



**HAL**  
open science

# Monte Carlo dosimetry of a realistic multicellular model of follicular lymphoma in a context of radioimmunotherapy

Julien Bordes, Sébastien Incerti, Erick Mora-ramirez, Jonathan Tranel, Cédric Rossi, Christine Bezombes, Julie Bordenave, Manuel Bardiès, Richard Brown, Marie-claude Bordage

## ► To cite this version:

Julien Bordes, Sébastien Incerti, Erick Mora-ramirez, Jonathan Tranel, Cédric Rossi, et al.. Monte Carlo dosimetry of a realistic multicellular model of follicular lymphoma in a context of radioimmunotherapy. *Med.Phys.*, 2020, mp.14370. 10.1002/mp.14370 . hal-02981284

**HAL Id: hal-02981284**

**<https://hal.science/hal-02981284>**

Submitted on 8 Nov 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Monte Carlo dosimetry of a realistic multicellular model of follicular lymphoma in a**  
2 **context of radioimmunotherapy**

3 Julien Bordes<sup>a,b\*</sup>, Sébastien Incerti<sup>c,d</sup>, Erick Mora-Ramirez<sup>a,b,e</sup>, Jonathan Tranel<sup>a,b</sup>, Cédric Rossi<sup>a,b,f</sup>, Chris-  
4 tine Bezombes<sup>a,b</sup>, Julie Bordenave<sup>a,b</sup>, Manuel Bardiès<sup>a,b</sup>, Richard Brown<sup>g</sup>, Marie-Claude Bordage<sup>a,b</sup>

5 <sup>a</sup>*CRCT, UMR 1037 INSERM, Université Paul Sabatier F-31037 Toulouse, France*

6 <sup>b</sup>*UMR 1037, CRCT, Université Toulouse III-Paul Sabatier, F-31037, France*

7 <sup>c</sup>*Université de Bordeaux, CENBG, UMR 5797, F-33170 Gradignan, France*

8 <sup>d</sup>*CNRS, IN2P3, CENBG, UMR 5797, F-33170 Gradignan, France*

9 <sup>e</sup>*Universidad de Costa Rica, Escuela de Física, CICANUM, San José, Costa Rica*

10 <sup>f</sup>*CHU Dijon, Hématologie Clinique, Hôpital François Mitterand, 21000 Dijon, France*

11 <sup>g</sup>*Institute of Nuclear Medicine, University College London, UK*

12

13 *\* Corresponding author at:*

14 Centre de Recherches en Cancérologie de Toulouse

15 INSERM UMR 1037 / Université Toulouse III Paul Sabatier

16 2 avenue Hubert Curien CS 53717

17 31037 TOULOUSE CEDEX 1 FRANCE

18

19 *E-mail addresses:*

20 [julien.j.bordes@gmail.com](mailto:julien.j.bordes@gmail.com)

21 [incerti@cenbg.in2p3.fr](mailto:incerti@cenbg.in2p3.fr)

22 [erick.mora@ucr.ac.cr](mailto:erick.mora@ucr.ac.cr)

23 [j.tranel@hotmail.fr](mailto:j.tranel@hotmail.fr)

24 [cedric.rossi@chu-dijon.fr](mailto:cedric.rossi@chu-dijon.fr)

25 [christine.bezombes@inserm.fr](mailto:christine.bezombes@inserm.fr)

26 [julie.bordenave@inserm.fr](mailto:julie.bordenave@inserm.fr)

27 [manuel.bardies@inserm.fr](mailto:manuel.bardies@inserm.fr)

28 [richard.brown@ucl.ac.uk](mailto:richard.brown@ucl.ac.uk)

29 [marie-claude.bordage@inserm.fr](mailto:marie-claude.bordage@inserm.fr)

30 *Keywords:*

31 Small-scale dosimetry, realistic biological data, rituximab, Auger electrons,  $\beta^-$  particles.

32 *Abstract:*

33 *Purpose.* Tumors geometry and radiopharmaceutical biodistribution impact the energy deposi-  
34 tion in targeted radionuclide therapy. However, small-scale dosimetry studies generally consider an ar-  
35 tificial environment where the tumors are spherical and the radionuclides are homogeneously biodis-  
36 tributed. To bring realism, we developed a dosimetric methodology based on a three-dimensional *in*  
37 *vitro* model of follicular lymphoma incubated with rituximab, an anti-CD20 monoclonal antibody used  
38 in the treatment of non-Hodgkin lymphomas, which might be combined with a radionuclide. The effects  
39 of the realistic geometry and biodistribution on the absorbed dose were highlighted by comparison with  
40 literature data. Additionally, to illustrate the possibilities of this methodology, the effect of different ra-  
41 dionuclides on the absorbed dose distribution delivered to the *in vitro* tumor were compared.

42 *Methods.* The starting point was a model named Multicellular Aggregates of Lymphoma Cells  
43 (MALC). Three MALCs of different dimensions and their rituximab biodistribution were considered. Ge-  
44 ometry, antibody location and concentration were extracted from selective plane illumination micros-  
45 copy. Assuming antibody radiolabeling with Auger electron ( $^{125}\text{I}$  and  $^{111}\text{In}$ ) and  $\beta^-$  particle emitters  
46 ( $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ ), we simulated energy deposition in MALCs using two Monte Carlo codes: Geant4-  
47 DNA with “CPA100” physics models for Auger electrons emitters and Geant4 with “Livermore” physics  
48 models for  $\beta^-$  particles emitters.

49           *Results.* MALCs had ellipsoid-like shapes with major radii,  $r$ , of  $\sim 0.25$ ,  $\sim 0.5$  and  $\sim 1.3$  mm. Ritux-  
50 imab was concentrated in the periphery of the MALCs. The absorbed doses delivered by  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  
51  $^{90}\text{Y}$  in MALCs were compared with literature data for spheres with two types of homogeneous biodistri-  
52 butions (on the surface or throughout the volume). Compared to the MALCs, the mean absorbed doses  
53 delivered in spheres with surface biodistributions were between 18% and 38% lower, while with vol-  
54 ume biodistribution they were between 15% and 29% higher. Regarding the radionuclides comparison,  
55 the relationship between MALC dimensions, rituximab biodistribution and energy released per decay  
56 impacted the absorbed doses. Despite releasing less energy,  $^{125}\text{I}$  delivered a greater absorbed dose per  
57 decay than  $^{111}\text{In}$  in the  $r \sim 0.25$  mm MALC ( $6.78 \cdot 10^{-2}$  vs.  $6.26 \cdot 10^{-2} \mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ ). Similarly, the absorbed  
58 doses per decay in the  $r \sim 0.5$  mm MALC for  $^{177}\text{Lu}$  ( $2.41 \cdot 10^{-2} \mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ ) and  $^{131}\text{I}$  ( $2.46 \cdot 10^{-2} \mu\text{Gy} \cdot$   
59  $\text{Bq}^{-1} \cdot \text{s}^{-1}$ ) are higher than for  $^{90}\text{Y}$  ( $1.98 \cdot 10^{-2} \mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ ). Furthermore, radionuclides releasing more  
60 energy per decay delivered absorbed dose more uniformly thorough the MALCs. Finally, when consid-  
61 ering the radiopharmaceutical effective half-life, due to the biological half-life of rituximab being best  
62 matched by the physical half-life of  $^{131}\text{I}$  and  $^{177}\text{Lu}$  compared to  $^{90}\text{Y}$ , the first two radionuclides delivered  
63 higher absorbed dose.

64           *Conclusion.* In the MALCs considered,  $\beta^-$  emitters delivered higher and more uniform absorbed  
65 dose than Auger electron emitters. When considering radiopharmaceutical half-lives,  $^{131}\text{I}$  and  $^{177}\text{Lu}$  de-  
66 livered absorbed doses higher than  $^{90}\text{Y}$ . In view of real irradiation of MALCs, such a work may be useful  
67 to select suited radionuclides and to help explain the biological effects.

## 68 1 Introduction

69 In targeted radionuclide therapy (TRT), a tumor-seeking agent is combined with an emitter of  
70 short-range radiation, such as Auger electrons (typically inferior to 1  $\mu\text{m}$  in water),  $\alpha$  particles (inferior  
71 to 100  $\mu\text{m}$ ) and  $\beta$ - particles (in the order of millimeters). The objective of this combination is to deliver  
72 in patients a lethal absorbed dose to tumor cells while minimizing the exposure of healthy tissues. Thus,  
73 the development and the evaluation of TRT requires small-scale dosimetry studies<sup>1,2</sup>. However, previous  
74 studies have been generally based on simplifying assumptions (*e.g.* <sup>3-8</sup>) owing to the lack of data at the  
75 submillimeter scale regarding the tumor geometry and the radionuclide biodistribution.

76 Firstly, tumors were generally supposed spherical, or, alternatively, ellipsoidal. Absorbed dose  
77 in spheres was found to be higher than in the ellipsoids of the same volume<sup>9</sup>, emphasizing the influence  
78 of the geometry. Secondly, small-scale dosimetry calculations have regularly assumed a homogeneous  
79 radionuclide biodistribution, either at the surface or within the volume of the tumor. In practice, the  
80 targeting of a tumor is not homogeneous, partly due to the heterogeneous distribution of the receptors  
81 (*i.e.* the antigens). Thus, mathematical models of heterogeneous radionuclide biodistributions within  
82 the tumors have been studied<sup>10-13</sup>. As could be expected, these models led to absorbed doses signifi-  
83 cantly less uniform within the tumor compared to homogeneous biodistributions. Therefore, given the  
84 impact of both tumor geometry and radionuclide biodistribution, there are clear calls for the develop-  
85 ment of small-scale dosimetry based on realistic biological data.

86 In the context of radioimmunotherapy (RIT), a single *in vitro* cultured carcinoma cell was con-  
87 sidered in order to reproduce the effect of its realistic geometry and monoclonal antibody biodistribu-  
88 tion on the dosimetry<sup>14</sup>. These biological data were extracted from microscope images. Assuming anti-  
89 body radiolabeling with iodine-125, the energy deposition in the cell was calculated with Monte Carlo  
90 simulations. However, the relevance of this work was limited since tumors targeted in RIT such as non-  
91 Hodgkin lymphoma (NHL) grow as multicellular three-dimensional structures. Thus, a single cell dosi-  
92 metric model neglects the absorbed dose delivered by the radiopharmaceutical bound to other cells of  
93 the tumor (crossfire effect). Furthermore, studies regarding unlabeled monoclonal antibody treatments  
94 highlighted that the biodistribution might be impacted by the tumor size and the extracellular matrix

95 (*i.e.* the assemblage of non-cellular molecules providing structural support for the surrounding cells),  
96 limiting the effectiveness of the treatment. Increasing tumor size is regarded as possibly reducing the  
97 monoclonal antibody concentration and decreasing survival of a mouse model<sup>15</sup> and patients<sup>16</sup>. Further-  
98 more, the extracellular matrix might hamper the monoclonal antibody penetration<sup>17</sup>. To address these  
99 questions, a three-dimensional *in vitro* culture system of follicular lymphoma (a NHL subtype) express-  
100 ing extracellular matrix was designed in a separate project at the Cancer Research Center of Toulouse<sup>18-</sup>  
101 <sup>21</sup>. The model was termed multicellular aggregate of lymphoma cells (MALC). The MALCs were incu-  
102 bated with rituximab. This chimeric monoclonal antibody targeting the membrane-associated CD20 an-  
103 tigen has been combined with radionuclides for RIT treatments (see clinical review of Read *et al.*<sup>22</sup>).

104 To improve the realism and the relevance of the single cell dosimetry, this paper presents a do-  
105 simetric model based on selective plane illumination microscopy (SPIM) images of three MALCs, of dif-  
106 ferent size, treated by rituximab. Data were extracted to define the tumor geometry and the radiophar-  
107 maceutical biodistribution. Rituximab was virtually radiolabeled with two types of electron emitters.  
108 The Auger electron emitters iodine-125 (<sup>125</sup>I) and indium-111 (<sup>111</sup>In) were studied because they proved  
109 their efficiency in destroying lymphoma cells *in vitro* in RIT-related research<sup>23,24</sup>.  $\beta^-$  particle emitters  
110 lutetium-177 (<sup>177</sup>Lu), iodine-131 (<sup>131</sup>I) and yttrium-90 (<sup>90</sup>Y) were also considered. For the RIT treatment  
111 of patients with relapsed / refractory NHL, <sup>177</sup>Lu-lilotomab satetraxetan is currently on trial<sup>25</sup>, while  
112 both <sup>131</sup>I-tositumomab (Bexxar) and <sup>90</sup>Y-ibritumomab tiuxetan (Zevalin) have been used<sup>26</sup>. In RIT, the  
113 dosimetry is highly impacted by the radiopharmaceutical effective half-life, a parameter that would de-  
114 pend on the rituximab radiolabeling. We studied the energy deposition patterns in MALCs with Monte  
115 Carlo modeling of radiation transport. Additionally, the effective half-life was accounted for by using the  
116 simulated data and a simple pharmacokinetic model.

117 As a first step, the relevance of implementing realistic biological data was highlighted by com-  
118 paring average absorbed doses to literature data. For the following step, to illustrate the possibilities  
119 offered by this new dosimetric model, we compared the radionuclides' absorbed fraction, average ab-  
120 sorbed dose and absorbed dose volume distribution. As a last step, the influence of the radiopharma-  
121 ceuticals half-life on the absorbed dose was studied.

## 122 2 Material and methods

### 123 2.1 Three-dimensional model of follicular lymphoma

124 The development of the MALCs as well as the microscopy acquisition were detailed in prior stud-  
125 ies<sup>18-21</sup>. This section focuses on information relevant to the present work.

