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A model for optimizing delivery of targeted radionuclide therapies into resection cavity margins for the treatment of primary brain cancers

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5 May 2017Raghu Raghavan^{1,4}, Roger W Howell² and Michael R Zalutsky³¹ Therataxis, LLC, JHU Eastern Complex, Suite B305, 1101 E. 33rd St., Baltimore MD 21218, United States of America² Division of Radiation Research, Department of Radiology, New Jersey Medical School Cancer Center. Rutgers, The State University of New Jersey, 205 S. Orange Ave, Newark, NJ 07103, United States of America³ Department of Radiology, Duke University Medical Center, 311 Research Drive, Durham, NC 27710, United States of America⁴ Author to whom any correspondence should be addressed.E-mail: raghu@therataxis.com**Keywords:** CED, targeted radiotherapy, brain cancer, advection-reaction, infiltrating tumor, MNT, radionuclide**Abstract**

Radionuclides conjugated to molecules that bind specifically to cancer cells are of great interest as a means to increase the specificity of radiotherapy. Currently, the methods to disseminate these targeted radiotherapeutics have been either systemic delivery or by bolus injection into the tumor or tumor resection cavity. Herein we model a potentially more efficient method of delivery, namely pressure-driven fluid flow, called convection-enhanced delivery (CED), where a device infuses the molecules in solution (or suspension) directly into the tissue of interest. In particular, we focus on the setting of primary brain cancer after debulking surgery, where the tissue margins surrounding the surgical resection cavity are infiltrated with tumor cells and the most frequent sites of tumor recurrence. We develop the combination of fluid flow, chemical kinetics, and radiation dose models needed to examine such protocols. We focus on Auger electrons emitting radionuclides (e.g. ⁶⁷Ga, ⁷⁷Br, ¹¹¹In, ¹²⁵I, ¹²³I, ^{193m}Pt, ^{195m}Pt) whose short range makes them ideal for targeted therapy in this setting of small foci of tumor spread within normal tissue. By solving these model equations, we confirm that a CED protocol is promising in allowing sufficient absorbed dose to destroy cancer cells with minimal absorbed dose to normal cells at clinically feasible activity levels. We also show that Auger emitters are ideal for this purpose while the longer range alpha particle emitters fail to meet criteria for effective therapy (as neither would energetic beta particle emitters). The model is used with simplified assumptions on the geometry and homogeneity of brain tissue to allow semi-analytic solutions to be displayed, and with the purpose of a first examination of this new delivery protocol proposed for radionuclide therapy. However, we emphasize that it is immediately extensible to personalized therapy treatment planning as we have previously shown for conventional CED, at the price of requiring a fully numerical computerized approach.

1. Introduction

Molecularly targeted radiotherapy is an attractive alternative to treatment with conventional external beam radiation and consists of two components—the targeting vehicle and the radionuclide. The former consists of a molecule that binds specifically to cancer cells, imparting tumor selectivity, while the latter provides a radiation source within the body for delivering a cytotoxic level of DNA damage to cancer

cells. Although the vast majority of targeted radionuclide therapy investigations have been carried out with radionuclides decaying by β -emission, more recent efforts have focused on the use of radionuclides that emit α particles or Auger electrons (Vertes *et al* 2011). These type of particulate radiations are attractive for targeted radiotherapy because they are of higher linear energy transfer (LET) than β -emitters, providing greater cytotoxic potency. In particular, the short range in tissue of the electrons from the Auger

emitters minimizes irradiation of normal tissues adjacent to tumor tissue. In settings where avoiding radiation toxicity to normal tissues is of paramount importance, such as the treatment of malignancies within the central nervous system, Auger electron emitters deserve serious consideration because their range of these electrons in tissue is generally less than 200 nm (Reilly 2010). However, because of the very circumscribed range of Auger electrons in tissue, their cytotoxicity is critically dependent on the use of a targeting vehicle that can deliver the radionuclide in close proximity to DNA within the nucleus of the cell (Kassis *et al* 1990). A variety of approaches have been investigated to achieve nuclear localization of Auger electron emitters, among them ^{125}I -iododeoxyuridine, cis- and trans- $^{195\text{m}}\text{Pt}$, carbo- $^{193\text{m}}\text{Pt}$, and other small molecules (Howell *et al* 1992, 1994, Reischung *et al* 2008). Tumor-targeted macromolecules including antibodies and antibody fragments that bind to internalizing receptors have also been investigated extensively (Cornelissen and Vallis 2010). Our own efforts in this regard have focused on ~ 75 kDa modular nanotransporters (MNT), which are poly-functional vectors that bind to cell surface receptors and are transported to the cell nucleus. We have shown that an MNT composed with epidermal growth factor (EGF) as the ligand domain markedly enhanced the cytotoxicity of the Auger emitters ^{125}I and ^{67}Ga for human tumor cells that express EGF receptors compared with a series of controls including correspondingly labeled EGF (Slastnikova *et al* 2006, Koumariou *et al* 2014).

Glioblastoma (GBM) is the most aggressive form of primary brain cancer and is associated with a dismal prognosis. Current standard-of-care treatment—a combination of surgery, radiation, and temozolomide—provides a median survival of only about 14 months (Stupp *et al* 2005). Thus there is an urgent need for the development of more specific and effective strategies for the treatment of malignant primary brain tumors (Johnson and O'Neill 2012). The potential benefit for targeted radionuclide therapy for GBM has been demonstrated in studies in which antitenascin-C antibodies labeled with the β -emitter ^{131}I was directly injected into a surgically created resection cavity (SCRC) after tumor removal (Reardon *et al* 2007). Although significantly prolonged median survival of GBM patients was observed compared with standard of care, this loco-regional therapy would be of little benefit in addressing the diffuse, infiltrative nature of GBM, which is the major barrier to achieving durable tumor control (Burger and Kleihues 1989, Giese *et al* 2003).

Because of their subcellular tissue range, Auger electrons have a much lower cytotoxicity when decaying outside the cell nucleus, i.e. on the cell surface or in the extracellular space. We hypothesize that Auger electron emitting targeted radiotherapeutics conjugated with localizing agents such as MNT might be

well suited to the treatment of infiltrative GBM when delivered by convection enhanced delivery (CED) (Bobo *et al* 1994) because the limited range of action of Auger electrons should minimize absorbed dose to normal brain tissue where decays should occur outside the cell. Herein we describe a model to evaluate this possibility for a variety of Auger emitters in order to determine which radionuclides might be best suited for this purpose. Calculations were performed for 12 Auger electron-emitting radionuclides that differ with regard to parameters that influence their radiation dosimetry: (a) physical half life (T_p), (b) number of Auger electrons emitted per decay, (c) range of emitted electrons, and (d) emission characteristics of any multi-cellular range radiations including x-rays, γ -rays and β -particles and conversion electrons. Calculations also were performed for two α -particle emitters, ^{213}Bi ($T_p = 60$ min) and ^{211}At ($T_p = 7.2$ h) because both have been evaluated in clinical studies as loco-regional therapies for treatment of primary brain cancers (Cordier *et al* 2008, Zalutsky *et al* 2008).

The objective of this study was to identify a pressure-driven infusion protocol and radionuclide that, when administered into the SCRC, would deliver a potentially therapeutic absorbed dose to infiltrating tumor cells within the 2 cm cavity margin (assumed to account for about 1 in 10 cells in this margin; (Burger and Kleihues 1989) while delivering 10-fold lower absorbed dose to neighboring normal cells. For the purposes of model development, EGFR was selected as the molecular target based on the overexpression of this receptor on 40%–50% of GBM (Frederick *et al* 2000), MNT with EGF as the ligand domain was selected as the targeted radiotherapeutic based on encouraging results demonstrating its ability to deliver Auger electron emitters to the nucleus of GBM cells with high cytotoxicity (Slastnikova *et al* 2006, Koumariou *et al* 2014).

2. Methods

2.1. General considerations

The hypothesis we investigate is that through appropriate modeling, one can design and execute a protocol of a pressure-driven infusion of radionuclide in solution (CED, Bobo *et al* 1994) into the 2 cm margin surrounding a SCRC in a patient with primary brain cancer such that (i) the tumor cells in this margin (approximately 1 in 10 cells) are killed, while (ii) neighboring normal cells remain intact. While studies both theoretical and experimental have been performed extensively on antibody-conjugated delivery of radionuclides (Weinstein and van Osdol 1992, Akabani *et al* 2006, references therein, and others), these have been for systemically delivered radiolabeled molecules, or such molecules administered as a bolus, so that the subsequent spread of the radiolabeled molecule is by diffusion, binding, and chemical

kinetics. The equations involved have been of the reaction-diffusion type, (a subject which also has a large mathematical literature), applied to the description of radionuclide distribution and corresponding absorbed dose (Fujimori *et al* 1990, 1991). As far as we are aware, the current study is the first to examine a delivery protocol that involves CED to target the delivery of protein-conjugated radionuclides, exemplified herein by MNT. The equations are of reaction-advection type, which has been much less studied mathematically. CED of antibodies (without consideration of conjugation to radionuclides and the absorbed dose resulting therefrom) has been performed, as have been such infusions of large proteins for radiotherapeutic purposes (Luther *et al* 2008, Mehta *et al* 2012).

In order to design a protocol to deliver cytotoxic radiation absorbed doses to cancer cells infiltrating normal tissue, our strategy was to consider agents that bind specifically to tumor cells, and then internalize efficiently, ultimately localizing to the nucleus, the most radiation-sensitive cell compartment. We have been evaluating EGFR-targeted MNT for this purpose (Slasnikova *et al* 2006), and for this reason, have chosen this vehicle for development and application of our model. In previous experimental studies we examined Auger emitters (Howell 1992) because they are high LET radiations with a sub-cellular emission component, as well as some alpha emitters. We examine these in our model in this paper. Our objective was to examine the requirements on the infusion protocol such that the non-targeted radionuclides do not destroy normal cells while those internalized after EGFR binding do kill the tumor cells. Finally, the MNT concentration must be such that the outer edge of the 2 cm annulus surrounding the SCRC margin can be reached in a reasonable time (see below) and there must exist an infusion device and clinically acceptable procedure that can reliably deliver the radiotherapeutic for this purpose.

