respectively, compared to 250 MBq, found in this study. It should be noticed that for longer range $\alpha$-particles, such as the ones emitted by $^{213}$Po ($E_{\alpha} = 5.8$ MeV), the main contributor to the $^{213}$Bi absorbed dose, an RBE $< 2$ could be postulated. The assumption of a solid bone structure for bone marrow calculations is not realistic and accounting for a more complex bone structure would lead to reduced absorbed doses [47] and RBE for $\alpha$-emitters bound in proximity of the bone marrow regions [48]. Modelling the bone structure is a complex task considering that even the simple determination of the bone marrow mass is virtually impossible, especially for mice. The EBRT derived MTD should be taken with caution because the biological response to exposures with different dose rates is not yet understood.

Nonetheless, identifying the bone marrow as the non-limiting organ for $^{213}$Bi-DOTATATE is in agreement with the MTD found for PC3-tumor bearing mice treated with $^{213}$Bi-DOTA-AMBA (25 MBq corresponding to 4 Gy in the blood). Therefore, restricting the maximum injected activity to 13.7 MBq, as in this paper based on kidney dosimetry, is actually a conservative choice.

The therapeutic efficacy of the radionuclides analyzed is then evaluated accounting for the toxicity in healthy organs (i.e., kidneys). In this study, we confirm the considerable potential of $\alpha$-emitting radionuclides for small tumors or micrometastases characterized by a rather uniform receptor expression [12]. The high potency (i.e., high LET and short half-life) of $^{213}$Bi warrants high dose rates even in low receptor expression areas, eradicating most of the tumor cells. Even if this finding is limited to preclinical therapy tumor models, characterized by a more uniform receptor expression than corresponding clinical tumor models, the use of multiple fractions of therapy in combination with $^{90}$Y- and $^{177}$Lu-DOTATATE [46,49] might overcome the limitations of $^{213}$Bi-DOTATATE treatment when administered for larger or more heterogeneous tumors. It is evident that either $^{177}$Lu or $^{161}$Tb would provide a more uniform dose delivery in smaller (1 to 3 mm) tumors, as analyzed in this study, whilst larger tumors (28 to 32 mm) would benefit from the longer $^{90}$Y-particles range [3]. In particular, the absorbed dose per decay of $^{161}$Tb is 44% higher than that of $^{177}$Lu, indicating a superior therapeutic potential for smaller tumor sizes. Despite accounting for the absorbed dose heterogeneity at microscale level (cell dimension), the in vivo survival is well predicted by average calculations assuming uniform dose distributions, as also postulated in clinical practice. This result may not come as a surprise considering that uniform radionuclide distributions are usually considered as acceptable model for internalized molecules via receptor mediated endocytosis [9].

It should be noted, however, that the lack of difference between

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**Table 9**

| Radionuclide | Dose rate (mGy/h) | Lowest survival |
|--------------|------------------|----------------|
| $^{177}$Lu $\alpha/\beta = 100$ Gy | 13.61 | 37.05% |
| $^{177}$Lu $\alpha/\beta = 10$ Gy | 10.77 | 18.60% |
| $^{161}$Tb $\alpha/\beta = 100$ Gy | 13.62 | 30.36% |
| $^{161}$Tb $\alpha/\beta = 10$ Gy | 10.86 | 12.43% |
| $^{90}$Y $\alpha/\beta = 100$ Gy | 13.29 | 60.10% |
| $^{90}$Y $\alpha/\beta = 10$ Gy | 9.90 | 47.89% |
| $^{213}$Bi $\alpha/\beta = 3.4$ | 2.18 | 2.42% |

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**Fig. 6.** In vivo survival comparison. A) In vivo survival curves for the analyzed radionuclides reported with dashed or continuous lines for an $\alpha/\beta = 100$ Gy and $\alpha/\beta = 10$ Gy, respectively. The other radiobiological parameters used to describe the in vivo survival according to the LQ model are: $\alpha = 0.14$ Gy$^{-1}$, $T_b = 60$ h and $T_D = 14.5$ d. A RBE $= 3.4$ is used for $^{213}$Bi calculations. B) Box plots indicating the in vivo survival distribution over time on different excised tissue sections (T1-T4) and uniform spherical phantom. The red “X” indicates the in vivo survival obtained by using a single average dose rate S-value. The whiskers correspond to 1.5 times the interquartile range.
heterogeneous and uniform in vivo survival could be caused by the use of a single SSTR2 image, at the beginning of treatment, to perform calculations. Indeed, a significant reduction of receptor expression along treatment may vary the outcome of the calculations [18]. Moreover, cells may show an adaptive response and somatostatin induced vasoconstriction may result in regional hypoxia and therefore radionuclides emitting high-LET radiations, such as $^{211}$Bi may be recommended in the later therapy cycles.

The fast re-growth observed in the in vivo survival curves ($T_90 = 14$ days) is also not representative of clinical models characterized by lower proliferation rates.

In order to achieve tumor control each and every cell should be eradicated. To do so the dose rate variation at the cellular level should be analyzed against biological phenomena such as DNA repair capacity, cell cycle progression and proliferation in order to further improve biophysical modeling of PRRT.

On the other hand, PRRT is acknowledged to be a palliative treatment with minimal adverse events. Dosimetry-guided treatment planning based on maximum tolerated absorbed dose to non-targeted organs obtained by modulating the activity per cycle while limiting the number of administrations can contribute to further reduce the tumor load, thus prolonging the life of patients with metastasized disease through repeated therapy cycles.

Conclusion

Out of the radionuclides studied, our results indicate that $^{211}$Bi has the highest potential for further improving therapeutic efficacy in tumors and metastases with mostly uniform (even if low) receptor expression after $^{177}$Lu or $^{90}$Y-DOTATATE therapies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmp.2022.02.021.

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