#### 126 2.1.1 MALC growth and labeling

127 To compare the antibody biodistribution in different tumor volumes, the MALCs, created with  
128 follicular lymphoma cells expressing green fluorescent protein (GFP), were cultured during 2, 5 or 10  
129 days (hereafter referred to as D2, D5 and D10). Then, they were treated by a saturating concentration  
130 of 10 µg/ml of CF555 labeled rituximab<sup>27</sup> for a duration of 24 h.

#### 131 2.1.2 SPIM imaging

132 Fluorescence microscopy technologies, such as SPIM detect the light emitted by fluorescent  
133 markers<sup>28</sup>. SPIM was particularly adapted to this study since it allows the observation of whole organ-  
134 isms or three-dimensional multicellular models over a few millimeters while keeping a low phototoxi-  
135 city<sup>29,30</sup>. 24 h after rituximab administration, z-stack of images were acquired. Each image corresponded  
136 to a slice of the MALC in the x-y plane. For example, Figure 1 displays a central slice of MALCs (a) D2, (b)  
137 D5 and (c) D10. Green signal corresponds to GFP 488 nm emission and reveals MALC geometry. The  
138 black hollow at the center of MALCs D5 and D10 are due to a lack of follicular lymphoma cells. Red signal  
139 from the 561 nm emission of CF555 represents the rituximab biodistribution. To optimize the contrast  
140 between follicular lymphoma cells and rituximab, the colors on the images differ from the real colors  
141 produced by the fluorescent markers. SPIM images were composed of voxels with (x, y and z) dimen-  
142 sions of  $3.7 \cdot 3.7 \cdot 3.1 \mu\text{m}^3$  and were stored into TIFF files.

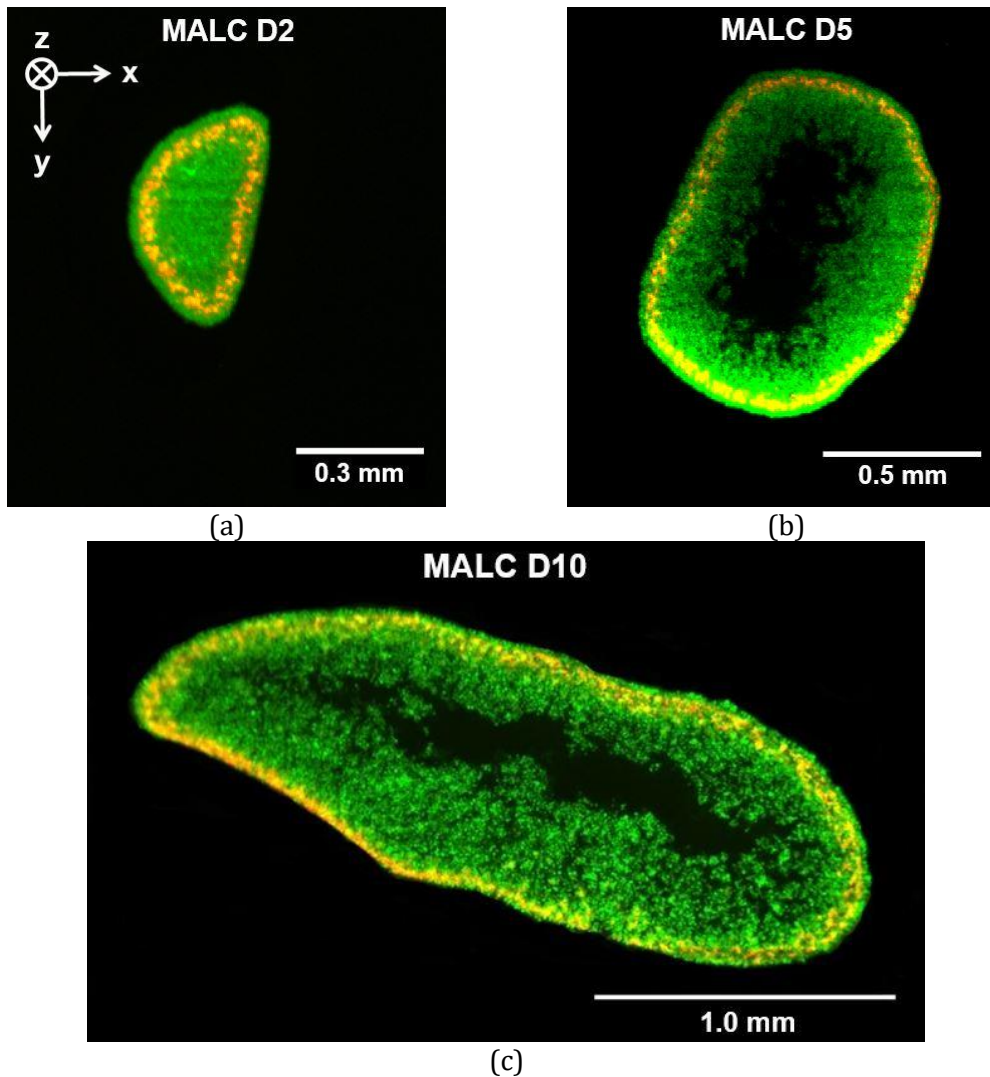


Figure 1. SPIM images of the central slices of the MALC: (a) D2, (b) D5 and (c) D10. Follicular lymphoma cell nuclei and rituximab appear in green and red, respectively.

## 143 2.2 Image processing

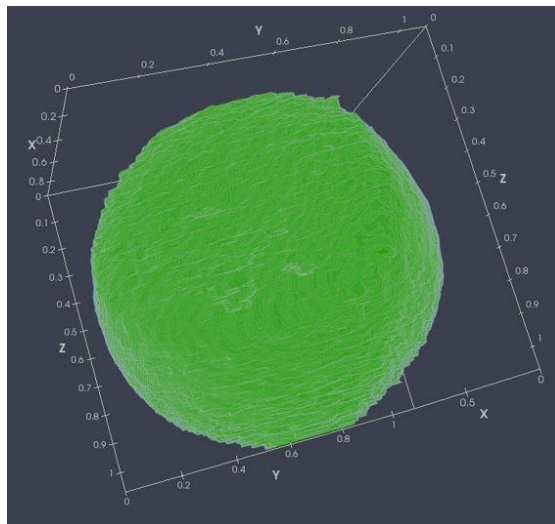
144 To extract the data required for the dosimetry of the MALCs, SPIM images were processed with  
 145 Fiji<sup>31</sup> (“Fiji Is Just ImageJ”, downloadable from [fiji.sc](http://fiji.sc)). This program has specific features for the  
 146 processing of microscope images of three-dimensional biological samples<sup>32</sup> and can read TIFF files. First,  
 147 the stacks of images were transferred to Fiji for 3D reconstructions (*e.g.* MALC D5 in Figure 2). Then,  
 148 two macros were developed and implemented in Fiji:

149 - The first one treated the green signal in order to extract the MALCs geometry. MALC voxels  
 150 were separated from the background by applying an intensity threshold. A clear delineation of the  
 151 MALCs boundary allowed a straightforward determination of the threshold value. Since the black hollow  
 152 sections of the MALC D5 and D10 were in the center, they were considered as a part of the volume of  
 153 interest (VOI). Finally, in view of the definition of the MALC geometry during Monte Carlo simulations,



154 the macro recorded different information into a “.dat” file; namely, the number of voxels, the voxel di-  
155 mensions along the three axes, and each voxel coordinate (x, y, z).

156 - The second macro treated the red signal from the rituximab CF555 label. It removed the back-  
157 ground noise, then recorded the voxel coordinates and intensity into a “.dat” file. During simulations,  
158 the source voxels were selected with a probability directly derived from their red signal intensity. It was  
159 supposed that the CF555 biodistribution would be identical to the radiopharmaceutical. This assumes  
160 that the CF555 labeling would neither detach from the antibody nor impact its biodistribution. To the  
161 best of our knowledge, no publication addresses these questions. This also assumes that the radiolabel-  
162 ing would not affect the rituximab biodistribution. This last point is supported by the requirement that,  
163 in nuclear medicine, the radiolabeling strategies preserve the biological properties of the proteins<sup>33</sup> such  
164 as rituximab.



165

166 Figure 2. 3D reconstruction of MALC D5 from SPIM images. The axes are given in millimeters.

167

## 167 2.3 Voxel-based dosimetry

### 168 2.3.1 General settings

169 Energy depositions were calculated with the Geant4 Monte Carlo open-source platform<sup>34-36</sup>  
170 since it enables the implementation of voxelized geometries and its physics models are well adapted to  
171 energies encountered in TRT. Furthermore, Geant4, being a well-established object-oriented code base,  
172 has a high degree of reuse meaning that little code must be written for a new application. The Geant4

173 “microbeam” example served as a basis. It was developed at the Centre d’Études Nucléaires de Bor-  
174 deaux-Gradignan (CENBG) to simulate the irradiation of individual cells with a microbeam of protons  
175 or  $\alpha$  particles<sup>37,38</sup>. In order to suit this example for TRT, we added the possibility of simulating the irra-  
176 diation of a VOI (in this work the MALC) by internalized radiopharmaceuticals. To reconstruct the  
177 MALCs, the class *G4PVParameterised* placed the SPIM images voxels ( $3.7 \cdot 3.7 \cdot 3.1 \mu\text{m}^3$ ) according to  
178 their coordinates stored in the output file of the first macro (see 2.2). The MALCs were positioned into  
179 a sphere of liquid water. This enabled electron backscattering into the MALCs with the consequence of  
180 an additional energy deposition. The simulation of the irradiation of each MALC by each radionuclide  
181 considered  $10^6$  decays. The activity was assumed homogeneously biodistributed within a voxel source.  
182 Accordingly, the coordinates of a decay site inside such a voxel were sampled from a uniform distribu-  
183 tion. The particles were then emitted isotropically. Depending on the type of electron emitter, energy  
184 depositions in the MALCs were simulated with different methods that will be presented in the following  
185 sections (2.3.2 and 2.3.3).

### 186 **2.3.2 Auger electron emitters**

187  $^{125}\text{I}$  and  $^{111}\text{In}$  mean emission spectra of monoenergetic electrons (Auger and internal conversion  
188 electrons, Figure 3) and photons (X-rays and  $\gamma$ -rays) were extracted from the publication of the Ameri-  
189 can Association of Physicists in Medicine<sup>39</sup> (AAPM), as it is still a reference for Auger electron emitters  
190 (*e.g.* <sup>14,40,41</sup>). Table 1 shows important properties of the decay of  $^{125}\text{I}$  and  $^{111}\text{In}$ . Half-lives were extracted  
191 from the Medical Internal Radiation Dose (MIRD) book<sup>42</sup>. The mean penetration corresponds to the  
192 length of the vector connecting the electron emission and stopping points. Hence, this distance is lower  
193 than the path length because the latter considers the direction changes of the electrons. Although nu-  
194 clear medicine dosimetric studies usually report the path length, in the present work the penetration  
195 was more relevant for analyzing the results.

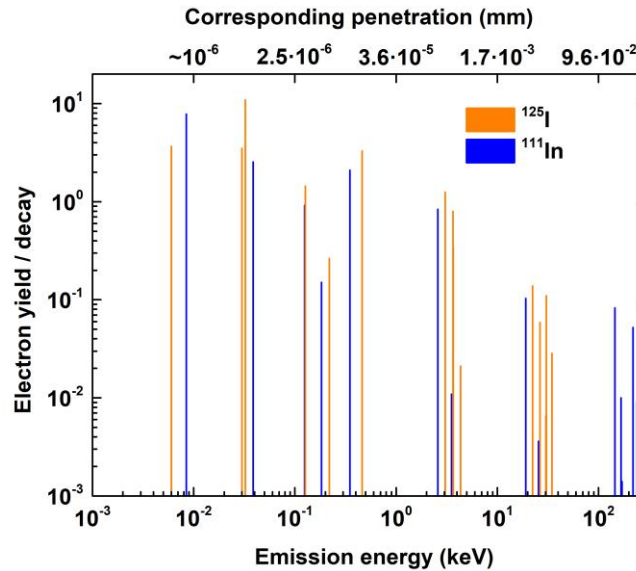


Figure 3. Mean electron emission spectra (Auger and internal conversion electrons) of  $^{125}\text{I}$  and  $^{111}\text{In}$  from the AAPM publication<sup>39</sup>. For each energy decade, the top x-axis provides the corresponding mean penetration according to Geant4-DNA.

Table 1. Some physical properties of the Auger electron emitters. Half-lives come from MIRD data<sup>42</sup>, spectra data were extracted from the AAPM publication<sup>39</sup> and mean penetrations were determined with Geant4-DNA.