Infusions into brain parenchyma may be performed clinically under two different conditions: intratumorally with the primary contrast-enhancing tumor present; or after the tumor has been removed and infusion is performed into the margins of the fluid-filled SCRC remaining after surgery. There are many variations of each of these scenarios: for example, the infusion can be through multiple catheters. In any case, the presence of a tumor with its attendant inhomogeneities in blood-brain barrier (BBB) leakiness and intratumoral interstitial pressures, makes the modeling more complex. So we shall begin with the simpler model of infusion into the margins occurring *after* the primary tumor mass has been resected. The margins upto 2 cm in from the cavity walls are where the tumor recurs in about 85% of cases following primary tumor resection in malignant GBM (Wong *et al* 2006). The approach that we study in this paper is to infuse these margins with active molecules that bind

to residual tumor cells assumed to comprise about 1:10 cells in the 2 cm annular region (Burger and Kleihues 1989). We assume for simplicity that binding occurs only to tumor cells. Variations on these assumptions can easily be incorporated into the model as we obtain experimental authority to improve these estimates. As stated, our goal is to develop a protocol that ensures that the absorbed dose of radioactivity is safe for normal cells and lethal for tumor cells within the 2 cm region surrounding the SCRC. We make further simplifying assumptions in our analysis to obtain solutions with a minimum of numerical or computer simulations. To avoid misunderstanding, we emphasize that these simplifying assumptions can be lifted at the expense of having to use a purely numerical software computation, and that we have already the basis for such computations (Raghavan and Brady 2011). Our purpose in this paper is to set up the mathematical framework to examine such issues, to examine feasibility of this potential targeted radiotherapy treatment method, and to suggest specific radionuclides for the purpose; we can later extend the methods to include more realistic assumptions.

The model we use is illustrated in figure 1. We shall assume complete spherical symmetry in this paper: considering the brain as a sphere of radius $b \approx 7$ cm, the sphere then having a volume roughly that of the human brain. The resection cavity is a concentric sphere at the center with a radius $a = 2$ cm. (Obviously, the calculation can be carried out with variations on these numerical values.) Infusion is carried out from within the cavity outward uniformly across the cavity wall, with a total flow rate Q . As a matter of fact, the flow rate will play no role in our calculations. What we assume for the most part is that the infusion is carried out for 400 min. We do vary this number to show the effects of much longer infusions: shorter infusions will be *more* efficacious as we shall discuss later. In separate work we are creating the requisite devices to ensure uniform infusions into the margins from within the cavity at a flow rate that reaches the outer margin 2 cm away from the cavity wall (as shown in the blown up version top center in figure 1). We should point out that current practice in clinical trials is *not* to employ such infusions, but rather, generally utilizes several catheters placed directly into the peritumoral interstitium (the tissue in the margins) (Mehta *et al* 2012). We return to this point in Discussion (second paragraph). Our goal is that the absorbed dose to normal cells over the course of the radioactivity residence time is less than D_{safe} while those to the tumor cells is at least D_{lethal} where we specify these limits below. Cells at the wall of the cavity are clearly exposed to the radioactivity for the longest time, since the infusion begins there. Thus we need to evaluate the absorbed dose to normal cells at the infused concentration of radioactivity, which we further assume to begin at a given activity concentration to examine the

conditions under which it stays less than D_{safe} . On the other hand, the absorbed dose at the outer margin where the radius is $a_+ = a + 2 \text{ cm}$ is clearly least exposed to radioactivity both because the exposure commences only at the end of the infusion time by definition and also because the initial radioactivity has decayed due to the physical half-life of the radionuclide. Thus we need to evaluate the absorbed dose to tumor cells at this location to check if it is greater than D_{lethal} . We therefore compute the absorbed doses (to both normal tissue and tumor cells) at these two extremities of the tissue margins.

There are two components to a model for calculating the radiation absorbed dose upon convective delivery. One part of the calculation is that of the CED process to compute the concentration of the MNT: free, bound (to the cell membrane), and internalized. We assume that cells are spheres with the nucleus an inner sphere (right side of figure 1). These, and other parameters and variables are defined in table 1. For the full set of calculations involving all the radionuclides under consideration, we shall fix the affinity and binding kinetics to have the parameters listed in table 1. We also introduce the fraction of ‘hot’ MNT (the MNT molecules that have a radioactive atom attached divided by the total number of MNT molecules) as f and the mean number of radionuclides that each ‘hot’ MNT carries as m . Throughout we choose $m = 1$, and the results that will be presented in

subsequent tables will be for $f = 0.01$, so that 99% of the MNT are cold, and required to saturate the binding sites (see discussion on the retardation factor below).

To indicate what these numbers mean for the number of binding sites on a cell for example, we recall that the number of binding sites per unit interstitial volume (see table 1 above) is $1 \mu\text{m}$. (The reason for this choice of reference volume is explained below.) Further as the table indicates, we take the fraction of total tissue volume that is interstitial to be $1/5$ so the cells occupy $4/5$ ths of the available tissue space (we ignore the small fraction $\sim 5\%$ that is occupied by the blood vessels). Thus the number binding sites per cell = $\# \text{ Binding sites l}^{-1} \div \# \text{ cells l}^{-1}$; and the latter is: (available volume for cells in a liter in cubic microns)/(volume of cell in cubic microns). Using the data in the table, our assumption thus becomes:

$$\begin{aligned} & \# \text{ Binding sites per cell} \\ &= \frac{\text{number of binding sites per liter of tissue}}{\left(\frac{\# \text{ cells per liter of tissue}}{\frac{4}{3} \times \pi \times \left(\frac{9}{10^4} \right)^3} \right)} \approx \frac{1}{2} \times 10^6. \quad (1) \end{aligned}$$

Thus not only is our assumption consistent with the same concentration assumed in table 2 of (Weinstein and van Osdol 1992) but it is equivalent, with other

Table 1. Variables and parameter used in the model.

Symbol	Meaning	Value
MNT	Modular nanotransporters	—
a, a_+	Radius of resection cavity/tumor, of outer cavity margin	2 cm, 4 cm
b	Outer radius of brain	7.5 cm
k_1	Linearized binding rate constant of MNT	$10^4 - 1.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$
k_2	Linearized unbinding rate constant of MNT	$1-3 \times 10^{-5} \text{ s}^{-1}$
K_{irr}	Irreversible endocytosis rate constant of MNT	10^{-5} s^{-1}
B	Number of binding sites/interstitial volume, assumed uniform in tissue	1 μm
Q	CED volumetric infusion rate	‘arbitrary’-see text
K_{trans}	Loss rate of MNT through capillaries	10^{-6} s^{-1}
ϕ	Interstitial volume fraction of peritumoral margin	0.2
$R_C (R_N)$	Radius of cell (nucleus)	9(7) μm
	Receptors per cell (from B, R_C, ϕ)	$\frac{1}{2} \times 10^6$
	Infusion volume = $\phi \times$ volume of margin	$\sim 50 \text{ cc}$ (equation(2))
T_i	Infusion time	400 min
D_{safe}	Absorbed dose considered safe for normal cells	$\sim 4 \text{ Gy}$
D_{lethal}	Absorbed dose considered lethal for tumor cells	$\sim 40 \text{ Gy}$
f	Fraction of MNT that are radiolabeled	0.01
m	Mean number of radionuclides per MNT	1.0
ϕ	Extracellular volume fraction	0.2
A_1	Rate constant used for non-dimensionalizing time	9 min^{-1} (equation (11))

parameters having the values just discussed, to there being about $\frac{1}{2} \times 10^6$ MNT binding sites per cell as just shown. Moreover, this is consistent with, for example, table 1 in (Akabani *et al* 2006), in which the numbers of binding sites per cell are in the order of 10^6 , based on prior experimental determination of binding site concentrations.

In table 2, we list some of the symbols we have used and indicated their meaning and where they are defined. In particular the rate constants and the concentration functions we calculate should be clearly understood to be dimensionless in the way specified by the text and indicated by the table. For example, as will be seen below, we initially define X as the concentration per unit reference volume (where the reference is the interstitial space) as a function of radial position r and time t . Subsequently, and thereafter and in all the numerical calculations, we redefine it to be non-dimensional by dividing by B (see previous table) and also we introduce the independent spatial variable to be ρ which will be defined in equation (7). Thus X is a quite different function of its arguments than it was. We carefully explain this in the text, so no confusion should be caused by this ‘overloading’ of the symbols c, b, X . The Summary in the next section will re-emphasize these points.

Besides computing the CED process resulting in the distribution of the MNT, the other component to the calculation computes the absorbed dose (energy per unit volume) to regions of a cell from radiations emitted by the radiolabeled MNT. The two models and computations can be treated separately and then brought together to complete the calculation. We do these in the next three sections. Most of the nuclides we consider are Auger emitters of potential interest for targeted radiotherapy and two clinically relevant alpha particle

emitters are also considered: ^{213}Bi and ^{211}At (which also emits Auger electrons). While the absorbed dose due to particulate radiations are described by using the details of the convective process and cellular dosimetry, we estimated the gamma ray contribution—with its much longer range—differently and in a worst case scenario: we expect a more careful calculation will yield smaller values for the gamma contributions. The methods are described below. Table 3 lists the radionuclides we have considered in this paper along with their physical half lives and the infusion activity (given to the nearest integer in MBq) at the start of the infusion. As noted above, we have fixed the starting concentration of the MNT as well as the fraction that are hot: then the activities are simply inversely proportional to the physical half life of the radionuclides.

We explain in detail how the activities were computed for the table. The activity quoted (in MBq) is just the $10^{-6} \times$ number of disintegrations per second in the infusion vial, where we have assumed a fresh supply of nuclides (this can obviously be corrected for disintegration during transport to the surgical suite). The number of radionuclides in the vial is then the fraction (0.01) of the MNT that are hot. In turn the concentration of MNT as noted above is the same as the binding site concentration. So we need to compute the volume infused and multiply that by the concentration to obtain the numbers. Now, the volume we are required to fill (called the volume of distribution) is the volume of the 2 cm margin which surrounds the tumor of radius 2 cm. However, in an ideal infusion with the infusate in the interstitial space, we will need only 1/5 of that volume of infusion to fill that. This assumption is not correct, and as will be explained later, *for the baseline values of the various infusion and MNT kinetic parameters in the table*, we will need to multiply this by

Table 2. Symbols used in the model.

Symbol	Meaning	Where defined
R_S	Source of radioactivity: cell nucleus, cell surface, or interstitium	Section 4
V_I	Volume of interstitial space per cell	Section 5.1
V_N	Volume of cell nucleus	"
V_B	Effective volume for absorbed dose from radionuclide conjugated to bound MNT	"
S_{eff}	Effective S Value	"
S_{self}	Self-dose S value	Section 4
S_{cross}	Cross-dose S value (out to 100 μm)	"
ξ	Fraction of cells in margin that are cancerous	0.1
ρ	'Rectified' radial position: really the time required to reach a given position	Equation (7)
$\alpha_{1,2}; \kappa_{1,2}; \beta$	Dimensionless versions of rate constants and decay coefficients	Equations (11)–(13)
$c(x, t)$	Non-dimensionalized concentration of free MNT at place x in brain at time t	"
$b(x, t)$	Non-dimensionalized concentration of bound MNT at place x in brain at time t	"
$X(r, t)$	Non-dimensionalized concentration of internalized MNT	Equation (14)

Table 3. Radionuclides, their half-lives, and their initial activities in the infusion volume.

Nuclide	Half-life (h)	Infusion activity (MBq)
^{51}Cr	664.8	36
^{67}Ga	78.24	306
^{77}Br	56	427
$^{99\text{m}}\text{Tc}$	6	3985
^{111}In	67.92	352
^{119}Sb	38.2	626
^{123}I	13.2	1811
^{125}I	1442.4	17
$^{193\text{m}}\text{Pt}$	73	328
$^{195\text{m}}\text{Pt}$	103.92	230
^{201}Tl	73	328
^{203}Pb	51.9	461
^{213}Bi	0.76	31463
^{211}At	7.2	3321

a factor of about 2.2 (see remark at the end of section 3). Thus, finally multiplying this number by the decay rate to obtain the number of decays per second, and putting it all together, we get

$$\text{Activity} = 10^{-6} \times \left(\frac{\text{decay rate in s}^{-1}}{\frac{T_{1/2}}{\ln 2} \times 3600} \right)^{-1} \times 0.01 \times \frac{\text{hot MNT per } l}{\text{volume of infusion}} \times 10^{24} \times 10^{-6} \times 0.2 \times \frac{\frac{4}{3} \times \pi \times (4^3 - 2^3) \times 0.2}{1000} \quad (2)$$

$$\times \frac{\text{factor, see Remark, end of section 3}}{2.2} \quad (3)$$

All the calculations below are linear with regard to radionuclide concentration *provided the MNT concentrations are kept fixed*. (The results are quite nonlinear in the latter.) So, if we wish to adjust the absorbed dose up or down based on the results developed in this paper, all we have to do is alter the fraction of MNT that are 'hot' (i.e. the specific activity). Thus, in the case of Ga-67, if we desire an activity of 30 MBq based on the resultant absorbed dose to the tissue (see later), we

would lower the fraction of hot MNT by a further factor of 10. To repeat, if we lowered the concentration of the MNT itself by a factor of 10, the results would be entirely different and in fact, such a choice will be a total failure as we shall describe later.

3. Convective delivery

Models of convective transport have been widely used (Morrison *et al* 1994, Raghavan and Brady 2011), and generally take the form

$$\frac{\partial}{\partial t} c(\mathbf{x}, t) = \text{Advection} + \text{Diffusion} + \text{Loss} + \text{Binding Kinetics}, \quad (4)$$

where $c(\mathbf{x}, t)$ is the concentration of *free* MNT per unit *interstitial* volume at place $\mathbf{x}$ in the brain (in our case) and time t . We shall give the mathematical form of the right-hand side below. (The references cited may be consulted for a more complete treatment and derivation.) Advection is the process by which the MNT molecule is carried by the fluid during the phase where there is infusion: this rate may be that of the fluid velocity in which case there is no *physical* retardation due to the size or physical adsorption of the MNT, or it may be lower due to size and other physical effects. For simplicity, we shall neglect diffusion as being of little account. For MNT, antibodies, or similar large proteins, this should be a good approximation. The term denoted *Loss* accounts for losses through the capillary walls; this will be usually small in the intact BBB presumed to be present in the peritumoral region. Finally, unlike in usual modeling treatments of CED, we consider binding kinetics, which are critical in our application. We simplify the chemical kinetic phenomena into a reversible exchange between the free and bound forms of the MNT, the latter meaning: bound to the cell membrane and hence capable of dissociation. In addition, we assume a certain rate of irreversible endocytosis or internalization of the bound form resulting in transport into the interior of the cell, indeed into the nucleus as a consequence of the MNT nuclear

localization sequence module. Denoting the concentration of the bound form in the interstitium by b , we then can write the equations in the form, (see the table 1 for the meaning of the coefficients),

$$\frac{\partial}{\partial t}c(\mathbf{x}, t) + \mathbf{v} \cdot \nabla c = -a_1c + k_2b - k_1(B - b)c, \quad (5a)$$

$$\frac{\partial}{\partial t}b(\mathbf{x}, t) = k_1(B - b)c - a_2b, \quad (5b)$$

$$a_1 := \nabla \cdot \mathbf{v} + K_{\text{trans}}, \quad (5c)$$

$$a_2 := k_2 + K_{\text{irr}}. \quad (5d)$$

If we compare these equations with the standard derivation in the references cited, it will be noticed that (i) all reaction rates are on an interstitial volume basis, and (ii) the velocity is also an ‘interstitial velocity’, i.e., it is the Darcy velocity divided by the interstitial volume fraction ϕ . As mentioned, the internalization process is assumed here to be one-way, i.e., there is no recycling of the radiolabeled MNT (or radiolabel itself if MNT is degraded), receptor mediated or otherwise, back to the cell surface. Denoting the number per unit interstitial volume of the internalized molecules to be $X(r, t)$ ⁵, the equation describing this (again with space and time arguments suppressed) is

$$\frac{dX}{dt} = K_{\text{irr}}b \implies \quad (6a)$$

$$X(r, t) = K_{\text{irr}} \int_0^t d\tau b(\mathbf{x}, \tau) \quad (6b)$$

because at the start of the infusion there are no internalized molecules. So, once we have computed b, c we can obtain the concentration of the internalized molecules by quadrature because we have decoupled this variable by our assumption of no recycling. The above equations pertain while the infusion persists ($0 \leq t \leq T_I$). After it ceases, the convection ceases as well, so we have the above equations without the convective term, as long as we continue to neglect diffusion. These equations are even simpler to solve. The integration for X is then extended out to infinite time, the result staying finite since b will at such times be exponentially decreasing. For the calculations in this paper, we further make the assumption of complete spherical symmetry.

Remark 1. Based on the specific activity (the radioactivity per unit mass in units of Bq kg^{-1} , for example), of labeled proteins generally encountered in radionuclide therapy (Vertes *et al* 2011), we expect (see below) that only a fraction of the MNT molecules will be conjugated with the radionuclide. Thus a fraction f of the concentrations computed will be radioactive and there is a further decay rate involved with these which will be accounted for, later. Also at equilibrium, $b/B = c/(c + K_d)$ where $K_d := k_2/k_1$ is the

dissociation constant. For the parameters we have chosen, the ratio is essentially unity. We also note the coefficient in front of the nonlinear term dominates the other reaction rates, so that the nonlinearity cannot be considered a perturbation. Unfortunately, neither can separation of time scales be justified, which would have allowed a fast kinetics to be treated separately from a slow advection.

3.1. Alternative forms

The equations (5a) and (5b) can be written in a number of alternative forms for different purposes. We describe these: a summary at the end of this section describes the meaning of the symbols in the form we use for calculation. We first focus on the advective flux $\mathbf{v}$ that by assumption here is both steady and entirely radial, the speed then being denoted $w(r)$, which can take various forms depending on the hydrodynamic and oncotic properties of the interstitial medium. It is simpler to change units to remove this from the equations, or rather, so that the radial speed is unity for all distances (in reality of course, the speed decreases with distance from the center roughly as $1/r^2$). In mathematical parlance, this is called ‘rectifying the (velocity) vector field’. Then defining the r -dependent time ρ required to reach a radial position r

$$\rho := \int_a^r dr'/w(r') \quad (7)$$

allows us to replace

$$\mathbf{v} \cdot \nabla \Leftarrow \partial/\partial \rho \quad (8)$$

the arrow indicating the direction of the replacement. We henceforth use the new independent variables t, ρ . It is just as easy to read off a solution at a desired spatial point when given as a function of ρ if we also provide the graph of ρ as a function of spatial position. (In certain simplified cases, we can provide analytic solutions directly as a function of r but because we do not need these for our current objective, we do not exhibit these.) It should be noted that b, c are of course now different functions of the new pair of variables t, ρ : we shall abuse notation and use the same symbols b, c to mean the new functions. No confusion should arise since the context should make clear what the independent variables are. The boundary/initial value conditions are that

$$c(t, \rho = 0) = C_0, T_I \geq t > 0; c(t = 0, \rho > 0) = 0, \quad (9)$$

$$b(t < \rho) = 0 \quad (10)$$

and we seek non-zero solutions within the sector $t \geq \rho > 0$. The limit at the boundary for c is only ‘right continuous’, and is discontinuous with the zero solution that one obtains for times before the infusion begins. In other words, the boundary conditions state that there is no MNT in tissue (bound or free) prior to the start of the infusion, which is taken to be time zero. The infused concentration is then held constant for

⁵ Admittedly this is an odd reference volume to use for an internalized molecule which is assumed in the nucleus and not in the extracellular space, but it does avoid introducing the interstitial volume fraction ϕ into the formulas at this point.

the duration of the infusion. Henceforth we further redefine the variables to non-dimensional form as follows. The concentrations c , b are measured in units of B , and the rate constants in units of

$$A_1 := a_1 + k_1 B \quad (11)$$

and times in units of $1/A_1$. Then

$$\frac{\partial}{\partial t} c(\rho, t) + \frac{\partial}{\partial \rho} c(\rho, t) = \kappa_2 b - \beta(1 - b)c - \alpha_1 c, \quad (12)$$

$$\frac{\partial}{\partial t} b(\rho, t) = \beta(1 - b)c - \kappa_2 b - \alpha_2 b, \quad (13)$$

where $\alpha_1 := a_1/A_1$, $\beta := k_1 B/A_1$ and similarly for all the rate constants. In these units, $b \leq 1$ always, and strictly speaking the factor $1 - b$ in the equations above should be replaced with $1 - b$, $0 \leq b \leq 1$, 0 otherwise. Note the continued abuse of notation for b , c . (We could as well have measured the concentration in units of C_0 , the initial concentration, which may be advantageous in certain circumstances.) Obviously, equation (6b) should then be rewritten

$$X(\rho, t) = \frac{K_{\text{irr}}}{A_1} \int_0^t d\tau b(\rho, \tau). \quad (14)$$

Here again, we are renaming the function: X is henceforth rendered dimensionless by dividing by B and is also a different function of its arguments, and is defined by (14).