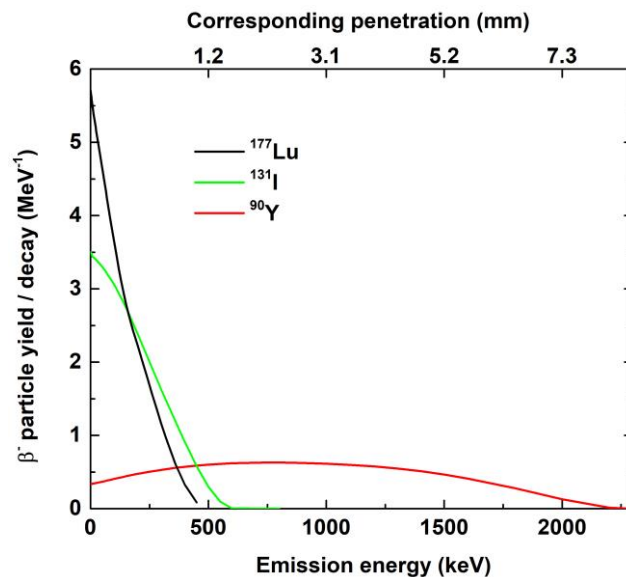
Auger electron emitter	$^{125}\text{I}$	$^{111}\text{In}$
Half-life (day)	59.4	2.8
Mean electron energy released (keV/decay)	19.5	34.7
Electron yield per decay	25.8	14.9
Electron mean energy $E_{e^- \text{ mean}}$ (keV)	0.8	2.3
Penetration at $E_{e^- \text{ mean}}$ (mm)	$2.5 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$
Maximum energy $E_{e^- \text{ max}}$ (keV)	34.7	245.0
Penetration at $E_{e^- \text{ max}}$ (mm)	$1.5 \cdot 10^{-2}$	$4.2 \cdot 10^{-1}$

Auger electrons induce energy deposition clusters localized at the nanometer scale<sup>43</sup>. Such phenomena can be studied with step-by-step Monte Carlo codes since they give the track structure of the particles down to the nanometer scale. Accordingly,  $^{125}\text{I}$  and  $^{111}\text{In}$  emissions were simulated with Geant4-DNA<sup>44-47</sup> ([geant4-dna.org](http://geant4-dna.org)). “CPA100” physics models (“option 6”)<sup>48,49</sup> were used. They describe ionization, electronic excitation and elastic scattering processes and are applicable from 256 keV down to 11 eV in liquid water.

210 For the simulations of photon interactions (Compton and Rayleigh scatterings as well as photo-  
 211 electric absorption), Geant4-DNA does not provide any physics models to-date. Thus, Geant4 “Liver-  
 212 more” physics models for photons were selected<sup>50,51</sup>. During simulations, electrons and photons could  
 213 produce vacancies in the atomic shells so atomic deexcitation emission was tracked (Auger electron  
 214 emission, Auger cascade and fluorescence).

### 215 2.3.3 $\beta^-$ particle emitters

216 AAPM’s publication<sup>39</sup> only provides data for Auger electron emitters. Therefore,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  
 217  $^{90}\text{Y}$  mean emission spectra of  $\beta^-$  particles (Figure 4), monoenergetic electrons and photons were ex-  
 218 tracted from the MIRD book<sup>42</sup>. Table 2 presents the main properties of the decay of  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ .



219

220 Figure 4.  $\beta^-$  particle emission spectra of  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$  from MIRD book<sup>42</sup>. The top x-axis provides the corre-  
 221 sponding Geant4 mean penetration every 500 keV.

222

223 Table 2. Some physical properties of the  $\beta^-$  particle emitters. Half-lives and spectra data come from MIRD book<sup>42</sup>  
 224 and mean penetrations were determined with Geant4.

$\beta^-$ particle emitter	<sup>177</sup> Lu	<sup>131</sup> I	<sup>90</sup> Y
Half-life (day)	6.6	8.0	2.7
Mean electron energy ( $\beta^-$ particles + monoenergetic electrons) released (keV/decay)	147.8	191.7	933.2
$\beta^-$ particle mean energy $E_{\beta^- \text{ mean}}$ (keV)	133.1	181.8	933.0
Penetration at $E_{\beta^- \text{ mean}}$ (mm)	$1.6 \cdot 10^{-1}$	$2.6 \cdot 10^{-1}$	2.9
$\beta^-$ particle maximum energy $E_{\beta^- \text{ max}}$ (keV)	497.8	806.9	2280.1
Penetration at $E_{\beta^- \text{ max}}$ (mm)	1.2	2.4	8.4

225

226 Part of the emission of <sup>177</sup>Lu, <sup>131</sup>I and <sup>90</sup>Y has an energy too high to be modeled with Geant4-DNA  
 227 physics models. Thus, simulations of the irradiation with these emitters were accomplished with Geant4  
 228 in a condensed history mode, as this provides physics models applicable up to GeV energies. “Liver-  
 229 more”<sup>52,53</sup> physics models were used to describe particles interactions.

230 The ionization process was simulated along with bremsstrahlung. This latter slightly increased  
 231 the stopping power of the electrons /  $\beta^-$  particles at these energies, thus marginally reducing their pen-  
 232 etration. “Livermore” electron models are defined down to about 10 eV. Nevertheless, in accordance  
 233 with guidelines, the cut-off was set to 250 eV. Energy production cuts for secondary particles was also  
 234 fixed at 250 eV. In order to limit the number of electrons crossing a voxel with no energy deposition,  
 235 step size limit was arbitrary chosen to 0.6  $\mu\text{m}$ , corresponding to less than 1/5 of the voxel dimensions.

## 236 2.4 Dosimetric quantities

### 237 2.4.1 Simulation results

238 Geant4 simulations generated output files containing the energy deposited in the MALCs voxels.  
 239 Firstly, different dosimetric quantities were directly calculated from these data: the absorbed fraction  
 240 and the absorbed dose.

241 - The absorbed fraction  $\phi$  represents the fraction of energy released from the source region that  
 242 is deposited in the target region. Here, the MALCs represent both the source and the target regions. The  
 243 results focused on electron absorbed fractions  $\phi_{e^-}$  (monoenergetic electrons and eventually  $\beta^-$  parti-  
 244 cles), because photon absorbed fractions are insignificant at this scale:

$$\phi_{e^- \text{ MALC}} = \frac{E_{e^-}}{E_{e^-_0}} \quad (1)$$

245 Where  $E_{e^-}$  is the deposited energy and  $E_{e^-_0}$  the electron-released energy. The absorbed fraction  
 246 is frequently reported in nuclear medicine dosimetry studies<sup>54</sup>.

247 - The energy deposited  $E_i$  (in J) in a VOI  $i$  (either a whole MALC or a voxel) characterized by a  
 248 mass  $m_i$  (in kg) was converted to absorbed dose  $D_i$  (in Gy):

$$D_i = \frac{E_i}{m_i} \quad (2)$$

249 This quantity was then divided by  $10^6$  to obtain the mean absorbed dose per decay (or per unit  
 250 of time-integrated activity, in  $\text{Bq} \cdot \text{s}$ , according to MIRD most recent terminology<sup>55</sup>). First, this quantity  
 251 was determined for the whole MALCs. However, the absorbed dose was not uniformly delivered within  
 252 the volume. In order to specify the volume fractions having received either low or high absorbed dose,  
 253 cumulative dose-volume histograms (cDVH) were plotted. They give the fraction of the volume (*i.e.* the  
 254 fraction of MALC voxels) in which an absorbed dose greater than or equal to a given value was delivered.  
 255 These graphs are routinely used in external radiotherapy, but also in some TRT studies<sup>56,57</sup>.

#### 256 2.4.2 Considering radiopharmaceutical effective half-life

257 Subsequently, in order to consider the effective half-life of the radiopharmaceutical X-rituximab  
 258 (X being one of the radionuclides), the MALCs and voxels absorbed doses per unit administered activity  
 259 ( $A_0$  in Bq) were calculated. In nuclear medicine, this quantity allows the assessment of the absorbed  
 260 dose delivered to a VOI for a given administered activity. It was calculated as the product of the absorbed  
 261 doses per decay (see 2.4.1) and the time-integrated activity coefficient  $\tilde{a}$  (also known as the residence  
 262 time and given in s). The value of  $\tilde{a}$  represents the total number of decays occurring in a MALC per unit  
 263 administered activity<sup>55</sup>:

$$\tilde{a} = \frac{1}{A_0} \int_0^{\infty} A(X - \text{rituximab}, t) \cdot dt \quad (3)$$

264 Where  $A(X - \text{rituximab}, t)$  is the time-dependent activity of the radiopharmaceutical in a MALC  
 265 (in Bq). Assuming that this radiopharmaceutical has an instantaneous uptake in a MALC (*i.e.* instanta-  
 266 neously bound to the MALCs according to the biodistribution extracted from the images) and its elimi-  
 267 nation is monoexponential:

$$\tilde{a} = \frac{1}{A_0} \int_0^{\infty} A_0 \cdot e^{-(\ln(2)/T_{\text{eff}}(X - \text{rituximab})) \cdot t} \cdot dt \quad (4)$$

$$\tilde{a} = 1.443 \cdot T_{\text{eff}}(X - \text{rituximab}) \quad (5)$$

268

269 Where  $T_{\text{eff}}(X - \text{rituximab})$ , the effective half-life of the radiopharmaceutical (in s), depends on  $T_{\text{phys}}(X)$   
 270 the physical half-life of the radionuclide X (in s) and on  $T_{\text{bio}}(\text{rituximab})$  the biological half-life of rituxi-  
 271 mab (in s):

$$T_{\text{eff}}(X - \text{rituximab}) = \frac{T_{\text{phys}}(X) \cdot T_{\text{bio}}(\text{rituximab})}{T_{\text{phys}}(X) + T_{\text{bio}}(\text{rituximab})} \quad (6)$$

272

273 The physical half-lives are well documented (see Table 1 and Table 2), but, rituximab biological  
 274 half-life in NHL is less-well established. In a clinical study, Scheidhauer *et al.* evaluated the effective half-  
 275 life of the radiopharmaceutical  $^{131}\text{I}$ -rituximab in NHL<sup>58</sup>. Biodistribution in 14 patients was determined  
 276 by gamma camera imaging. Our work focused on the 3.4 days mean effective half-life determined by  
 277 Scheidhauer *et al.* We emphasize, however, that its precision is limited. It is associated to a standard  
 278 deviation of 1.1 day. Also, Scheidhauer *et al.* reported a high inter-patient variability, with effective half-  
 279 lives ranging from 1.7 to 5.5 days. The gamma camera imaging introduces further uncertainties on the  
 280 dosimetry<sup>59-60</sup>. According to equation (6), the 3.4 days effective half-life corresponds to a rituximab bio-  
 281 logical half-life of 5.8 days. Using this value and the equation (6), the effective half-lives of rituximab  
 282 radiolabeled with  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$  or  $^{90}\text{Y}$  were deducted.

## 283 3 Results

### 284 3.1 MALC and rituximab biodistribution characteristics

285 Table 3 presents the dimensions of the three MALCs along x-, y-, z- axes and their masses in  
 286 Geant4 simulations which were used to determine the absorbed doses according to equation (2). The  
 287 masses were evaluated by multiplying the number of voxels in a MALC by the mass of a voxel filled with  
 288 liquid water (as simulations were performed in this material). Figure 1 shows that MALCs considered  
 289 in this study have ellipsoid-like shape. For MALC D2, D5 and D10, major radii are  $\sim 0.25$  mm,  $\sim 0.5$  mm  
 290 and  $\sim 1.3$  mm, respectively. Rituximab is heterogeneously biodistributed inside a shell-like volume at  
 291 the periphery of the MALCs (Figure 1). Generally, the shell-like volumes are characterized by a thickness  
 292 and a distance to the surface of the MALCs both of the order of a few hundredths of a millimeter. It was  
 293 previously demonstrated that the extracellular matrix limits the diffusion of therapeutic agents in tu-  
 294 mors<sup>17</sup>. It was also shown that MALCs expressed extracellular matrix proteins<sup>20</sup>. Accordingly, in an on-  
 295 going MALC-based study, the possibility that the rituximab peripheral location is due to the extracellular  
 296 matrix is being investigated.

297 Table 3. Sizes along x-, y- and z-axes of the MALCs D2, D5 and D10.

MALC	Dimensions x · y · z (mm <sup>3</sup> )	Mass (mg)
D2	0.34 · 0.53 · 0.50	$4.6 \cdot 10^{-2}$
D5	0.96 · 1.09 · 1.07	$5.5 \cdot 10^{-1}$
D10	2.54 · 1.23 · 2.26	2.8

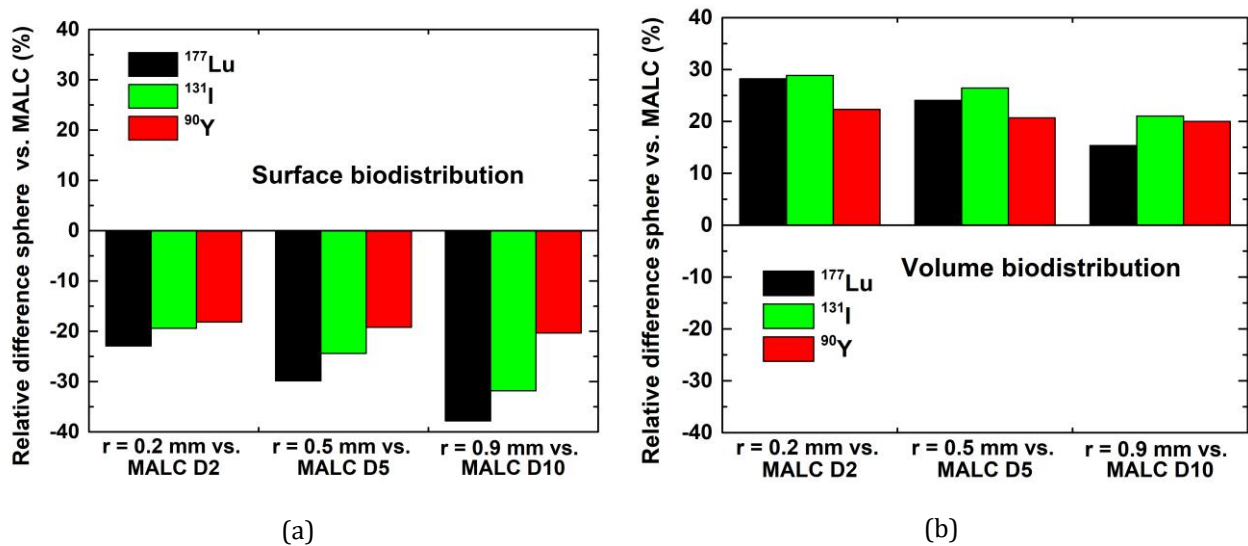
### 298 3.2 Dosimetric quantities per decay

#### 299 3.2.1 Comparison with literature data obtained in a simplified model

300 Figure 5 displays the relative difference (%) of absorbed dose per decay between the reference  
 301 data of Bardiès and Chatal<sup>3</sup> for spheres with either (a) a surface or (b) a volume biodistribution and  
 302 MALCs calculations (relative difference =  $100 \cdot (D_{\text{sphere}} - D_{\text{MALC}}) / D_{\text{MALC}}$ ). In this previous study, calcula-  
 303 tions were carried out by an analytical method based on scaled dose-point kernels in spheres with ra-  
 304 dius ranging from 0.01 to 22 mm. For a valid comparison, the results for a MALC were compared to the  
 305 results of the sphere having the closest mass. Accordingly, absorbed doses for MALCs D2, D5 and D10



306 were compared to the values for spheres with radius,  $r$ , of 0.2 mm, 0.5 mm and 0.9 mm, respectively.  
 307 Furthermore, only  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$  were considered because Bardiès and Chatal focused their study  
 308 on  $\beta^-$  particle emitters. The peripheral biodistribution of rituximab (discussed in section 3.1) can be seen  
 309 as an intermediate between the surface and volume biodistributions assumed by Bardiès and Chatal.  
 310 Hence, this is coherent that the absorbed doses per decay for MALCs lie between the values for these  
 311 two theoretical situations. It is also noticeable that results with these two biodistributions own a similar  
 312 degree of agreement with results for the MALCs. This indicates that for the purpose of absorbed dose  
 313 calculations, in these particular cases, the surface and the volume biodistribution are both equally rele-  
 314 vant. Furthermore, non-negligible discrepancies between the theoretical model and realistic data were  
 315 observed. These results highlight the need to account of realistic biological data.



316 Figure 5. Relative differences (%) between absorbed dose per decay calculated in spheres of various radii with  
 317 either a (a) surface or (b) a volume biodistribution<sup>3</sup> and MALCs (taken as reference). Comparison spheres vs.  
 318 MALCs are as follow:  $r = 0.2 \text{ mm vs. MALC D2}$  (left),  $r = 0.5 \text{ mm vs. MALC D5}$  (middle) and  $r = 0.9 \text{ mm vs. MALC}$   
 319  $\text{D10}$  (right).

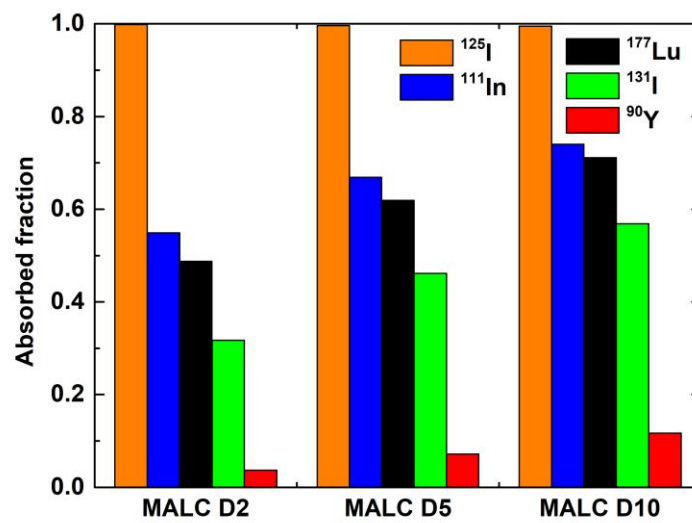
320

### 321 3.2.2 Comparison of the radionuclides

322 For clarity, the results for each radionuclide are reported by ascending order of energy released  
 323 per decay, that is:  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ .

324 Figure 6 displays, for each radionuclide, the electron absorbed fraction in the MALCs and Table  
 325 4 shows the corresponding numerical values. As MALCs get bigger, the electrons /  $\beta^-$  particles deposit

326 more energy inside before exiting them, increasing the absorbed fractions. Assuming a homogeneous  
 327 surface biodistribution of rituximab (which is at first sight the closer approximation of the real biodis-  
 328 tribution as seen on Figure 1), for very large MALCs the absorbed fraction would tend toward 0.5 (semi-  
 329 infinite medium condition). However, for MALC D5 and D10, the values become greater than 0.5 for all  
 330 radionuclides but  $^{90}\text{Y}$  (presumably, absorbed fraction of  $^{90}\text{Y}$  would become greater than 0.5 in bigger  
 331 MALCs). This can be explained by the rituximab penetration inside the MALCs, enabling the electrons /  
 332  $\beta^-$  particles to systematically deposit energy in the VOI, regardless their trajectories. This point is critical  
 333 since it further demonstrates the importance of using realistic biological data.



334 Figure 6. Absorbed fractions in MALCs D2, D5 and D10 for  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ .  
 335  
 336

337 Table 4. Absorbed fraction in the MALCs for the five radionuclides.

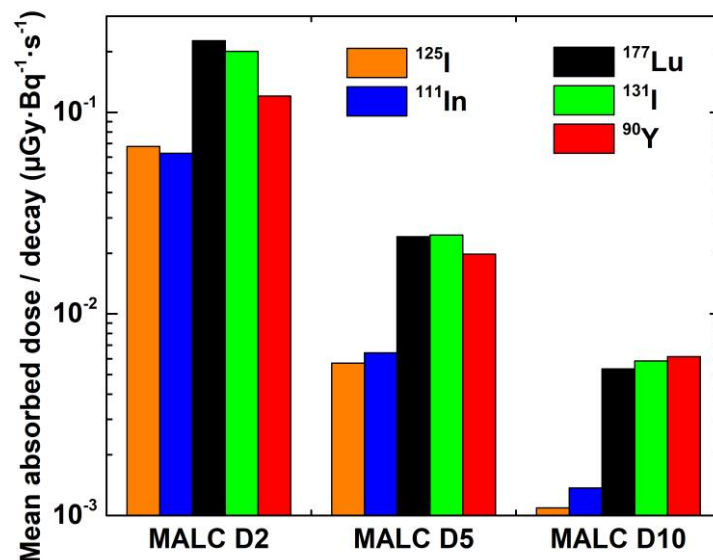
Radionuclide	Absorbed fraction		
	MALC D2	MALC D5	MALC D10
$^{125}\text{I}$	1.00	1.00	1.00
$^{111}\text{In}$	0.55	0.67	0.74
$^{177}\text{Lu}$	0.49	0.62	0.71
$^{131}\text{I}$	0.32	0.46	0.57
$^{90}\text{Y}$	0.04	0.07	0.12

338

339 As radionuclides release more energy per decay, the penetration of the electrons /  $\beta^-$  particles  
 340 increase (see Table 1 and Table 2). Thus, more energy is deposited outside the MALCs, and in turn the

341 absorbed fractions decrease.  $^{125}\text{I}$  deposits nearly its whole electron energy within the three MALCs. The  
 342 electrons it emits are unlikely to deposit energy outside the MALCs since their penetrations are limited  
 343 to  $1.5 \cdot 10^{-2}$  mm while the rituximab is mostly located at more than  $\sim 3 \cdot 10^{-2}$  mm from the edge. Note that  
 344 the real values are inferior to 0.998 (rounded to 1.00 for consistency), due to scarce rituximab biodis-  
 345 tribution closer to or at the surface. The absorbed fractions for  $^{111}\text{In}$ , lying between 0.55 and 0.74, are  
 346 notably lower than that of  $^{125}\text{I}$ . The reason is that  $\sim 80\%$  of the electron energy released per decay of  
 347  $^{111}\text{In}$  is taken away by emissions of at least 145.0 keV, characterized by penetrations above  $1.8 \cdot 10^{-1}$  mm  
 348 (right-hand side of Figure 3). Due to this property, the absorbed fractions for  $^{111}\text{In}$  are remarkably close  
 349 to  $^{177}\text{Lu}$ , as their emissions have similar penetrations. Furthermore, the absorbed fractions for  $^{131}\text{I}$  are  
 350 higher than for  $^{177}\text{Lu}$  as its penetration are higher (by  $1.0 \cdot 10^{-1}$  mm at  $E_{\beta^- \text{ mean}}$ ). Finally, the absorbed  
 351 fractions for  $^{90}\text{Y}$ , ranging between 0.04 and 0.12, are by far the lowest. Such low values are due to the  
 352 penetrations of its emissions (up to 8.4 mm) being significantly superior to the size of the MALCs.

353 Figure 7 displays the mean absorbed dose per decay delivered to the MALCs by each radionu-  
 354 clide and Table 5 shows the corresponding numerical values. These results are impacted by the energy  
 355 released by the radionuclides and the absorbed fractions.



356

357

358

Figure 7. Mean absorbed dose per decay in MALC D2, D5 and D10 for  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ .

359

360

Table 5. Mean absorbed dose per decay in the MALCs for the five radionuclides.

Radionuclide	Mean absorbed dose per decay ( $\mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ )		
	MALC D2	MALC D5	MALC D10
$^{125}\text{I}$	$6.78 \cdot 10^{-2}$	$5.69 \cdot 10^{-3}$	$1.10 \cdot 10^{-3}$
$^{111}\text{In}$	$6.26 \cdot 10^{-2}$	$6.41 \cdot 10^{-3}$	$1.37 \cdot 10^{-3}$
$^{177}\text{Lu}$	$2.27 \cdot 10^{-1}$	$2.41 \cdot 10^{-2}$	$5.34 \cdot 10^{-3}$
$^{131}\text{I}$	$2.01 \cdot 10^{-1}$	$2.46 \cdot 10^{-2}$	$5.84 \cdot 10^{-3}$
$^{90}\text{Y}$	$1.21 \cdot 10^{-1}$	$1.98 \cdot 10^{-2}$	$6.15 \cdot 10^{-3}$

361

362

363

364

Despite  $^{111}\text{In}$  (34.7 keV/decay) releasing more energy than  $^{125}\text{I}$  (19.5 keV/decay), the former delivered a lower absorbed dose in MALC D2 due to its lower absorbed fraction. Nevertheless, for MALC D5 and D10, the absorbed fraction for  $^{111}\text{In}$  increases, leading to greater absorbed doses than for  $^{125}\text{I}$ .

365

366

367

368

369

370

371

372

373

In each MALC, absorbed doses for  $\beta^-$  particle emitters are greater than for Auger electron emitters, because they release considerably more energy. For example, absorbed doses for  $^{177}\text{Lu}$  are greater than that of  $^{111}\text{In}$  by a factor  $\sim 4$ . Both have similar absorbed fractions, but  $^{177}\text{Lu}$  (147.8 keV/decay) releases  $\sim 4$  times more energy. For  $\beta^-$  particle emitters,  $^{177}\text{Lu}$  and  $^{131}\text{I}$  (191.7 keV/decay) results are close. Lower energy released by  $^{177}\text{Lu}$ , is compensated by its higher absorbed fractions. Among  $\beta^-$  particle emitters,  $^{90}\text{Y}$  (933.2 keV/decay) emits by far the more energy, yet the absorbed doses delivered are lowest in MALCs D2 and D5 due to extremely low absorbed fractions. In MALC D10, however, it delivered the highest absorbed doses because, compared to MALC D2, absorbed fractions were increased by a factor 3.