Summary 2. All quantities are dimensionless in the equations (12) and (13) that describe the CED process for MNT delivery. The concentrations c , b of the free and bound MNT, respectively, are measured in units of B , the number of binding sites per unit interstitial volume. The times t , ρ are measured in units of A_1^{-1} , where A_1 , given by equation (11) is a rate which evaluates to about 9 min^{-1} with the numbers in table 1. The time ρ is really a measure of the distance reached by the fluid in the time ρ . (Thus the actual distance is a very nonlinear function of ρ , increasing like $\rho^{1/3}$ in the absence of losses.) With our assumptions of spherical symmetry, there is a unique correspondence between the radial distance of the place reached by the MNT and time. All the reaction rates are measured in units of A_1 . As discussed below, the initial concentration of MNT is set equal to the concentration of the total number of binding sites, assumed to be uniform throughout the 2 cm SCRC margin.

We shall in a separate paper discuss the behavior of the equations (12) and (13) in detail. Both for numerical stability and for theoretical purposes, it is better to transform the independent variables to $s := t - \rho$ and $y = \rho$ because that will transform into coordinates that move with the advective current. However, in this paper we shall retain the variables shown since they are easier to interpret visually. We also point out that the steady-state solutions are readily obtained. Setting the right-hand side of (13) to zero gives us $c(\rho, t \rightarrow \infty)$ in terms of $b(\rho, t \rightarrow \infty)$, which, then,

substituted into (12) will give us an implicit equation for $b(\rho, t \rightarrow \infty)$ by quadrature. It should be noted that

$$K_{\text{irr}} \int_0^\infty b(\rho, t \rightarrow \infty) = c_0 \quad (15)$$

since the right-hand side is just the flux of particles entering the tissue (recall our remarks on the definition of ρ). We do not pause to describe these solutions but content ourselves with solving the equations as given above with one of the methods provided in MathematicaTM, and display direct the solutions of the above equations. We performed our calculations in Mathematica Version 10, and we used the program NDSolve to compute the solutions to both b , c from the simultaneous equations given above, with the boundary conditions as stated. For the parameter values chosen in table 1, and for infusions that last just a few hours (we have chosen 400 min for the infusion in most of the results presented), the loss of free MNT due to transcapillary transport is negligible. Then c will rise to the infused value and b , the fraction of the available binding sites that are bound, will rise to one eventually. On the other hand, the time at which a substantial fraction of the MNT infused reaches a given location is quite different from ρ . In the cases of bolus and systemic delivery of antibody-conjugated radionuclides, Weinstein and his collaborators have extensively studied the effects of this, calling it, evocatively, the ‘binding site barrier’, (van Ossdol and Weinstein 1991, Weinstein and van Ossdol 1992, Saga et al 1995). However, at least in the CED context (see appendix A of Morrison et al 1994), it is invariably treated from a linearization of the equations, resulting in an incorrect picture of the phenomenon. The nonlinearity plays an essential and important role. In figure 2(a), we show the concentration of free and bound radionuclides as a function of time and one may see the behavior postulated above. Figure 2(b) on the right shows the internalized MNT concentration: by our assumptions these never decay although of course the radionuclide conjugated to the MNT will be subject to radioactive decay. The original free and bound concentrations shown in figure 2(a) are also displayed in the figure as the much smaller magnitude curves.

In order to illustrate the retardation factor and the nonlinear behavior with initial concentration, we present figure 3. The main figure displays the relative concentration as a function of time at a particular place. This place has been chosen such that the fluid particles reach there from the inner boundary of the cavity at radius a in five units of time. It is seen that when the infused concentration of MNT is ten times the binding site concentration, then essentially there is no retardation of convection of the MNT. On the other hand when the initial concentration is only 1/10 of the binding site concentration, there is a strong binding site barrier, and the concentration reaches its peak value only after a time about 10 times as long. This factor which varies between 1 and ∞ is displayed in the inset of figure 3 as a function of the infused

(a) Free and membrane-bound MNT concentration with time.

(b) The internalized MNT concentration along with the previously displayed concentrations.

Figure 2. Concentration of free, bound, and internalized MNT as a function of time. Note that the concentration is per liter of interstitial volume: to obtain the number of moles per liter of total tissue volume, one must multiply by 0.2: see text for discussion.

Figure 3. The retardation factor as a function of initial MNT concentration (inset) as derived from the concentration rise at a particular place as a function of time in arbitrary units (main illustration). This shows that the retardation factor and the binding site barrier are both due to nonlinearities in the differential equations.

concentration (in units of binding site concentration). As mentioned above, we have chosen the infused concentration of MNT to be equal to B , so that the retardation factor is about four.

Remark 3. We also note that, for the values chosen for the coefficients and the boundary values in the equations, we have a total concentration of about 2.2 (in units of binding site concentration) for most of the time. Thus to obtain the final absorbed dose to target as obtained in the calculations below, we must allow for this factor in the activity of the infusate listed in table 3.

4. Absorbed dose: S-values

In this section, we discuss the calculation of S-values (n.d.a.). This is the first step in integrating the calculation above with energy deposition associated

with the radiations emanating from the radionuclides to obtain the radiation absorbed doses. Nothing in this section is new from a radiation dosimetry perspective, the material being a summary principally taken from (Howell and Rao 1989, Goddu *et al* 1994a, 1994b) as well as other references that will be cited. However, this discussion is included here to make the paper understandable to the non-specialist in radiation dosimetry or nuclear medicine, just as, we hope, the material on the convective delivery of MNT was understandable to the non-specialist in biomechanics and fluid flow. Of course, one must consult secondary and primary sources for the justification and derivation of the formulas we use here. An S-value, specific to a radionuclide, is the energy per unit mass that is deposited in target region R_T per disintegration of a radionuclide in source region R_S ; we may write this symbolically as $S(R_T \leftarrow R_S)$. Thus to compute the energy deposited at a place we need the S-value as well

as the number of disintegrations that occur at the place R_S over time, and to integrate over all source locations R_S . We are interested in total absorbed doses to volumes (cytoarchitectural regions), and here we assume to a good enough approximation that the density of tissue is 1 g ml^{-1} to allow us to convert mass to volume. This energy is also a sum over all the radiations emitted by the radionuclide which can include charged particles such as electrons and alpha particles, as well as gamma and x-ray photons. In our study, radionuclides can be either be bound to the cell surface membrane, translocated to the nucleus of a cancer cell (we assume for simplicity that all radionuclides that become internalized are immediately transported from the cytoplasm to the nucleus), or free in the interstitial space: these are the various source regions R_S . The nucleus of the cell is the most radiation sensitive region and thus it is considered to be the target R_T as is usually done in dosimetry calculations performed at the cellular level. We assume that if we avoid destruction of the nucleus, the rest of the cell will be viable: and that destroying the nucleus will destroy the cell. We recognize that this may be an oversimplification as studies have shown that cytoplasmic irradiation can be modestly radiotoxic (Wu *et al* 1999). In order to compute if the normal tissue will be spared, we further localize the target R_T to be at the wall of the resection cavity itself (since that is the worst case scenario for our evaluation as mentioned before). On the other hand, to evaluate if we have sufficient energy to destroy all the tumor cells within the 2 cm SCRC margin, we choose the place R_T now to be the outer margin, which in this study we have chosen to be 2 cm beyond the outer boundary of the SCRC wall, and exposed to the infusate for the least time within the target region. We will also give the absorbed doses with these places switched, i.e., evaluating the absorbed dose to a tumor cell at the SCRC wall and a normal cell at the outer margin—these may be considered ‘best case’ scenarios.

Remark 4. We now make some important remarks to avoid confusion about how we use spatial as well as temporal information, and some of the approximations involved.

In the previous section a location r , or its ‘rectified’ version ρ , has been treated as a continuous quantity. However, in treating the tissue as a continuum, we are obviously smoothing over large numbers of cells, and so in the calculations below, the location of a cell nucleus, cell surface, or a neighboring cell will all possess the same concentration value coming from the calculations above for the MNT. In other words, while we use a more microscopic lens in computing the cellular S -values (see below), when it comes to integrating with the MNT calculation, the coordinate of place is the same for all the sources for a given target. The furthest we go out to in calculating absorbed doses deposited to a

given cell is about $100 \mu\text{m}$. The resolution for the concentration calculations (say a mm) has therefore to be considerably coarser than this for this approximation to be justifiable. (These concentrations of course depend on whether we are considering free, bound, or internalized MNT, but not within a $100 \mu\text{m}$ neighborhood of any location.) The calculation for photons does *not* assume this because they have considerably longer range that results in the traversal of many resolution elements of, say, an MR image (mm), so that source and target may have quite different macroscopic coordinates. However, we do acknowledge that neither the cellular S -value calculations nor the organ S -value calculations completely capture the cases where the range of the particle emissions are in the intermediate range. Thus ^{111}In for example has a large number of conversion electrons with ranges of $200 \mu\text{m}$ or more. Such ranges require, in our opinion, new methods for effective evaluation of the S -values intermediate between the methods in use for the organ and the cellular levels. The method we use for summing up the contributions from cells within a certain range becomes increasingly inaccurate with increasing range. Further, such intermediate ranges more or less invalidate the separation of scales that we are using just mentioned. The time that it takes for the infusion to traverse a $\frac{1}{2}$ mm can be of the order of an hour, which means that neither the same spatial value nor the same time value of the concentration can be used over the entire range. The standard *Mathematica* routines we have used here are not stable enough to support this. We postpone to the future the development of better methods than we have used here to sum the space-time dependent concentration weighted by the appropriate S -values to obtain a total absorbed dose for such intermediate range particles. We will not be unique in ignoring conversion electrons of larger range: for example, a recent evaluation of such Auger emitters carries the calculations out to only $30 \mu\text{m}$ (Falzone *et al* 2015). In the Discussion, we return to this issue and estimate the errors involved and their impact on the conclusions we obtain.