374

375

376

377

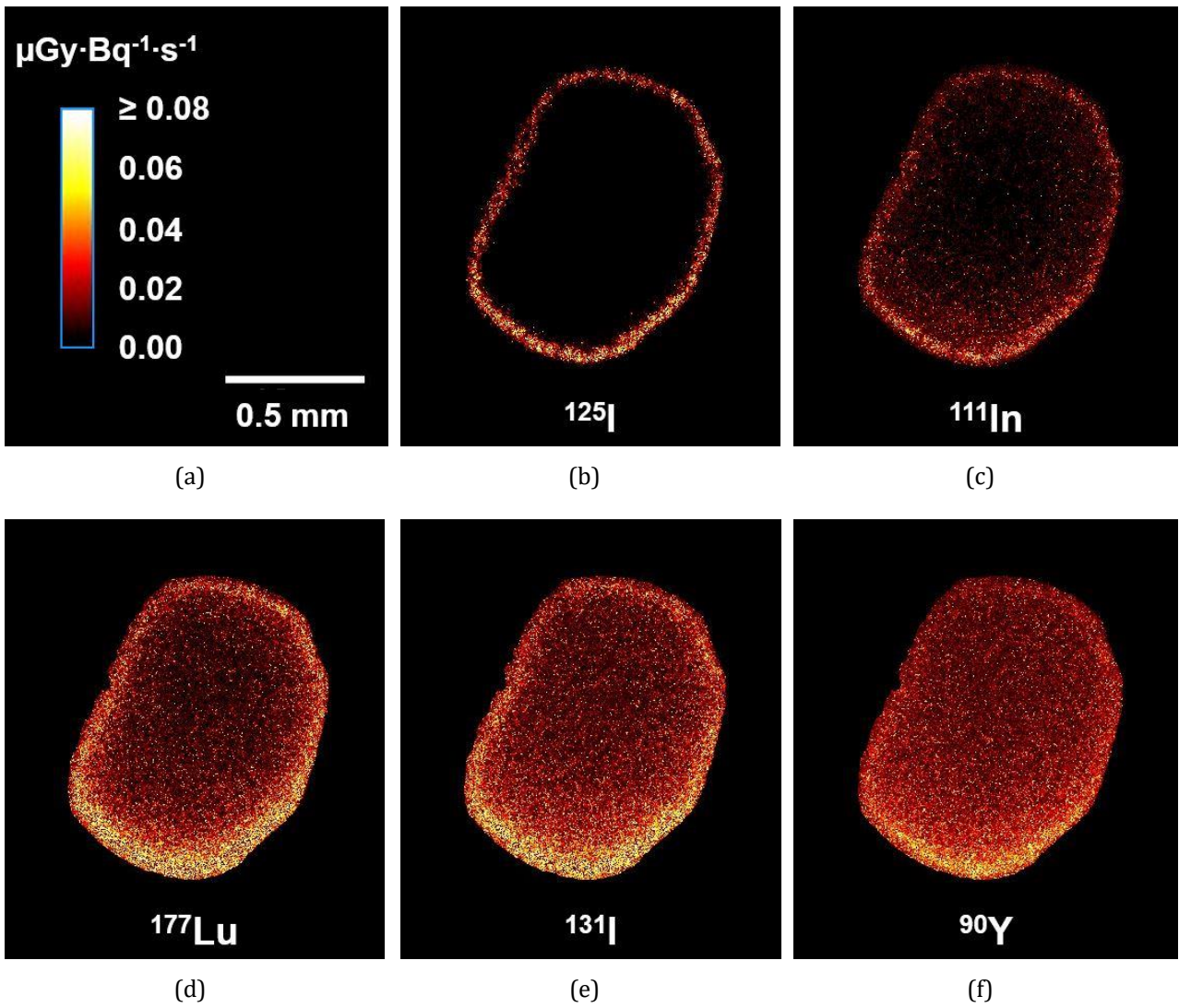
378

379

Figure 8 illustrates, as an example the distributions of the absorbed dose per decay (in  $\mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ ) inside the voxels of the slice of MALC D5 shown in Figure 1 (b). Note that  $0.01 \mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$  corresponds to a mean energy deposited per decay inside a voxel of  $2.6 \cdot 10^{-3} \text{ eV} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ . Figure 9 displays the cDVH (in  $\mu\text{Gy} \cdot \text{Bq}^{-1} \cdot \text{s}^{-1}$ ) of MALCs (a) D2, (b) D5 and (c) D10 for  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ . Globally, when radionuclides release more energy, the absorbed dose is delivered more uniformly within the MALCs because of the increasing penetration of the emission.

380 Figure 8 (b) highlights the peripheral irradiation of  $^{125}\text{I}$ , with a pattern very similar to the ritux-  
381 imab biodistribution. However, this radionuclide delivered high absorbed dose as revealed by the less-  
382 steep gradient of its cDVH. These observations are consistent with  $^{125}\text{I}$  emitting low energy electrons,  
383 which deposit most of the energy in a small distance from the decay site. The highly localized absorbed  
384 dose delivered by  $^{125}\text{I}$  was further observed at the cellular level by Arnaud *et al.*<sup>14</sup>. Unlike this radionu-  
385 clide,  $^{111}\text{In}$  sparsely delivered absorbed dose in the central part of the MALCs (Figure 8 (c)) due to the  
386 higher penetrations of its emission. For both Auger electron emitters, the low absorbed dose delivered  
387 in the central part of the MALCs (or the lack thereof) is linked to the rituximab peripheral biodistribu-  
388 tion. Nevertheless, the limited penetration of rituximab inside the MALCs can be counterbalanced by a  
389 radiolabeling with one of the  $\beta^-$  particle emitters.

390 This benefit, illustrated by Figure 8 (d), (e) and (f), was brought by the higher penetration of  
391 their emission. It translates to higher cDVH values on the left-hand side (Figure 9). Among  $\beta^-$  particle  
392 emitters,  $^{90}\text{Y}$  energy deposition was the most uniform throughout the MALCs. In comparison,  $^{177}\text{Lu}$  and  
393  $^{131}\text{I}$  deposited less energy in the central part but more in the periphery. The improved absorbed dose  
394 uniformity of  $^{90}\text{Y}$  is further expressed by steeper gradients in its cDVH. It is linked to the variation of  
395 penetration and stopping power of  $\beta^-$  particles with their energy.  $\beta^-$  particles emitted by  $^{90}\text{Y}$  having  
396 higher penetrations than for  $^{177}\text{Lu}$  and  $^{131}\text{I}$ , were more likely to reach the center of the MALCs. This dif-  
397 ference is more pronounced as MALCs get bigger. Compared to  $^{177}\text{Lu}$  and  $^{131}\text{I}$ , emissions of  $^{90}\text{Y}$  have lower  
398 stopping powers (according to NIST database<sup>61</sup>, at  $E_{\beta^- \text{ mean}}$ : 0.34, 0.29 and 0.19 keV/ $\mu\text{m}$ , respectively),  
399 thus they deposited less energy close to the decay site.



400 Figure 8. Absorbed dose per decay ( $\mu\text{Gy}\cdot\text{Bq}^{-1}\cdot\text{s}^{-1}$ ) delivered to the voxel of the slice of MALC D5 shown in Figure 1  
 401 (b), for (b)  $^{125}\text{I}$ , (c)  $^{111}\text{In}$ , (d)  $^{177}\text{Lu}$ , (e)  $^{131}\text{I}$  and (f)  $^{90}\text{Y}$ . The corresponding color scale is displayed in (a).

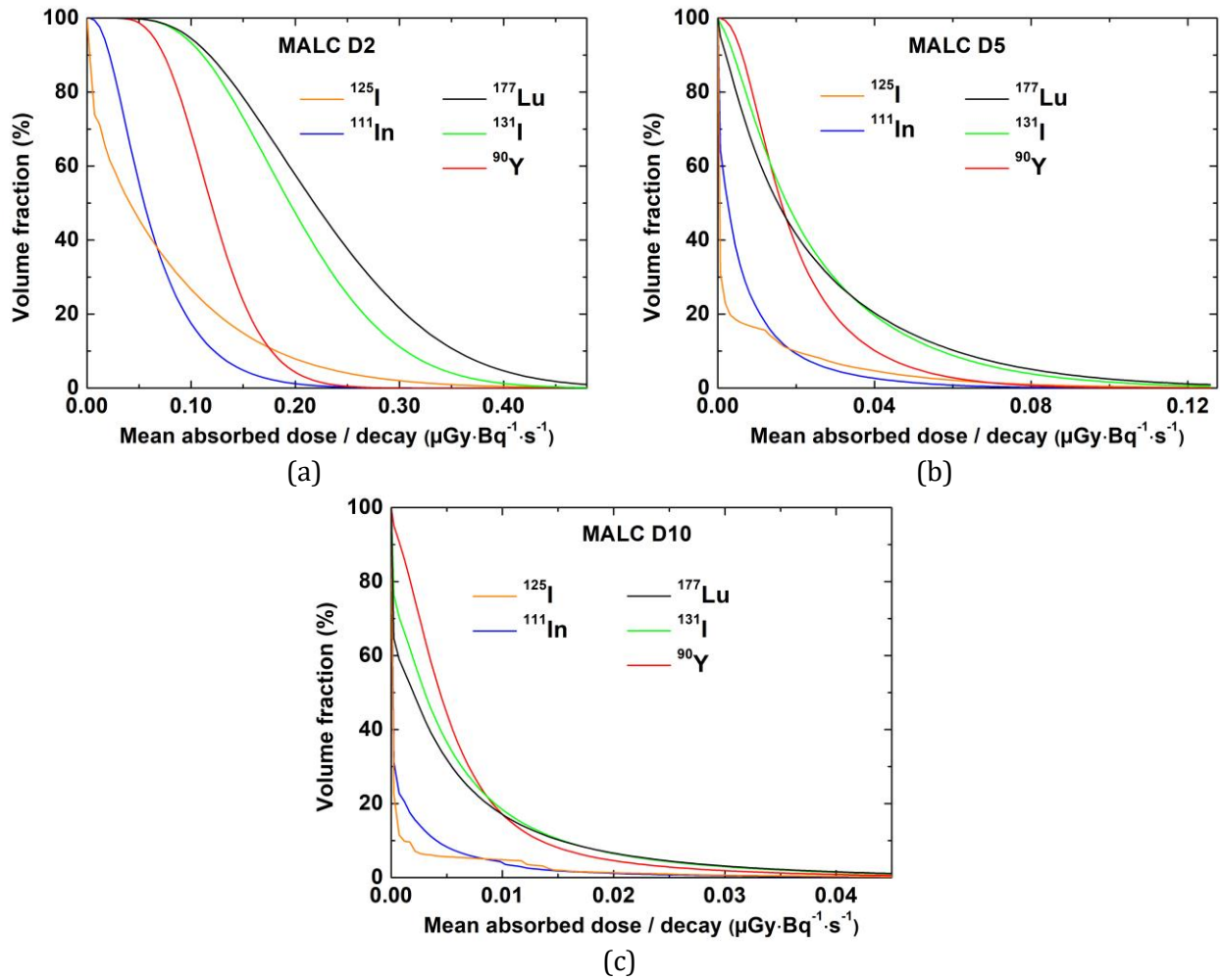


Figure 9. cDVH of MALC (a) D2, (b) D5 and (c) D10 per decay for  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ .

402  
403

### 404 3.3 Absorbed dose per unit administered activity

405 The influence of the radiolabeled rituximab effective half-life on the absorbed dose is presented.  
406 Table 6 summarizes the effective half-lives for radiolabeling with  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  or  $^{90}\text{Y}$  as calculated  
407 from equation (6). The differences between the physical half-lives of the radionuclides were decreased  
408 by the introduction of the biological half-life of the rituximab (5.8 days).

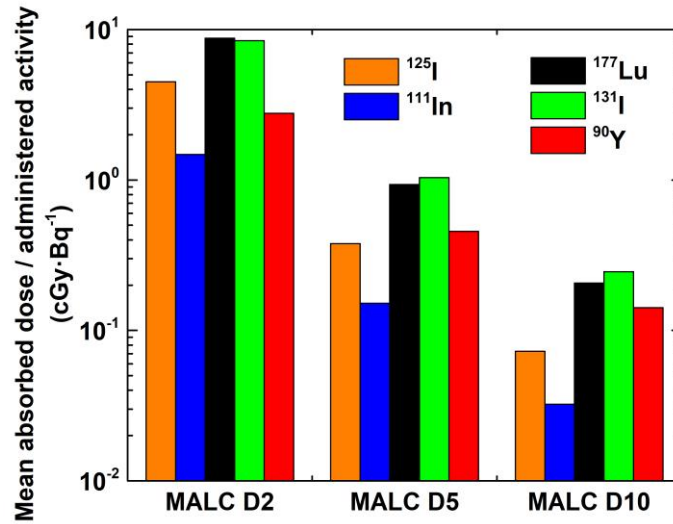
409 Table 6. Effective half-lives of rituximab radiolabeled with one of the five radionuclides in NHL, determined with  
 410 equation (6).

<b>Radionuclide X</b>	<b>Effective half-life of X-rituximab (day)</b>
<sup>125</sup> I	5.3
<sup>111</sup> In	1.9
<sup>177</sup> Lu	3.1
<sup>131</sup> I	3.4
<sup>90</sup> Y	1.8

411

412 For each radionuclide, Figure 10 displays the mean absorbed dose per unit administered activity  
 413 within the MALCs and Table 7 shows the corresponding numerical values. Regarding Auger electron  
 414 emitters, the effective half-life of <sup>125</sup>I-rituximab was 2.8 times higher than <sup>111</sup>In-rituximab. Therefore,  
 415 despite lower absorbed dose delivered per decay in MALC D5 and D10, <sup>125</sup>I delivered more absorbed  
 416 dose per unit administered activity than <sup>111</sup>In. For  $\beta^-$  particle emitters, the effective half-life of <sup>177</sup>Lu-  
 417 rituximab and <sup>131</sup>I-rituximab were 1.7 and 1.9 times greater than that of <sup>90</sup>Y-rituximab. As a result, ab-  
 418 sorbed dose per unit administered activity for <sup>177</sup>Lu and <sup>131</sup>I were more than 3 times higher in MALC D2  
 419 and more than 2 times higher in MALC D5 than that of <sup>90</sup>Y. Furthermore, in MALC D10, despite higher  
 420 absorbed dose per decay, <sup>90</sup>Y delivered less absorbed dose per unit administered activity than <sup>177</sup>Lu and  
 421 <sup>131</sup>I. As discussed by Scheidhauer *et al.*<sup>58</sup>, the relatively long biological half-life of rituximab (5.8 days in  
 422 NHL) is best matched by <sup>131</sup>I physical half-life (8.0 days) than by <sup>90</sup>Y short physical half-life (2.7 days).  
 423 <sup>177</sup>Lu physical half-life of 6.6 days is also well adapted to the rituximab biological half-life.





424

425 Figure 10. Mean absorbed dose per unit administered activity to MALCs D2, D5 and D10 for <sup>125</sup>I, <sup>111</sup>In, <sup>177</sup>Lu, <sup>131</sup>I  
 426 and <sup>90</sup>Y.

427

428

Table 7. Mean absorbed dose per unit administered activity to the MALCs for the five radionuclides.