We also point out another approximation we have made. To obtain order of magnitude estimates for the cellular S -values out to the $100 \mu\text{m}$ range mentioned, we assume all cells are equally sized spheres with radius of $R_C = 9 \mu\text{m}$ (Akabani *et al* 2006) and closely packed in the usual face centered cubic lattice configuration. This simplifies these calculations considerably since otherwise we would have to account for the microscopic distribution of cell sizes and shapes in the brain of the particular patient—clearly an impossibility. An alternative, namely a random packing of cells with perhaps varied shapes would involve a far more numerically heavy calculation with perhaps no better accuracy of approximation to the real disposition of the cells. However, in keeping with all of the literature on CED and on interstitial spaces, we

assume that the interstitial space is 20% (Syková *et al* 1994). This number comes into some of the calculations (see below). Cells are neither rigid nor spheres and are easily deformed in shape, therefore the actual packing density of cells is in fact higher than the maximum allowed for spheres of equal fixed size (about 26% for the void space). Thus although it is inconsistent to assume the 20% fraction along with that of fixed volume spheres, we tolerate this inconsistency, and the symmetric disposition of the spheres allows for easier calculations. The overall error introduced by this inconsistency is far less than some of the other approximations introduced. The nucleus is considered as a $R_N = 7 \mu\text{m}$ sphere within and concentric to the cell.

We resume our main thread of calculations. The radionuclides under consideration have been listed in table 3. The S-value for a radionuclide is usually expressed as

$$S(R_T \leftarrow R_S) = \frac{1}{M_T} \sum_i \phi_i(R_T \leftarrow R_S) \Delta_i, \quad (16)$$

where M_T is the mass of the target, $\phi_i(R_T \leftarrow R_S)$ is the fraction of energy emitted from the source R_S that is absorbed in the target R_T for the i th radiation component of the radionuclide, and Δ_i is the mean energy emitted per disintegration for that component. The absorbed fraction $\phi_i(R_T \leftarrow R_S)$ depends on the geometry of the source and target regions as well as the type and energy of the radiation component, because the emitted charged particles and photons deposit their energies in different manners and over different distances.

Cellular S-values for charged particles

Given a decay mode with charged particle emission, the absorbed fraction is written as

$$\phi_i(R_T \leftarrow R_S) = \int_0^\infty \psi_{R_T \leftarrow R_S}(x) \cdot \frac{1}{E_i} \frac{dE}{dx} \Big|_{X(E_i)-x} dx \quad (17)$$

where E_i is the initial energy, $\frac{dE}{dx}$ and $X(E_i)$ are the stopping power and the range with given E_i for the charged particles, respectively. The stopping power and the range of alpha particles are imported from the NIST website (n.d.b.). However, the NIST data is inadequate for the low ranges of electron energy we encounter for the Auger emitters, so we use the empirical relationship discussed in (Howell and Rao 1989, Goddu *et al* 1994) in which references to earlier work may be found. The energy—range relationship is given as

$$E_e = 5.9(X + 0.007)^{0.565} + 0.00413X^{1.33} - 0.367, \quad (18)$$

where E is in keV and X in μm . The initial energies of the radiations emitted by the radionuclides we are considering were obtained from MIRDCell (n.d.a.), and so $X(E_i)$ is obtained by solving equation (18) for X . The derivative obtained from equation (18) is evaluated, as indicated, at $X(E_i) - x$, the ‘residual

range’ left to the particle after having traversed a distance x . The geometric factor $\psi_{P \leftarrow R}(x)$ in both absorbed fractions is defined as the mean probability of a randomized directed vector with length x that starts at a random point in the source R and ends at a random point in the target P . Therefore, it is highly dependent on the geometry of the source and target (Goddu *et al* 1994). Consider a particular target, say a tumor cell nucleus. For the self dose, the source is either the nucleus (N) or the surface (S) of the same cell. The radionuclides are assumed to distribute uniformly within these regions. The geometric factors for these, given as $\psi_{S0}(x)$ and $\psi_{N0}(x)$ are listed in (Goddu *et al* 1994) as equations (A6) and (A8). We recall that we also have to compute the absorbed dose from the free radionuclides in the interstitium. However, we partition the space so that any portion of the interstitium is assigned to the cell that it surrounds, and we assume that absorbed dose per disintegration is the same as that from the cell surface with which it is contiguous so we have not computed an S-value for the interstitium separately. In fact, the interstitial widths have been estimated to be of the order of 50 nm (Thorne and Nicholson 2006); therefore our approximation should have a significant impact on the absorbed dose to the cell nucleus only from electrons with ranges of the order of the thickness of the cell cytoplasm. These limited cases will not affect the trends we have presented. A nuclide in the interstitium is essentially ‘on the (nearest) cell’ in the terms of its distance to the target. The geometric factors for the cross dose, call them $\psi_S(x)$ and $\psi_N(x)$ are listed in (Goddu *et al* 1994) as equations (A1) and (A2) there. We do not reproduce those formulas here, but refer the reader to the cited sources. Therefore, the absorbed dose per cumulative activity can be calculated with specific parameters of the cell radius $R_C = 9 \mu\text{m}$, the nucleus radius $R_N = 7 \mu\text{m}$. We have calculated the total cross dose under the following assumption: the spherical cells have been arranged in a regular close packed face centered cubic lattice with spherical cells and we have computed the cross dose upto the 32nd neighbor of the target cell, that distance being just over 100 μm with our choice of cell size. (Within this distance from a central cell there are 1060 cells in the fcc lattice.) We have already referred above to this approximation: we postpone further examination of its consequences to the Discussion. The radiation types and the corresponding Δ_i (g-rad/mCi-h) and E_i (MeV) of each radionuclide are available in the data tables from (n.d.a.). We have also used the following conversions that 1rad/ $\mu\text{Ci-h} = 0.27 \text{ Gy/MBq-h}$ and 1 g-rad/ $\mu\text{Ci-h} = 0.469 \text{ MeV}$. Apart from ^{213}Bi and ^{211}At (Akabani *et al* 2006), the radionuclides are Auger electron emitters. *The alpha emitters have entries that call for special discussion and explanation below.* The results are shown in table 4.

Remark 5. The self-doses and cross-doses are computed as described above: $S_{\text{self}}(S)$ for example is the absorbed dose to the nucleus per disintegration

Table 4. Radionuclides and their cellular S -values in units of J/kg/disintegration; in other words gray/(Bq-sec).

Nuclide ^a	$S_{\text{self}}(S)$	$S_{\text{self}}(N)$	$S_{\text{cross}}(S)$	$S_{\text{cross}}(N)$
⁵¹ Cr	7.59×10^{-9}	3.99×10^{-4}	1.51×10^{-7}	6.99×10^{-8}
⁶⁷ Ga	2.84×10^{-5}	7.26×10^{-4}	8.21×10^{-4}	3.82×10^{-4}
⁷⁷ Br	1.32×10^{-5}	5.20×10^{-4}	3.46×10^{-5}	1.53×10^{-5}
^{99m} Tc	1.21×10^{-5}	3.14×10^{-4}	1.66×10^{-4}	7.59×10^{-5}
¹¹¹ In	5.68×10^{-5}	5.97×10^{-4}	2.30×10^{-4}	9.21×10^{-5}
¹¹⁹ Sb	1.23×10^{-4}	3.43×10^{-4}	5.14×10^{-4}	2.12×10^{-4}
¹²³ I	4.93×10^{-5}	6.27×10^{-4}	2.84×10^{-4}	1.21×10^{-4}
¹²⁵ I	9.20×10^{-5}	1.38×10^{-3}	2.11×10^{-4}	8.03×10^{-5}
^{193m} Pt	1.46×10^{-4}	2.32×10^{-3}	1.57×10^{-3}	7.17×10^{-4}
^{195m} Pt	5.55×10^{-4}	3.86×10^{-3}	2.54×10^{-3}	1.05×10^{-3}
²⁰¹ Tl	9.51×10^{-5}	1.59×10^{-3}	7.37×10^{-4}	3.29×10^{-4}
²⁰³ Pb	2.90×10^{-5}	9.87×10^{-4}	2.59×10^{-4}	1.19×10^{-4}
²¹³ Bi	3.95×10^{-4}	1.26×10^{-3}	5.26×10^{-3}	2.40×10^{-3}
²¹³ Bi + daughters	1.19×10^{-2}	3.71×10^{-2}	0.30	0.14
²¹¹ At	6.6×10^{-3}	2.05×10^{-2}	8.41×10^{-2}	3.83×10^{-2}
²¹¹ At + daughters	1.4×10^{-2}	4.31×10^{-2}	0.24	0.11

^a It should be remembered that the fraction of tumor cells in the margin is taken to be 1/10, so that the contribution to the cross dose from radionuclides on MNT that are internalized or bound takes into account this fraction as explained in the text.

occurring on the surface or in the neighboring interstitium of the same cell. Since we compute doses only to the cell nucleus, we have dispensed with denoting the target structure: only the source regions need to be specified. Thus, we no longer use the notation $S_{\text{self}}(N \leftarrow S)$ since the nucleus (at a location specified in the tables) is always the target. The cross doses, as just mentioned, include these respective contributions from all cells within 110 μm from the center of the target nucleus.

The alpha emitters. The two alpha emitters (in contrast to the Auger emitters) have unstable daughter nuclei, and so a further calculation is required to obtain the total absorbed doses. The contribution of the daughters makes an enormous difference to the total absorbed dose, particularly for the bismuth nuclide, but essentially no difference at all to the *ratio* of the absorbed doses in a tumor versus a normal cell. Although in reality the parent comes with the daughter, we feel it is instructive, in a calculation, to show the results with and without the daughters' contributions to illustrate this. Thus, we have included two rows for each alpha emitter. The first row shows the result without the contribution of the daughter radionuclides, and the second row, includes them and all of their emissions. (As mentioned in the text, their gamma decays are handled separately: only the charged particle emissions are included in the table.) The daughters are assumed to be in equilibrium with the parent, and as can be seen, the daughters makes an almost two orders of magnitude difference for ²¹³Bi and a factor of two difference for the ²¹¹At. Later in connection with table 8, we shall discuss that this makes no difference to the absorbed dose ratio as just mentioned.