Radionuclide	Mean absorbed dose per unit administered activity (cGy · Bq <sup>-1</sup> )		
	MALC D2	MALC D5	MALC D10
<sup>125</sup> I	4.50	3.78 · 10 <sup>-1</sup>	7.27 · 10 <sup>-2</sup>
<sup>111</sup> In	1.48	1.51 · 10 <sup>-1</sup>	3.23 · 10 <sup>-2</sup>
<sup>177</sup> Lu	8.76	9.33 · 10 <sup>-1</sup>	2.06 · 10 <sup>-1</sup>
<sup>131</sup> I	8.45	1.04	2.46 · 10 <sup>-1</sup>
<sup>90</sup> Y	2.78	4.56 · 10 <sup>-1</sup>	1.42 · 10 <sup>-1</sup>

429

430 Figure 11 illustrates, as an example the isodoses (in cGy·Bq<sup>-1</sup>) inside the voxels of the slice of  
 431 MALC D5 shown by Figure 1 (b). Only the β- particle emitters were represented since the absorbed dose  
 432 distribution of Auger electron emitters were very heterogeneous and did not allow for a clear represen-  
 433 tation of isodoses. Figure 12 shows the cDVH of MALC (a) D2, (b) D5 and (c) D10 per unit administered  
 434 activity for <sup>125</sup>I, <sup>111</sup>In, <sup>177</sup>Lu, <sup>131</sup>I and <sup>90</sup>Y.

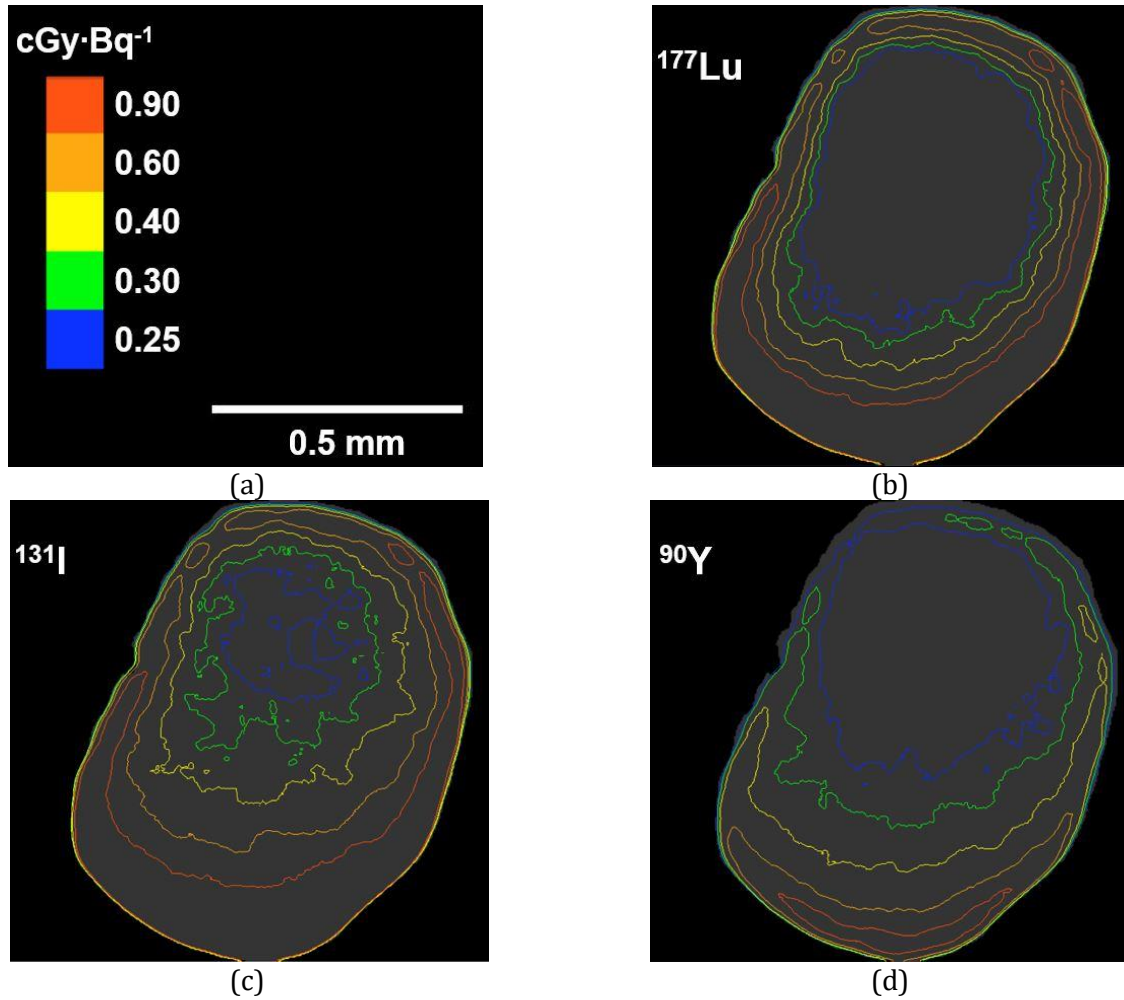
435

436

437

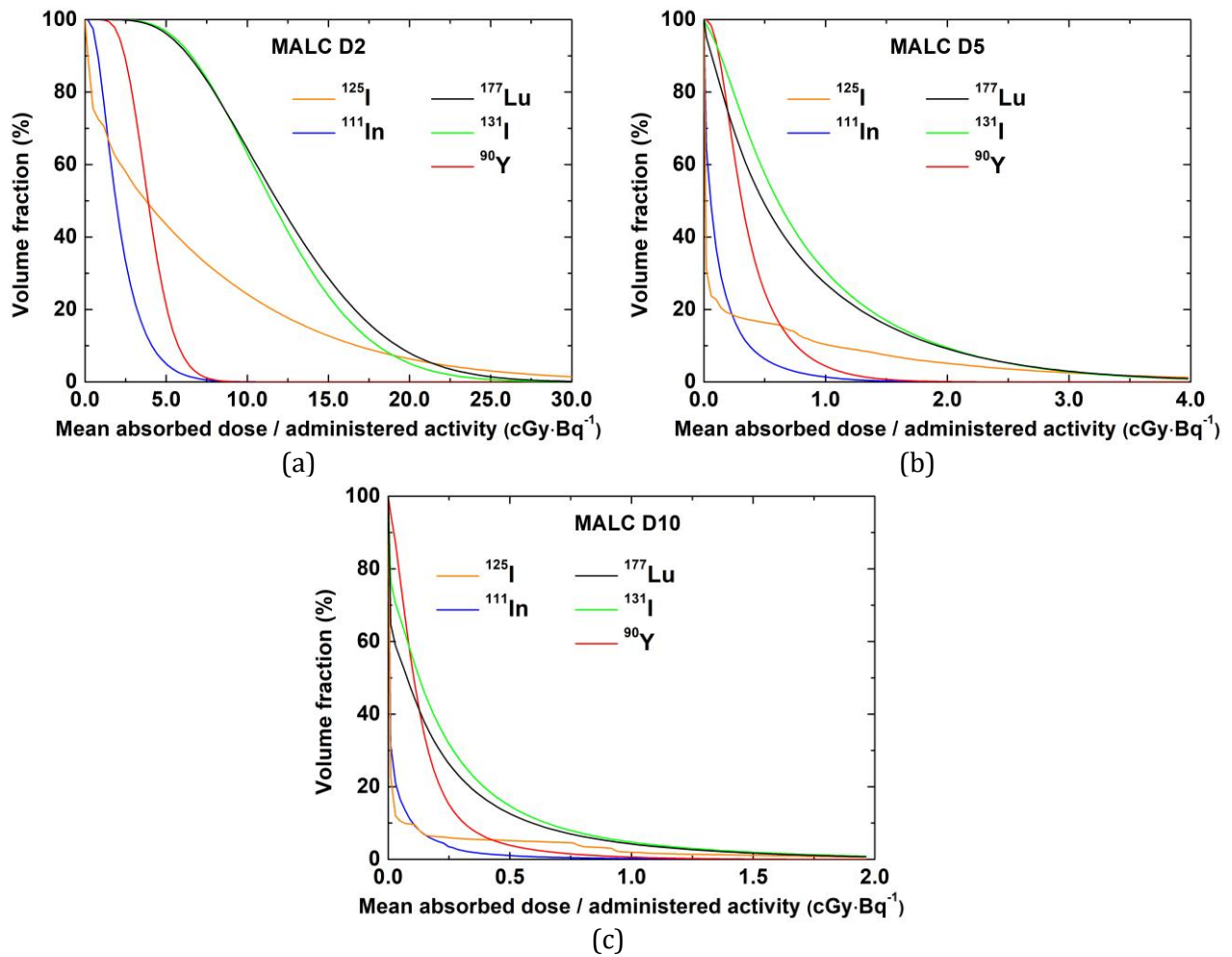
When the effective half-life of radiolabeled rituximab increases, the absorbed doses per admin-  
 istered activity increase. As a consequence, the region covered by high absorbed dose is largest for <sup>131</sup>I  
 and smallest for <sup>90</sup>Y as illustrated by the isodoses (Figure 11). Furthermore, the higher the effective half-

438 life, the greater the shift of the cDVH curves toward higher absorbed doses. Accordingly, in the region of  
 439 high absorbed doses, cDVH values of  $^{125}\text{I}$  become significantly higher than that of  $^{111}\text{In}$ . In this region,  
 440 cDVH values of  $^{177}\text{Lu}$  and  $^{131}\text{I}$  are noticeably higher than those of  $^{90}\text{Y}$ .



441 Figure 11. Isodose distributions in the slice of MALC D5 shown in Figure 1 (b), per unit administered activity of  
 442 (b)  $^{177}\text{Lu}$ , (c)  $^{131}\text{I}$  and (d)  $^{90}\text{Y}$  (cGy·Bq<sup>-1</sup>). The corresponding color scale is displayed in (a).

443



444 Figure 12. cDVH per unit administered activity of MALC (a) D2, (b) D5 and (c) D10 for  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  
 445  $^{90}\text{Y}$ .

### 446 3.4 Discussion

447 Our methodology allowed the observation of the absorbed dose in an *in vitro* tumor model which  
 448 is more realistic than the spherical tumor with uniform biodistribution simplification. It is recognized,  
 449 however, that the realism of this work is limited with respect to clinical context. It is therefore essential  
 450 to review the degree of relevance of the biological and physical aspects of this work.

451 The present study focused on three MALCs with dimensions of the order of millimeter. On one  
 452 hand, such a sample did not reproduce the diversity of tumor geometry and rituximab pharmacokinetics  
 453 in patients, which are critical for dosimetry. For example, in clinical routine, the size of the diagnosed  
 454 follicular lymphomas ranges from a few millimeters to several centimeters<sup>62</sup>. Additionally, multiple  
 455 studies have highlighted the need for dosimetry in smaller follicular lymphomas (down to  $\sim 10\ \mu\text{m}$ )<sup>63-65</sup>.  
 456 Our study did not extend to the biggest and the smallest of the follicular lymphomas. It is expected that,  
 457 with the increasing / decreasing size of the tumor model, radionuclides with higher (*e.g.*  $^{90}\text{Y}$ ) / lower

458 (e.g.  $^{125}\text{I}$ ) penetration emissions, would become more favored from a dosimetric perspective. On the  
459 other hand, previous studies have highlighted the ability of three-dimensional aggregates to mimic the  
460 physiological *in vivo* aspects of tumors, enabling the identification of new targets and drugs<sup>66-68</sup>. In par-  
461 ticular, this tool has long been recognized as very effective to characterize the penetration of antibod-  
462 ies<sup>69-70</sup>. These previous works give confidence in the clinical relevance of the rituximab peripheral dis-  
463 tribution in the MALCs, a key point of the present study.

464         The accurate Monte Carlo tracking of the radionuclide emissions in the MALCs relied largely on  
465 the choice of the physics models. Geant4-DNA provides two sets of physics models able to entirely sim-  
466 ulate the electron emissions of  $^{125}\text{I}$  and  $^{111}\text{In}$ : the default (“option 2”) and the CPA100 (“option 6”) sets.  
467 The latter set was selected following two main arguments. Differential cross sections for ionization and  
468 cross section for electronic excitation of “option 6” are in better agreement than that of “option 2” with  
469 experimental data. Furthermore, for low energy electrons, dose-point kernels generated by “option 2”  
470 are considered unrealistic because they are very diffusive<sup>71</sup>, while those calculated by “option 6” are less  
471 so<sup>72</sup>. Concerning  $\beta^-$  particle emitters, “Livermore” were considered more relevant than “PENELOPE-  
472 2008”<sup>73</sup> electron physics models based on two arguments. Firstly, to be in accordance with Geant4-DNA  
473 “option 6”, the simulations were realized in liquid water. For this material, “Livermore” ionization cross  
474 sections are in better agreement with experimental data<sup>36</sup>. Secondly, regarding deexcitation processes,  
475 “Livermore” simulates every atomic shell, while “PENELOPE-2008” only takes into account K, L and M  
476 shells<sup>73</sup>.

477         Monte Carlo simulations were informed with data from one SPIM acquisition, showing biodis-  
478 tribution in MALCs after 24 h of incubation. To approximate the time-dependent activity in the MALCs,  
479 we used the straightforward assumptions of a radiopharmaceutical instantaneous uptake and monoex-  
480 ponential elimination. In clinical studies, a more detailed description of the uptake and elimination is  
481 provided. Typically, 3 to 4 SPECT or PET acquisitions are performed, the first a few hours and the last  
482 between a few tenth of hours and nearly 200 h after administration<sup>25,56,74-75</sup>. Generally, these data are  
483 fitted by a mono/biexponential function. The instantaneous uptake does not account for the decays oc-  
484 ccurring outside the tumor during the uptake in a clinical context. Hence, the absorbed dose to the tumor

485 delivered by radionuclides with short half-lives, such as  $^{111}\text{In}$  and  $^{90}\text{Y}$ , is overestimated compared to  
486 those with longer half-life. We note, however, that SPECT acquisitions of mice and patients showed early  
487 uptake of rituximab in NHL<sup>76-77</sup> (a few hours after administration while  $^{111}\text{In}$  and  $^{90}\text{Y}$  physical half-lives  
488 are  $\sim 2$  days). Regarding the elimination of the rituximab in NHL, no data were found in the literature.  
489 Implementation of more sophisticated pharmacokinetic models in our methodology would be an im-  
490 portant next step for a more relevant dosimetry. Ideally, these models should consider rituximab data  
491 in patients' NHL, upon availability.