We used our own *Mathematica*TM programs for this calculation, being unaware at the time of (Vaziri et al 2014), (n.d.a.). Our results for the self doses agree well with the results produced by MIRDcell v2.0.15 except for ²¹¹At where ours are up to 16% lower. One of us (RWH) is investigating this discrepancy and anticipates releasing a new version of MIRDcell to correct the problem.

5. Absorbed dose: putting it together

In section 3, we have already calculated the concentrations χ of MNT in different compartments as functions of location r and time t , while in section 4 we discussed the calculation of the absorbed dose per disintegration based on methods available from the literature. We now compute the radiation dose parameters needed to assess the therapeutic potential of the radionuclides listed in table 3. (Recall the discussion on the dual use of coordinates following the previous remark.)

We assume that spherical symmetry still holds, and the volume of the source at the location r_0 is V_S . We recall the fraction of 'hot' MNT is denoted as f and the mean number of radionuclides that each 'hot' MNT carries is m . Within a duration of T_i , the total number of disintegrations *per unit volume* at a source point S for each MNT population (free, bound to cell surface, internalized to the cell nucleus) is given as

$$n^\chi(r_S) = mf \int_0^{T_i} \lambda e^{-\lambda t} \chi(r_S, t) dt, \quad (19)$$

where λ is the decay constant of the radionuclides. The concentrations χ are of course computed for the three different MNT populations (free, cell surface, nucleus). (So χ is one of c , b , or X .) We compute these at the two

places in the annular region (inner and outer edge relative to SCRC) discussed previously. The wall of the cavity where we compute the inner absorbed dose has $\rho = 0$, and there

$$c = C_0, 0 < t < T_I, \quad (20)$$

where T_I is the infusion time. The solution for b from equation (13) is then immediate, from which in turn the density of the internalized MNT population is obtained by the integration in (6b). Explicitly we have, for $0 \leq t \leq T_I$ and with $\zeta := \alpha_2 + \beta$,

$$b(t) = \beta C_0 [1 - \exp(-\zeta t)] / \zeta, b(t) \leq 1 \quad (21)$$

$$= 1 \text{ otherwise} \quad (22)$$

$$X(t) = \left[\beta C_0 t - \frac{b(t)}{\zeta} \right] / \zeta. \quad (23)$$

After time T_I , the equations for b, c (without the advection term) are solved with the initial value of b being $b(T_I)$ from the above and that of c being C_0 . On the other hand, to calculate the ‘worst case’ at the outer margin 2 cm away from the SCRC wall, where the least total exposure to radioactivity occurs, we recall that our principal protocol is such that the MNT just arrives there at the conclusion of the infusion. (In a variation of the protocol, we shall also allow the infusion to continue so that the fluid moves past the 2 cm region so as to decrease normal tissue absorbed dose from the unbound MNT.) The numerical solution of the differential equations as described previously is used to obtain the time dependent concentrations of the MNT and then integrated numerically. The time to which the concentrations of the MNT are computed is either $5 \times$ the half life of the radionuclide in question or 150 h, whichever is greater. In all cases, a constant concentration has been reached well before that time, due to the degradation or eventual capture of the free MNT, leaving only the inescapably (by our assumption) internalized MNT. However, in computing the absorbed dose from the radionuclide (see immediately below) we essentially integrate over all time, so that long lived radionuclides are allowed to do the maximum damage possible.

5.1. Dose calculation

We can now combine our calculations of MNT distribution due to the infusions culminating in the evaluation of equation (19) for the total number of disintegrations $n_d^X(R)$ with the S -value calculations to finally obtain the absorbed dose at the place P with radial coordinate r as defined above.

Charged particle (Auger and alpha) emissions.

To compute the absorbed dose from charged particles emitted by these radionuclides, we need to define what we call effective S -values. We recall our assumption that the fraction of cells in the margins that are cancerous is $\xi = 0.1$. For the absorbed dose to a cancer cell nucleus, from cancer cell nuclei, we define

$$S_{\text{eff}}(TN) := S_{\text{self}}(N) + \xi \times S_{\text{cross}}(N), \quad (24)$$

where the self and cross doses have been shown in table 4. For the absorbed dose to a cancer cell nucleus from the bound radionuclides at the surface of cancer cells, denoted $S_{\text{eff}}(TS)$, we use the same formula *mutatis mutandis*:

$$S_{\text{eff}}(TS) = S_{\text{self}}(S) + \xi \times S_{\text{cross}}(S). \quad (25)$$

However, the absorbed dose from the interstitium to a cell nucleus, be it the nucleus of a cancerous or a normal cell in tissue, is simply

$$S_{\text{eff}}(I) = S_{\text{self}}(S) + S_{\text{cross}}(S). \quad (26)$$

(See the section on S -values for why the input S -values remain the same.) Now when we compute the other numbers for the absorbed dose to a *normal* cell, namely $S_{\text{eff}}(N)$ and $S_{\text{eff}}(S)$, say, we simply omit the self-doses, because the normal cell by our assumption has no bound or internalized MNT. We reiterate that the reason we can simply add up all these contributions is that spatial resolution of our concentration calculations is of the order of millimeters, about the size of a voxel in current MRI. Within one voxel, there are of the order of 10^5 cells, while we have gone out to a tenth of that distance in computing the cross doses, so all the disintegrations are evaluated ‘at the same point’ as we have mentioned. What remains is to identify the volumes of the sources so that the total energy deposited at the target (per unit volume or unit mass there) may be evaluated, because in the above, we have indicated only the number of disintegrations per unit volume of the source. For the absorbed dose from a cell nucleus the volume V_N is that of a nucleus, namely a sphere of radius $r_N = 7 \mu\text{m}$. For the free MNT in the interstitium, the volume V_I is that of the interstitial space allotted to one cell which by our assumption is just $\frac{1}{4}$ of the volume of a cell V_C , a sphere of radius $r_C = 9 \mu\text{m}$. (We have assumed that the interstitial space occupies $1/5$ th of the total space. Neglecting the small contribution of the blood vessels, the remaining $4/5$ th of the space is thus assumed to be occupied by the cells.) Finally for the bound MNT/radionuclide conjugate which is actually distributed over the *area* of the cell surface, we infer an effective volume V_B from the fact that the total number of disintegrations if the concentration were B and the time infinite is simply the number of binding sites in a cell. In other words, recalling that the concentrations were per unit interstitial (and not per unit tissue) volume, we have $V_B = V_I$ as well. We can now multiply and add to obtain the desired results. We thus have for the absorbed dose to the cancer cell nucleus:

$$D_{\text{tumor}}(r) = S_{\text{eff}}(TN) n^X(r) V_N + S_{\text{eff}}(TS) n^b(r) V_B + S_{\text{eff}}(I) n^c(r) V_I.$$

Similarly, the absorbed dose to the nucleus of a normal cell is

$$D_{\text{normal}}(r) = S_{\text{eff}}(N) n^X(r) V_N + S_{\text{eff}}(S) n^b(r) V_B + S_{\text{eff}}(I) n^c(r) V_I.$$

Photon (gamma) emission. The primary purpose of this paper is to evaluate charged particle emitters, in particular Auger emitters, for their use in a novel delivery approach for treating the infiltrating component brain tumors. However, we also offer an upper bound to the gamma absorbed dose to the neighboring normal brain tissue to evaluate how important that may be. Because the gamma rays have considerably longer tissue range, we return to earlier point-source kernel methods (Howell and Rao 1989, with earlier references cited therein) which results in the following expression for the absorbed fraction

$$\phi_i(T \leftarrow S) = \int_0^\infty \psi_{T \leftarrow S}(r) \cdot \mu_{\text{en}} e^{-\mu x} B_{\text{en}}(\mu r) dx, \quad (27)$$

where μ and μ_{en} are the linear attenuation coefficient and the linear absorption coefficient, respectively, both of which can also be imported as functions of photon energy from the NIST website (n.d.c.), and B_{en} is the buildup factor accounting for the contribution of the secondary scatterings (Spencer and Simmons 1973) and can be calculated based on the NIST data. (The density of brain is taken as $\rho = 1 \text{ g cm}^{-3}$.) We use the geometric factors $\psi_{T \leftarrow S}(r)$ as given in (Howell and Rao 1989). We then compute a crude bound to the total photon absorbed dose by (i) taking the target region to be unit volume at $r = a$ (the SCRC wall) and (ii) assuming the equilibrium concentration values for the MNT during infusion, with irreversible slow loss thereafter, and (iv) integrating over all time. The actual absorbed dose to the normal tissue will be less, particularly because of assumption (ii), but we have not yet attempted a more careful evaluation. As will be seen, the gamma absorbed doses are negligible in most cases.

6. Results

We first list the absorbed doses, calculated with the above assumptions and with the baseline values for the kinetic parameters listed in table 1, for the outer margin of the 2 cm thick annular region surrounding the SCRC, where the absorbed doses are least due to shortest exposure to the infused radiolabeled MNT in these regions. Thus, the numbers in table 5 represent the worst case scenario for radiation absorbed dose to tumor (the second column). The last column showing the activities is reproduced from table 3 for the reader's convenience. For targeted radiotherapy, there are at least two factors to consider; achieving sufficient absorbed dose to all regions of the tumor to have a therapeutic effect, as well as minimizing the absorbed dose received by normal cell populations. The numbers in table 5, and in subsequent tables, are shown to the last significant place and reflect the absorbed dose imparted by particulate radiations only. As one might predict based on the shorter range of their emissions in tissue, the absorbed dose profile for Auger electron

Table 5. Radiation dose from charged particle emissions under baseline conditions delivered to the outer region of the cavity margin (2 cm from SCRC wall). The infusion parameters are as in tables 1–3.

Radionuclide	Tumor cells (Gy)	Normal cells (Gy)	Infusion activity (MBq)
^{51}Cr	58	0	36
^{67}Ga	90	9.5	306
^{77}Br	52	0.51	427
$^{99\text{m}}\text{Tc}$	10	4.2	3985
^{111}In	66	2.9	352
^{119}Sb	39	8.5	626
^{123}I	34	7.0	1811
^{125}I	208	1.3	17
$^{193\text{m}}\text{Pt}$	293	17	328
$^{195\text{m}}\text{Pt}$	484	28	230
^{201}Tl	181	9	328
^{203}Pb	98	3.6	461
^{213}Bi	12	11	31 463
$^{213}\text{Bi} +$ daughters	642	611	"
^{211}At	2760	2142	3321
$^{211}\text{At} +$ daughters	7325	6007	"

Table 6. Radiation dose under baseline conditions at the SCRC wall (the innermost region of the 2 cm thick margin).

Radionuclide	Tumor cells (Gy)	Tissue cells (Gy)	Infusion activity (MBq)
^{51}Cr	63	0	36
^{67}Ga	100	12	306
^{77}Br	58	0.6	427
$^{99\text{m}}\text{Tc}$	15	7	3985
^{111}In	74	4	352
^{119}Sb	46	11	626
^{123}I	43	10	1811
^{125}I	225	1	17
$^{193\text{m}}\text{Pt}$	325	20	328
$^{195\text{m}}\text{Pt}$	537	34	230
^{201}Tl	202	11	328
^{203}Pb	110	4.5	461
^{213}Bi	277	257	31 463
$^{213}\text{Bi} +$ daughters	15 040	14 432	"
^{211}At	4344	3480	3321
$^{211}\text{At} +$ daughters	11 595	9755	"

emitters is more favorable that for the alpha emitters with regard to the tumor specificity of dose deposition. Also, with the exception of 6 h half-life $^{99\text{m}}\text{Tc}$ and 13.2 h half life ^{123}I , all of the Auger electron emitters would provide tumor absorbed doses of 39 Gy or more with reasonably acceptable absorbed doses to normal tissue. (The results for the alpha emitters in tables 5, 6, and 8 are shown for both excluding and including the daughter nuclides to illustrate the effects of the latter are: recall the discussion on table 4.)

The absorbed doses calculated at the inner margin, at the wall of the SCRC, where these are the greatest, are shown in table 6. Thus the absorbed doses are now the worst case scenario for the dose to normal cells. It

Table 7. Absorbed dose from the gamma rays and x-rays; these must be added to the absorbed doses from particle emissions presented in tables 4 and 5 to dose. The double star superscript means daughter nuclides are included.

Nuclide	Δ in 10^{-14} J/dis	Dose inner (Gy)	Dose outer (Gy)
^{51}Cr	0.54	1	0.45
^{67}Ga	2.48	4	1.8
^{77}Br	5.17	9	4
$^{99\text{m}}\text{Tc}$	2.02	2	1
^{111}In	6.49	12	5.5
^{119}Sb	0.03	0.2	<0.1
^{123}I	2.76	4	1.8
^{125}I	0.67	4	1.8
$^{193\text{m}}\text{Pt}$	0.2	0.2	<0.1
$^{195\text{m}}\text{Pt}$	1.22	2	0
^{201}Tl	1.48	2	0.9
^{203}Pb	5.01	8	3.6
$^{213}\text{Bi}^{**}$	2.13	0.7	<0.1
$^{211}\text{At}^{**}$	0.63	0.6	<0.1

Note. The asterisks in table 7 on the alpha emitters indicates that the values include those of the daughter nuclides. See discussion above.

is therefore important to determine that these will not be too high. Again, the Auger electron emitters provide a more tumor specific dose profile than the alpha particle emitters. Indeed, the same radionuclides that provided good results for tumor killing at the outer margin, also would provide reasonably acceptable absorbed doses to normal cells at the inner edge of the 2 cm thick treatment region. Note that the absolute doses, to either increase tumor dose or decrease normal cell dose, can be modulated by changing the fraction of MNT molecules that are labeled. We shall return to this issue (i.e., selecting the amount or activity of the radionuclide to be ‘effective’ and ‘safe’) further below.

As mentioned, the approach utilized to calculate the radiation doses summarized in the above two tables considered only the absorbed dose due to the charged particles because these are of subcellular to a few cell diameter range, requiring treatment of energy deposition in small dimensions. We now turn to the radiation absorbed dose from gamma rays and x-rays, which as a consequence of their considerably longer tissue range, can be evaluated by an organ-level dosimetric calculation. In table 7, the first column of numbers shows the total energy available due to the gamma rays and x-rays from each radionuclide. This energy is often large compared to the energy carried by the charged particles (e.g. ^{111}In), however the absorbed fractions are very small, so that we may expect the absorbed doses from the gamma rays to be relatively small as well. Here, we cannot distinguish between the dose to tumor cells and to normal cells because the mean free path of the gamma rays and x-ray is considerably greater than the diameter of single cells or groups of cells, exceeding the diameter of the skull in most cases. The results in table 7 show that the

Table 8. Tumor:normal cell radiation dose ratios considering both particle and photon emissions.

Nuclide	Average dose ratio	Worst case dose ratio	Infusion activity for 44 Gy (MBq)
^{51}Cr	84	58	27
^{67}Ga	7.2	5.7	150
^{77}Br	8.7	5.8	361
$^{99\text{m}}\text{Tc}$	2	1.2	17534
^{111}In	6.4	4.5	235
^{119}Sb	4.3	3.5	706
^{123}I	3.6	2.5	2343
^{125}I	54	42	3.6
$^{193\text{m}}\text{Pt}$	16.6	14.5	34.5
$^{195\text{m}}\text{Pt}$	16	13.4	22.5
^{201}Tl	16.85	14	80
^{203}Pb	11.1	8.1	207
^{213}Bi	1.07	0.05	4997
$^{213}\text{Bi} +$ daughters	1.04	0.04	501
^{211}At	1.25	0.8	53
$^{211}\text{At} +$ daughters	1.2	0.75	20

absorbed dose from gamma rays are indeed small compared to that from the charged particles in tumor cells; however, particularly with ^{77}Br , ^{111}In , and ^{203}Pb , the absorbed dose from gamma rays is a significant component of the total absorbed dose received by normal cells. Further, we see that the total energy Δ emitted in the form of photons is ordered in magnitude in roughly the same way as are the absorbed doses in the subsequent columns. This is not surprising given the large mean free paths of these radiations. The values for the alpha emitters includes the photon radiations from the daughter nuclides: in contrast to the previous tables on the charged particle emissions, we do not give the values omitting these since it makes a difference of less than 0.1 Gy.

Next, in table 8, we summarize parameters that should be helpful in determining the potential utility of the various radionuclides as targeted therapeutics in this clinical setting. We present two measures of the radiation absorbed dose delivered to tumor and normal brain from both charged particles and photons, as well as the infused activity needed to deliver 44 Gy to tumor, a benchmark based on clinical studies (Reardon *et al* 2007). An explanation of how this was done follows. The ‘average dose’ ratio is the ratio of: (1) the sum of all the entries for a radionuclide in the columns pertaining to the tumor dose in tables 5 and 6 as well as the entries for both in table 7; to: (2) the same parameters for the normal cell dose in tables 5 and 6 as well as these same photon absorbed doses (taken as identical for tumor and normal cells). Similarly, in the second column we first add the dose to tumor from table 5 and the outer dose column in table 7. This is the minimum absorbed dose to tumor as we have explained. Then we take the sum of the dose to normal

Table 9. Radiation dose calculated for ^{77}Br under alternate conditions.

Parameter	Tumor dose minimum (Gy)	Tissue dose maximum (Gy)	Dose ratio
Baseline	52	0.6	87
Infusion time $\times 10$	82	1.6	51
$C_0 = 0.1; f = 1$	0	3.2	0
$K_{\text{irr}} \times 10$	85	0.7	121
$k_1 \times 10$	460	6.5	71
$k_2 \times 10$	68	0.6	113
$k_1 \div 10$	5	0	—
$k_2 \div 10$	52	0.6	87
$\{k_1, k_2\} \times 10$	582	6	97
$\{k_1, k_2\} \div 10$	7	0	—
Washout	33	0.1	330

cells from table 6 and the inner dose column in table 7. This represents the maximum dose absorbed by normal cells. Thus, by comparing the minimum dose to tumor in the 2 cm margin to the maximum dose to normal cells, we have the ‘worst case’ dose ratio, and this is summarized in table 8. Finally, the third column is simply the activity needed to guarantee that the minimum dose to the tumor be 44 Gy. Let us take the case of ^{77}Br as an example. From table 3, based on our initial assumption about the fraction of MNT that are labeled, have a ^{77}Br activity of 427, and from table 5, we see this delivers at least 52 Gy to the tumor cells (ignoring the gamma dose here). Hence, the activity level of ^{77}Br required to deliver at least 44 Gy to tumor cells would be $427 \times 44/52 = 361$ MBq. When dose from gamma and x-rays is included, average tumor : normal brain dose ratios for ^{203}Pb , ^{201}Tl , $^{193\text{m}}\text{Pt}$ and $^{195\text{m}}\text{Pt}$ are all greater than 10:1 and those for all the other Auger electron emitters under consideration except ^{123}I , $^{99\text{m}}\text{Tc}$, and ^{119}Sb are greater than 5:1. On the other hand, average tumor:normal absorbed dose ratios for the two short-lived alpha emitters ^{213}Bi and ^{211}At are less than 1.25:1, suggesting that they would be ineffective for targeted radiotherapy in this context. We also see that the daughter radiations which make so much difference to the total absorbed dose make essentially no difference in calculated tumor-to-normal cell absorbed dose ratios. This is of course due to the relatively long particulate range of the alpha particles, and is a further indication of the unsuitability of these nuclides for the therapeutic application in the setting considered here.