#### 492 **4 Conclusion**

493 We have developed a Monte Carlo dosimetric model based on realistic NHL geometry and ritux-  
494 imab biodistribution. It was employed to describe the absorbed dose that would be delivered at the  
495 microscopic scale by Auger electron emitters ( $^{125}\text{I}$  and  $^{111}\text{In}$ ) and  $\beta$ -particle emitters ( $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ )  
496 in the context of RIT.

497 A comparison between the mean absorbed dose per decay for realistic geometry and biodistri-  
498 bution with literature data for a sphere with either a surface or a volume biodistribution demonstrated  
499 significant differences. Then, for the five radionuclides, the absorbed fractions, the mean absorbed dose  
500 and its associated distribution were compared. This comparison further highlighted the importance of  
501 both the realistic MALC geometry and rituximab biodistribution as they often impacted the outcomes.

502 Aside from these two parameters, the decay properties - the electron /  $\beta$ -particle energy re-  
503 leased per decay and penetration - had an important impact. The uniformity of the absorbed dose deliv-  
504 ered throughout the MALCs increased for radionuclides releasing more energy per decay (in ascending  
505 order:  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{177}\text{Lu}$ ,  $^{131}\text{I}$  and  $^{90}\text{Y}$ ). The mean absorbed dose of  $\beta$ -particle emitters was higher than that  
506 of Auger electron emitters.  $^{177}\text{Lu}$  and  $^{131}\text{I}$  mean absorbed doses and were higher than  $^{90}\text{Y}$  in the two  
507 smallest MALCs. Furthermore, when considering the radiolabeled rituximab effective half-life, the ab-  
508 sorbed dose for  $^{177}\text{Lu}$  and  $^{131}\text{I}$  remained close to each other and considerably greater than that of  $^{90}\text{Y}$  in  
509 the three MALCs.

510 The methodology developed may be applied to various situations of TRT. Voxelized images of  
511 others tumoral / healthy tissue models and tumor-seeking agents can be considered. It is possible to

512 simulate the emission of other radionuclides of interest such as potential theranostic Auger electron  
 513 emitters (*e.g.* copper-64<sup>74</sup>, useful for PET imaging) and  $\beta^-$  particle emitters (*e.g.* terbium-161<sup>78-79</sup>, useful  
 514 for SPECT imaging) or therapeutic  $\alpha$  particle emitters (*e.g.* actinium-225<sup>80-81</sup>). To further improve the  
 515 realism of the simulations, future work may integrate the temporal variation of the vector biodistribu-  
 516 tion and the growth of MALC. Such work could be useful in the case of real irradiation experiment to  
 517 select relevant radionuclides and assess biological effects.

## 518 5 References

- 519 1. Strand SE, Jönsson BA, Ljungberg M, Tennvall J. Radioimmunotherapy dosimetry-a review. *Acta*  
 520 *Oncol.* 1993;32(7-8):807-817. doi:10.3109/02841869309096140
- 521 2. Roeske JC, Aydogan B, Bardiès M, Humm JL. Small-scale dosimetry: challenges and future  
 522 directions. *Semin Nucl Med.* 2008;38(5):367-383. doi:10.1053/j.semnuclmed.2008.05.003
- 523 3. Bardiès M, Chatal JF. Absorbed doses for internal radiotherapy from 22 beta-emitting  
 524 radionuclides: beta dosimetry of small spheres. *Phys Med Biol.* 1994;39(6):961-981.  
 525 doi:10.1088/0031-9155/39/6/004
- 526 4. Falzone N, Fernández-Varea JM, Flux G, Vallis KA. Monte Carlo evaluation of Auger electron-  
 527 emitting theragnostic radionuclides. *J Nucl Med.* 2015;56(9):1441-1446.  
 528 doi:10.2967/jnumed.114.153502
- 529 5. Champion C, Quinto MA, Morgat C, Zanotti-Fregonara P, Hindié E. Comparison between three  
 530 promising  $\beta^-$ -emitting radionuclides, <sup>67</sup>Cu, <sup>47</sup>Sc and <sup>161</sup>Tb, with emphasis on doses delivered to  
 531 minimal residual disease. *Theranostics.* 2016;6(10):1611-1618. doi:10.7150/thno.15132
- 532 6. Hindié E, Zanotti-Fregonara P, Quinto MA, Morgat C, Champion C. Dose deposits from <sup>90</sup>Y, <sup>177</sup>Lu,  
 533 <sup>111</sup>In, and <sup>161</sup>Tb in micrometastases of various sizes: implications for radiopharmaceutical therapy.  
 534 *J Nucl Med.* 2016;57(5):759-764. doi:10.2967/jnumed.115.170423
- 535 7. Amato E, Italiano A, Baldari S. An analytical model to calculate absorbed fractions for internal  
 536 dosimetry with alpha, beta and gamma emitters. *Pericol Classe Sci Fis Mat Nat.* 2014;92(1):A1.  
 537 doi:10.1478/AAPP.921A1
- 538 8. Amato E, Lizio D, Baldari S. Absorbed fractions for electrons in ellipsoidal volumes. *Phys Med Biol.*  
 539 2011;56(2):357-365. doi:10.1088/0031-9155/56/2/005
- 540 9. Rahman Z, Mirza SM, Arshed W, Mirza NM, Ahmed W. Influence of thyroid volume reduction on  
 541 absorbed dose in <sup>131</sup>I therapy studied by using Geant4 Monte Carlo simulation. *Chin Phys C.*  
 542 2014;38(87):056201. doi:10.1088/1674-1137/38/5/056201
- 543 10. Bao A, Zhao X, Phillips WT, et al. Theoretical study of the influence of a heterogeneous activity  
 544 distribution on intratumoral absorbed dose distribution. *Med Phys.* 2005;32(1):200-208.  
 545 doi:10.1118/1.1833151
- 546 11. Spaic R, Ilic R, Dragovic M, Petrovic B. Generation of dose-volume histograms using Monte Carlo  
 547 simulations on a multicellular model in radionuclide therapy. *Cancer Biother Radio.*  
 548 2005;20(3):320-324. doi:10.1089/cbr.2005.20.320

- 549 12. Uusijärvi H, Bernhardt P, Forssell-Aronsson E. Tumour control probability (TCP) for non-uniform  
550 activity distribution in radionuclide therapy. *Phys Med Biol.* 2008;53(16):4369-4381.  
551 doi:10.1088/0031-9155/53/16/010
- 552 13. Howell RW , Rajon D, Bolch BE. Monte Carlo simulation of irradiation and killing in three-  
553 dimensional cell-populations with lognormal cellular uptake of radioactivity. *Int J Radiat Biol.*  
554 2012;88(1-2):115-122. doi:10.3109/09553002.2011.602379
- 555 14. Arnaud FX, Paillas S, Pouget JP, Incerti S, Bardiès M, Bordage MC. Complex cell geometry and  
556 sources distribution model for Monte Carlo single cell dosimetry with iodine 125  
557 radioimmunotherapy. *Nucl Instrum Methods Phys Res B.* 2016;366:227-233.  
558 doi:10.1016/j.nimb.2015.11.008
- 559 15. Daydé D, Ternant D, Ohresser M, et al. Tumor burden influences exposure and response to  
560 rituximab: pharmacokinetic-pharmacodynamic modeling using a syngeneic bioluminescent  
561 murine model expressing human CD20. *Blood.* 2009;113(16):3765-3772. doi:10.1182/blood-  
562 2008-08-175125
- 563 16. Meignan M, Cottreau AS, Versari A, et al. Baseline metabolic tumor volume predicts outcome in  
564 high-tumor-burden follicular lymphoma: a pooled analysis of three multicenter studies. *J Clin*  
565 *Oncol.* 2016;34(30):3618-3626. doi:10.1200/JCO.2016.66.9440
- 566 17. Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK. Role of extracellular matrix assembly in  
567 interstitial transport in solid tumors. *Cancer Res.* 2000;60(9):2497-2503.
- 568 18. Gravelle P, Jean C, Valleron W, Laurent G, Fournié JJ. Innate predisposition to immune escape in  
569 follicular lymphoma cells. *Oncoimmunology.* 2012;1(4):555-556. doi:10.4161/onci.19365
- 570 19. Gravelle P, Jean C, Familiades J, et al. Cell growth in aggregates determines gene expression,  
571 proliferation, survival, chemoresistance, and sensitivity to immune effectors in follicular  
572 lymphoma. *Am J Pathol.* 2014;184(1):282-295. doi:10.1016/j.ajpath.2013.09.018
- 573 20. Decaup E, Jean C, Laurent C, et al. Anti-tumor activity of obinutuzumab and rituximab in a follicular  
574 lymphoma 3D model. *Blood Cancer J.* 2013;3(8):e131. doi:10.1038/bcj.2013.32
- 575 21. Rossi C, Gravelle P, Decaup E, et al. Boosting  $\gamma\delta$  T cell-mediated antibody-dependent cellular  
576 cytotoxicity by PD-1 blockade in follicular lymphoma. *Oncoimmunology.* 2019;8(3):1554175.  
577 doi:10.1080/2162402X.2018.1554175
- 578 22. Read ED, Eu P, Little PJ, Piva TJ. The status of radioimmunotherapy in CD20+ non-Hodgkin's  
579 lymphoma. *Target Oncol.* 2015;10(1):15-26. doi:10.1007/s11523-014-0324-y
- 580 23. Griffiths GL, Govindan SV, Sgouros G, Ong GL, Goldenberg DM, Mattes MJ. Cytotoxicity with Auger  
581 electron-emitting radionuclides delivered by antibodies. *Int J Cancer.* 1999;81(6):985-992.  
582 doi:10.1002/(SICI)1097-0215(19990611)81:6<985::AID-IJC23>3.0.CO;2-W
- 583 24. Ong GL, Elsamra SE, Goldenberg DM, Mattes MJ. Single-cell cytotoxicity with radiolabeled  
584 antibodies. *Clin Cancer Res.* 2001;7(1):192-201.
- 585 25. Blakkisrud J, Løndalen A, Martinsen ACT, et al. Tumor-absorbed dose for non-Hodgkin lymphoma  
586 patients treated with the anti-CD37 antibody radionuclide conjugate  $^{177}\text{Lu}$ -lilotomab satetraxetan.  
587 *J Nucl Med.* 2017;58(1):48-54. doi:10.2967/jnumed.116.173922
- 588 26. Goldsmith SJ. Radioimmunotherapy of lymphoma: Bexxar and Zevalin. *Semin Nucl Med.*  
589 2010;40(2):122-135. doi:10.1053/j.semnuclmed.2009.11.002
- 590 27. Bezombes C, Grazide S, Garret C, et al. Rituximab antiproliferative effect in B-lymphoma cells is  
591 associated with acid-sphingomyelinase activation in raft micodomains. *Blood.* 2004;104(4):1166-  
592 1173. doi:10.1182/blood-2004-01-0277

- 593 28. Huisken J, Stainier D YR. Selective plane illumination microscopy techniques in developmental  
594 biology. *Development*. 2009;136(12):1963-1975. doi:10.1242/dev.022426
- 595 29. Ichikawa T, Nakazato K, Keller PJ, et al. Live imaging and quantitative analysis of gastrulation in  
596 mouse embryos using light-sheet microscopy and 3D tracking tools. *Nat Protoc*. 2014;9(3):575-  
597 585. doi:10.1038/nprot.2014.035
- 598 30. Weber M, Mickoleit M, Huisken J. Multilayer mounting for long-term light sheet microscopy of  
599 zebrafish. *J Vis Exp*. 2014;(84):e51119. doi:10.3791/51119
- 600 31. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open source platform for biological image  
601 analysis. *Nat Methods*. 2012;9(7):676-682. doi:10.1038/nmeth.2019
- 602 32. Eliceiri KW, Rueden C. Tools for visualizing multidimensional images from living specimens.  
603 *Photochem Photobiol*. 2005;81(5):1116-1122. doi:10.1562/2004-11-22-IR-377
- 604 33. Sugiura G, Kühn H, Sauter M, Haberkorn U and Mier W. Radiolabeling strategies for tumor-targeting  
605 proteinaceous drugs. *Molecules*. 2014;19(2):2135-2165. doi:10.3390/molecules19022135
- 606 34. Agostinelli S, Allison J, Amako K, et al. Geant4—a simulation toolkit. *Nucl Instrum Methods Phys Res*  
607 *A*. 2003;506(3). doi:10.2172/799992
- 608 35. Allison J, Amako K, Apostolakis J, et al. Geant4 developments and applications. *IEEE Trans Nuc. Sci*  
609 2006;53(1):270-278. doi:10.1109/TNS.2006.869826
- 610 36. Allison J, Amako K, Apostolakis J, et al. Recent developments in Geant4. *Nucl Instrum Methods Phys*  
611 *Res A* 2016;835:186-225. doi:10.1016/j.nima.2016.06.125
- 612 37. Incerti S, Seznec H, Simon M, Barberet P, Habchi C, Moretto P. Monte Carlo dosimetry for targeted  
613 irradiation of individual cells using a microbeam facility. *Radiat Prot Dosim*. 2009;133(1):2-11.
- 614 38. Barberet P, Vianna F, Karamitros M, et al. Monte-Carlo dosimetry on a realistic cell monolayer  
615 geometry exposed to alpha particles. *Phys Med Biol*. 2012;57(8):2189-2207. doi:10.1088/0031-  
616 9155/57/8/2189
- 617 39. Howell RW. Radiation spectra for Auger electrons emitting radionuclides: Report No. 2 of AAPM  
618 Nuclear Medicine Task Group No 6. *Med Phys*. 1992;19(6) 1371-1383. doi:10.1118/1.596927
- 619 40. Taborda A, Benabdallah N, Desbrée A. Dosimetry at the sub-cellular scale of Auger-electron emitter  
620 <sup>99m</sup>Tc in a mouse single thyroid follicle. *Appl Radiat Isot*. 2016;108:58-63.  
621 doi:10.1016/j.apradiso.2015.12.010
- 622 41. Piroozfar B, Raisali G, Alirezapour B, Mirzaii M. The effect of <sup>111</sup>In radionuclide distance and Auger  
623 electron energy on direct induction of DNA double-strand breaks: a Monte Carlo study using  
624 Geant4 toolkit. *Int J Radiat Biol*. 2018;94(4):385-393. doi:10.1080/09553002.2018.1440329
- 625 42. Eckerman K, Endo A. *MIRD: Radionuclide data and decay schemes*. Reston, VA: Society for Nuclear  
626 Medicine; 2008.
- 627 43. Kassis AI. The amazing world of Auger electrons. *Int J Radiat Biol*. 2004;80(11-12):789-803.  
628 doi:10.1080/09553000400017663
- 629 44. Bernal MA, Bordage MC, Brown JMC, et al. Track structure modeling in liquid water: A review of  
630 the Geant4-DNA very low energy extension of the Geant4 Monte Carlo simulation toolkit. *Phys Med*.  
631 2015;31(8):861-874. doi:10.1016/j.ejmp.2015.10.087
- 632 45. Incerti S, Ivanchenko A, Karamitros M, et al. Comparison of GEANT4 very low energy cross section  
633 models with experimental data in water. *Med Phys*. 2010;37(9):4692-4708.  
634 doi:10.1118/1.3476457