Using ^{77}Br as an example, we have evaluated altering some of the baseline parameter values to determine their effect on absorbed doses from the charged particles (table 9). One or two parameter values are altered from their baseline values as listed in the first column, and the effects on calculated absorbed dose to tumor and to normal cells was determined. Because the absorbed gamma dose does not depend on the small scale location of the MNT (free, bound or

internalized), where the radioactive decay occurs, it is essentially unaffected by these variations except for those that impact the infusion protocol itself. Increasing k_1 and k_2 , the binding and dissociation constants for the protein, would increase absorbed dose to tumor by an order of magnitude with alteration in dissociation rate achieving this without increasing dose absorbed by normal cells. The last row (‘washout’) was computed under the following scenario. Following the infusion as described in the text, infusion without MNT or radionuclide (e.g., with saline buffered to be isotonic with interstitial brain fluid) was continued till the advection carried it a further 2 cm radially out so that the margins are ideally free of MNT/radionuclide at the end of this further infusion. This reduces the exposure of these regions to the radionuclide. The result is that the dose to both tumor and normal cells are lowered, though the ratio may be considered even more favorable (330:1) compared with the baseline case ($\sim 85:1$).

7. Discussion

Extensive studies, both theoretical and experimental, have been previously performed to model the distribution of radionuclides delivered to cancer cells both *in vitro* and *in vivo* (Weinstein and van Osdol 1992, Akabani *et al* 2006, and references therein). However, *in vivo* models have been for systemically delivered radiolabeled molecules, or such molecules deposited as a bolus, so that the subsequent spread of the therapeutic is by diffusion, binding, and chemical kinetics. The equations involved in those studies are of the reaction-diffusion type, much studied in mathematics as well. Further, CED of antibodies (without consideration of conjugation to radionuclides and the radiation dose resulting therefrom) has been performed (Luther *et al* 2008), as have been such infusions of large proteins for therapeutic purposes (Mehta *et al* 2012). To the best of our knowledge, this is the first study that examines CED to target the delivery of protein-conjugated radionuclides, exemplified by MNT, to cancer cells. The equations are of reaction—advection type, which has been much less studied mathematically.

In these calculations, we have assumed that the CED of the radiolabeled protein will be performed using an inside-out approach, described in figure 4, in which we assume a uniform infusion from the entire surface wall of the SCRC into surrounding brain tissue. However, we note that this procedure is not employed currently (see, for example Mehta *et al* 2011, for how catheters are currently placed in brain tumor infusions) and in fact no device is currently available that could perform such an infusion. Moreover, it would not be possible to cover the entire SCRC margin (a spherical shell in our example) with even four catheters placed around the cavity, and the spherically

Figure 4. An illustration of the ‘inside-out’ infusion model adopted in this paper. We have assumed an infusion that spreads evenly out from the wall surrounding the SCRC into surrounding brain tissue. Devices to enable such infusions in clinical settings are in progress. See text for further discussion. Illustration provided by Dr John Sampson, Department of Neurosurgery, Duke University Medical Center.

symmetric infusion envisaged in our model is a simplification of the asymmetrical tumor geometries found in GBM patients. While we and others are working on devices making this possible, we caution that these are not yet available. Nonetheless, even though this infusion strategy is not yet practical and the model is an oversimplification of the clinical problem, these calculations should allow cautious consideration of the possibility of treating infiltrating tumor cells around the SCRC with Auger electron and alpha emitter tagged proteins delivered by CED.

Planning the infusion is important because we have an extraordinarily rich parameter space that defines the problem including the properties of the internalizing protein (binding rate, dissociation rate, internalization rate), infusion protocol (catheter placement, flow rate, infusion volume), the labeled protein preparation (fraction of molecules labeled, total protein concentration) and the radionuclide. Before turning to a discussion of the choice of radionuclide, which has been the main focus of this paper, we comment briefly on some of these other variables. We can vary the infusion protocols themselves that affect the distribution of the MNT, and the concentration of the MNT itself to alter its time of arrival at locations of interest (the retardation factor as discussed). As shown in table 9, increasing the infusion time by a factor of 10 increased the minimum dose absorbed by tumor cells from 52 to 82 Gy; however, this would also increase maximum dose to normal cells by an even greater ratio (0.6–1.6 Gy). The model also shows that alterations in the binding parameters of the macromolecule could have a significant effect on radiation dosimetry. This could be a useful tactic because these are either known or could be measured before selection of the macromolecule for this targeted radiotherapy approach. Even for proteins directed against the same molecular

target, the association and dissociation rates for cell binding can vary by more than an order of magnitude as has been reported by Vaneycken *et al* (2006) for a series of VHH single domain antiHER2 antibody fragments.

The main goal of this study was to determine whether it would be feasible to consider combining CED with radionuclides as a treatment for tumor cells infiltrating normal brain in the region surrounding the cavity created after resection of distinguishable primary GBM. Beta emitters with medium and high energy electrons were not considered because their multi-mm average tissue range would not permit selective dosing of tumor cells scattered through normal brain. Our results show that even with alpha particle emitters, which have considerably shorter tissue ranges of only about 50–100 μm , unacceptable tumor-to-normal brain ratios would occur. On the other hand, six Auger-electron emitters (^{51}Cr , ^{125}I , ^{203}Pb , ^{201}Tl , $^{193\text{m}}\text{Pt}$, $^{195\text{m}}\text{Pt}$) met our original criteria of delivering tumor:normal tissue dose ratios $>10:1$, three others (^{77}Br , ^{67}Ga , and ^{111}In) had average tumor:normal brain dose ratios $>5:1$ with ^{123}I , $^{99\text{m}}\text{Tc}$, and ^{119}Sb having less favorable dose ratios.

From a purely dosimetric perspective, ^{51}Cr (84:1) and ^{125}I (54:1) would be optimal choices; however, their long physical half lives would be challenging both from a patient management perspective as well as the need to develop a labeling chemistry that would hold the radionuclide in place for months. Moreover, our calculations did not consider dose rate issues and for rapidly growing cancers including GBM, the slow rate of dose deposition compared with tumor doubling time could be problematic. ^{201}Tl shows the next highest tumor dose selectivity and has a reasonable half life and clinical pedigree as an imaging agent but unfortunately, its mono-cationic nature is not conducive to

stable labeling of proteins such as MNT. With the exception of ^{203}Pb , the 6 other Auger electron emitters with the most favorable dosimetry have all been evaluated in preclinical models as potential radiotherapeutics in other settings (Cornelissen and Vallis 2010, Reilly 2010), providing some additional rationale for pursuing their investigation as CED delivered therapeutics. Moreover, both ^{67}Ga and ^{111}In have been administered to thousands of patients for nuclear medicine imaging purposes, which should facilitate their clinical translation for other applications such as that proposed herein.

It is important to point out that the tumor to normal absorbed dose ratio may represent the minimum advantage afforded by these Auger electron emitters. It is well documented that when Auger electron emitters are attached to DNA in the cell nucleus, their relative biological effectiveness (RBE) for killing can be even higher than those observed for alpha particles (Rao *et al* 1989, Howell *et al* 1991). RBE values for cell killing by Auger emitters can have values as high as about 10 depending on their subcellular distribution. Interstitial or cytoplasmic localization of Auger emitters generally exhibits RBE values of about 1. Recent data indicates that localizing Auger emitters on the cell membrane can be highly radiotoxic (Pouget *et al* 2008, Paillas *et al* 2016). The highest RBE values have generally been observed when the Auger emitter is integrated into DNA in the cell nucleus (Humm *et al* 1994). Interestingly, Kassis and colleagues showed that the thymidine analogs $^{77}\text{BrUdR}$, $^{123}\text{IUdR}$, and $^{125}\text{IUdR}$ are similarly incorporated into the DNA and yield similar RBE values of about 7–9 (reviewed in Humm *et al* 1994). These radionuclides emit about 7, 15, and 25 Auger electrons per decay, respectively. However, ^{77}Br and ^{123}I required about 3.1 and 2.3 times more decays than ^{125}I to achieve 37% survival. Accordingly, this makes the prolific Auger emitters ^{125}I , $^{193\text{m}}\text{Pt}$, and $^{195\text{m}}\text{Pt}$, and ^{203}Pb particularly attractive candidates with their mean number of Auger electrons emitted per decay being 25, 26, 33, and 23, respectively (Howell 1992).

The above consideration also means that the errors we have introduced by our cutoff of range for the charged particles to 100 μm may not be of much import. It is true that the numbers will change somewhat. To ascertain this, we have carried out a partially corrected calculation for ^{111}In which has a large fraction of long range secondary electrons. We have taken the range out to 200 μm but we have retained our approach of using the same concentration values in space and time for all the sources despite their distance from the target nucleus. We have already explained that removing this restriction involves a new set of numerical methods which is beyond the scope of this paper. Referring to tables 5 and 6, we see that the maximum absorbed doses to the tumor and to normal cells are 66 and 3.6 Gy, respectively (for the conditions stated there). Under those same conditions, we find that

extending the range twice as far for ^{111}In results in the numbers increasing to 69 and 7.2 Gy, respectively. Thus the non-specific absorbed dose to normal cells (with no MNT internalized to the nucleus or bound to the cell surface by our assumption) does double with doubling the range (which means bringing in about 2^3 as many sources as before from the increased volume). Nevertheless the Auger emitter retains its excellent dose ratio, and particularly in light of the preceding paragraph showing we may be significantly underestimating the biological effectiveness of self doses, the overall conclusions remain hardly affected.

In conclusion, we have demonstrated that there is a range of Auger electrons emitters suitable for the destruction of the infiltrated margins left after a brain cancer resection. Internalization of these Auger emitters into the tumor cells and attaching them to DNA in the nucleus can turn on an RBE switch that can magnify their destructive power nearly ten-fold. Meanwhile, only a small dose is delivered to normal cells primarily from conversion electrons and photons which have RBE values of only 1. The problem therefore becomes one of an effective delivery protocol which depends on fluid flow in the brain. (We also remark that the problem of destroying a tumor mass this way is a much easier problem in dosimetry since we do not have to worry about the concomitant destruction of normal tissue.) For the application discussed here, the rich parameter space deserves exploration in order to optimize the effectiveness of targeted radionuclide therapy. Attempting to optimize these empirically would be very difficult because of the lack of animal models that would reasonably reflect the infiltrating nature of GBM, and that in a species of sufficient size to create both the cavity that might be left after a resection and the size of the margins that would need to be treated in a GBM patient. Given the clinical importance of treating patients with this dread disease, this calls for a development of the theory outlined above but with full incorporation of the inhomogeneities and physiology of brain tissue as we have begun in [J49], and are continuing to enhance. We therefore believe that models such as the one described herein are important so that the treatment planning and the pre-clinical and clinical development of these therapies may proceed hand-in-hand.

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