- 635 46. Incerti S, Baldacchino G, Bernal M, et al. The Geant4-DNA project. *Int J Model Simul Sci Comput.*  
636 2010;1(2):157-178. doi:10.1142/S1793962310000122
- 637 47. Incerti S, Kyriakou I, Bernal MA, et al. Geant4-DNA example applications for track structure  
638 simulations in liquid water: a report from the Geant4-DNA Project. *Med Phys.* 2018;45(8):e722-  
639 e739. doi:10.1002/mp.13048
- 640 48. Bordage MC, Bordes J, Edel S, et al. Implementation of new physics models for low energy electrons  
641 in liquid water in Geant4-DNA. *Phys Med.* 2016;32(12):1833-1840.  
642 doi:10.1016/j.ejmp.2016.10.006
- 643 49. Bordes J. Low-energy electron transport with alternative physics models within Geant4-DNA code  
644 and radioimmunotherapy applications [dissertation]. Toulouse, France: Toulouse III-Paul Sabatier  
645 University; 2017.
- 646 50. Cullen DE, Hubbell JH, Kissel L, Laboratory LLN. EPDL97: the evaluated photon data library `97  
647 version. *Livermore, CA: Lawrence Livermore National Laboratory.* 1997;Report UCLR--50400 Vol.6-  
648 Rev.(5). doi:10.2172/295438
- 649 51. Ivanchenko VN, Incerti S, Francis Z, et al. Combination of electromagnetic physics processes for  
650 microdosimetry in liquid water with the Geant4 Monte Carlo simulation toolkit. *Nucl Instrum*  
651 *Methods Phys Res B.* 2012;273:95-97. doi:10.1016/j.nimb.2011.07.048
- 652 52. Perkins ST, Cullen DE, Seltzer SM. Tables and graphs of electron-interaction cross sections from 10  
653 eV to 100 GeV derived from the LLNL Evaluated Electron Data Library (EEDL), Z = 1--100.  
654 *Livermore, CA: Lawrence Livermore National Laboratory.* 1991;Report UCLR--50400 Vol. 31:21-24.  
655 doi:10.2172/5691165
- 656 53. Perkins ST, Cullen DE, Chen MH, Rathkopf J, Scofield J, Hubbell JH. Tables and graphs of atomic  
657 subshell and relaxation data derived from the LLNL Evaluated Atomic Data Library (EADL), Z= 1--  
658 100. *Livermore, CA: Lawrence Livermore National Laboratory.* 1991;Report UCLR--50400 Vol. 30.  
659 doi:10.2172/10121422
- 660 54. Loevinger R, Budinger TF, Watson EE. *MIRD primer for absorbed dose calculations.* New York:  
661 Society of Nuclear Medicine; 1988.
- 662 55. Bolch WE, Eckerman KF, Sgouros G, Thomas SR. MIRD Pamphlet No. 21: a generalized schema for  
663 radiopharmaceutical dosimetry—standardization of nomenclature. *J Nucl Med.* 2009;50(3):477-  
664 484. doi:10.2967/jnumed.108.056036
- 665 56. Ljungberg M, Sjögreen-Gleisner K. The accuracy of absorbed dose estimates in tumours determined  
666 by quantitative SPECT: a Monte Carlo study. *Acta Oncol.* 2011;50(6):981-989.  
667 doi:10.3109/0284186X.2011.584559
- 668 57. Marcatili S, Villoing D, Mauxion T, McParland BJ, Bardiès M. Model-based versus specific dosimetry  
669 in diagnostic context: comparison of three dosimetric approaches. *Med Phys.* 2015;42(3):1288-  
670 1296. doi:10.1118/1.4907957
- 671 58. Scheidhauer K, Wolf I, Baumgartl HJ, et al. Biodistribution and kinetics of <sup>131</sup>I-labelled anti-CD20  
672 MAB IDEC-C2B8 (rituximab) in relapsed non-Hodgkin's lymphoma. *Eur J Nucl Med Mol Imaging.*  
673 2002;29(10):1276-1282. doi:10.1007/s00259-002-0820-7
- 674 59. Gustafsson J, Brolin G, Cox M, Ljungberg M, Johansson L and Sjögreen Gleisner K. Uncertainty  
675 propagation for SPECT/CT-based renal dosimetry in <sup>177</sup>Lu peptide receptor radionuclide therapy.  
676 *Phys Med Biol.* 2015;60(21):8329-8346. doi:10.1088/0031-9155/60/21/8329
- 677 60. Gear JI, Cox MG, Gustafsson J, et al. EANM practical guidance on uncertainty analysis for molecular  
678 radiotherapy absorbed dose calculations. *Eur J Nucl Med Mol Imaging.* 2018;45(13):2456-2474.  
679 doi:10.1007/s00259-018-4136-7

- 680 61. Berger MJ, Coursey JS, Zucker MA, Chang J. ESTAR, PSTAR, and ASTAR: computer programs for  
681 calculating stopping-power and range tables for electrons, protons, and helium ions (version  
682 1.2.3). Gaithersburg, MD: NIST. 2005; Technical Report NISTIR 4999.  
683 <https://www.nist.gov/pml/stopping-power-range-tables-electrons-protons-and-helium-ions>.  
684 Accessed October 9, 2018.
- 685 62. Wilder RB, Jones D, Tucker SL, et al. Long-term results with radiotherapy for stage I-II follicular  
686 lymphomas. *Int J Radiat Oncol Biol Phys*. 2001;51(5):1219-1227. doi:10.1016/s0360-  
687 3016(01)01747-3
- 688 63. Bousis C, Emfietzoglou D, Hadjidoukas P and Nikjoo H. Monte Carlo single-cell dosimetry of Auger-  
689 electron emitting radionuclide. *Phys Med Biol*. 2010;55(9):2555-2572. doi:10.1088/0031-  
690 9155/55/9/009
- 691 64. Bousis C, Emfietzoglou D and Nikjoo H. Monte Carlo single-cell dosimetry of I-131, I-125 and I-123  
692 for targeted radioimmunotherapy of B-cell lymphoma. *Int J Radiat Biol*. 2012;88(12):908-  
693 915. doi:10.3109/09553002.2012.666004
- 694 65. Fourie H, Newman RT and Slabbert JP. Microdosimetry of the Auger electron emitting <sup>123</sup>I  
695 radionuclide using Geant4-DNA simulations. *Phys Med Biol*. 2015;60(8):3333-3346.  
696 doi:10.1088/0031-9155/60/8/3333
- 697 66. Duval K, Grover H, Han LH, et al. Modeling physiological events in 2D vs. 3D cell culture. *Physiology*.  
698 2017;32(4):266-277. doi:10.1152/physiol.00036.2016
- 699 67. Thoma CR, Zimmermann M, Agarkova I, Kelm JM and Krek W. 3D cell culture systems modeling  
700 tumor growth determinants in cancer target discovery. *Adv Drug Deliv Rev*. 2014;69-70:29-41.  
701 doi:10.1016/j.addr.2014.03.001
- 702 68. Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W and Kunz-Schughart LA.  
703 Multicellular tumor spheroids: An underestimated tool is catching up again. *J Biotechnol*.  
704 2010;148(1):3-15. doi:10.1016/j.jbiotec.2010.01.012
- 705 69. Sutherland R, Buchegger F, Schreyer M, Vacca A and Mach JP. Penetration and binding of  
706 radiolabeled anti-carcinoembryonic antigen monoclonal antibodies and their antigen binding  
707 fragments in human colon multicellular tumor spheroids. *Cancer Res*. 1987;47(6):1627-1633.
- 708 70. Minchinton AI, Tannock IF. Drug penetration in solid tumors. *Nat Rev Cancer*. 2006;6(8):583-592.  
709 doi:10.1038/nrc1893
- 710 71. Kyriakou I, Incerti S, Francis Z. Technical Note: Improvements in GEANT4 energy-loss model and  
711 the effect on low-energy electron transport in liquid water. *Med Phys*. 2015;42(7):3870-3876.  
712 doi:10.1118/1.4921613
- 713 72. Bordes J, Incerti S, Lampe N, Bardiès M, Bordage MC. Low-energy electron dose-point kernel  
714 simulations using new physics models implemented in Geant4-DNA. *Nucl Instrum Methods Phys  
715 Res B*. 2017;398:13-20. doi:10.1016/j.nimb.2017.02.044
- 716 73. Salvat F, Fernández-Varea JM, Sempau J. PENELOPE-2008: a code system for Monte Carlo  
717 simulation of electron and photon transport. *Issy-les-Moulineaux, France: OECD-NEA*. 2009; Report  
718 6416. <https://www.oecd-nea.org/science/pubs/2009/nea6416-penelope.pdf>. Accessed  
719 Decembre 1<sup>st</sup>, 2019.
- 720 74. Avila-Rodriguez MA, Rios C, Carrasco-Hernandez J, et al. Biodistribution and radiation dosimetry  
721 of [<sup>64</sup>Cu]copper dichloride: first-in-human study in healthy volunteers. *EJNMMI Res*. 2017;7(1).  
722 doi:10.1186/s13550-017-0346-4

- 723 75. Santoro L, Mora-Ramirez E, Trauchessec D, et al. Implementation of patient dosimetry in the  
724 clinical practice after targeted radiotherapy using [<sup>177</sup>Lu-[DOTA0, Tyr3]-octreotate. *EJNMMI Res.*  
725 2018;8(1):103. doi:10.1186/s13550-018-0459-4
- 726 76. Camacho X, Machado CL, García MF, et al. Technetium-99m- or Cy7-labeled rituximab as an imaging  
727 agent for non-Hodgkin lymphoma. *Oncology.* 2017;92(4):229-242. doi:10.1159/000452419
- 728 77. Stopar TG, Fettich J, Zver S, et al. <sup>99m</sup>Tc-labelled rituximab, a new non-Hodgkin's lymphoma imaging  
729 agent: First clinical experience. *Nuc Med Commun.* 2008;29(12):1059-1065.  
730 doi:10.1097/MNM.0b013e3283134d6e
- 731 78. Müller C, van der Meulen NP, Benešová M, Schibli R. Therapeutic radiometals beyond <sup>177</sup>Lu and <sup>90</sup>Y:  
732 production and application of promising  $\alpha$ -particle,  $\beta$ -particle, and Auger electron emitters. *J Nucl*  
733 *Med.* 2017;58(Supplement 2):91S-96S. doi:10.2967/jnumed.116.186825
- 734 79. Müller C, Domnanich KA, Umbricht CA, van der Meulen NP. Scandium and terbium radionuclides  
735 for radiotheranostics: current state of development towards clinical application. *Br J Radiol.*  
736 2018;91(1091):20180074. doi:10.1259/bjr.20180074
- 737 80. Kratochwil C, Bruchertseifer F, Rathke H, et al. Targeted  $\alpha$ -therapy of metastatic castration-  
738 resistant prostate cancer with <sup>225</sup>Ac-PSMA-617: dosimetry estimate and empiric dose finding. *J*  
739 *Nucl Med.* 2017;58(10):1624-1631. doi:10.2967/jnumed.117.191395
- 740 81. Robertson AKH, Ramogida CF, Schaffer P, Radchenko V. Development of <sup>225</sup>Ac  
741 radiopharmaceuticals: TRIUMF perspectives and experiences. *Curr Radiopharm.* 2018;11(3):156-  
742 172. doi:10.2174/1874471011666180416161908

743

## 744 **6 Acknowledgments**

745 We would like to express our gratitude to Prof. Steve Archibald (PET Research Centre, University  
746 of Hull) and Dr Jean-Luc Gerner (ESA/ESTEC, retired) for helpful advice. We thank Lise Teyssedre  
747 (ITAV/CNRS) for fruitful discussion of SPIM imaging.

## 748 **7 Conflict of interest statement**

749 The authors declare that there is no conflict of interest regarding the publication of this article.