Interstitial diffusion and the relationship between compartment modelling and multi-scale spatial-temporal modelling of $^{18}$F-FLT tumour uptake dynamics

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Abstract

Tumour cell proliferation can be imaged via positron emission tomography of the radiotracer 3′-deoxy-3′-18F-fluorothymidine (18F-FLT). Conceptually, the number of proliferating cells might be expected to correlate more closely with the kinetics of 18F-FLT uptake than with uptake at a fixed time. Radiotracer uptake kinetics are standardly visualized using parametric maps of compartment model fits to time-activity-curves (TACs) of individual voxels. However the relationship between the underlying spatiotemporal accumulation of FLT and the kinetics described by compartment models has not yet been explored.

In this work tumour tracer uptake is simulated using a mechanistic spatial-temporal model based on a convection-diffusion-reaction equation solved via the finite difference method. The model describes a chain of processes: the flow of FLT between the spatially heterogeneous tumour vasculature and interstitium; diffusion and convection of FLT within the interstitium; transport of FLT into cells; and intracellular phosphorylation. Using values of model parameters estimated from the biological literature, simulated FLT TACs are generated with shapes and magnitudes similar to those seen clinically.
Results show that the kinetics of the spatial-temporal model can be recovered accurately by fitting a 3-tissue compartment model to FLT TACs simulated for those tumours or tumour sub-volumes that can be viewed as approximately closed, for which tracer diffusion throughout the interstitium makes only a small fractional change to the quantity of FLT they contain. For a single PET voxel of width 2.5–5 mm we show that this condition is roughly equivalent to requiring that the relative difference in tracer uptake between the voxel and its neighbours is much less than one.

Keywords: spatial-temporal model, compartment model, diffusion

(Some figures may appear in colour only in the online journal)

1. Introduction

3'-deoxy-3'-18F-flurorothymidine (18F-FLT) is a radiotracer used for positron emission tomography (PET) imaging of cellular proliferation. FLT accumulates in proliferating tumour cells, a potential target for radiotherapy dose-painting (Ling et al 2000, Bentzen 2005, Bussink et al 2011), as a result of intracellular phosphorylation by thymidine kinase-1 (TK1) which is selectively expressed in the S, G2 and M phases of the cell cycle (Sherley and Kelly 1988, Rasey et al 2002, Salskov et al 2007, Plotnik et al 2010).

The dynamics of FLT accumulation in tumours (perfusion, cellular uptake and phosphorylation) have been investigated using conventional 2-tissue compartment models. However these models assume that the tracer is always distributed uniformly within each compartment, whereas in reality after flowing into the tumour extracellular matrix through capillary walls, FLT diffuses through the interstitium before entering cells and being phosphorylated—a process that depends on the spatial distribution of vasculature, cells and extracellular medium. While the spatially varying dynamics of the transport of radiotracers and contrast agents such as 18F-fluoromisonidazole (FMISO, a PET tracer of hypoxia) and Iodixanol (Visipaque, used in CT perfusion imaging) have previously been investigated (Kelly and Brady 2006, Mönnich et al 2011, Peladeau-Pigeon and Coolens 2013), similar studies have not been carried out for FLT whose uptake mechanism and physical properties differ from FMISO.

In this work we simulate FLT dynamics using a 2D multi-scale spatial-temporal (2Dst) model based on the convection-diffusion-reaction equation, taking model parameter values from the biological literature. The primary purpose of the study is to explore the ability of compartment models to accurately recover the FLT kinetics from spatially varying uptake data generated by the spatial-temporal model, informing the proper interpretation of kinetic parameters obtained by fitting compartment models to uptake curves obtained from tumour regions in clinical dynamic PET images. The following processes are included in the 2Dst model

(a) After injection FLT permeates through capillary walls and moves through the tumour interstitium via strong diffusion and weak interstitial fluid convection. Thus FLT initially flows into interstitial regions adjacent to capillaries and gradually travels to less well vascularised areas.

(b) From the interstitial matrix, FLT enters cells via active transport and passive diffusion across the cell membrane (Paprroski et al 2010, Plotnik et al 2010). Within the intracellular space FLT is phosphorylated to FLT-monophosphate (FLT-MP) by TK1 (Muzi et al 2005a) and then further phosphorylated, but the monophosphorylation step is modelled as being rate-limiting (Muzi et al 2005b).
Near-uniform pressure profiles within tumours (DiResta et al 1993) mean that convective transport is often much less effective than diffusion. Here the relative importance of the two processes is determined for FLT by dimensional analysis and detailed computational modelling, and tumour FLT time-activity-curves (TACs) are simulated using the spatial-temporal model. The simulated TACs work out similarly to TACs obtained from clinical PET images of head-and-neck squamous cell carcinomas (HNSCC), supporting the proposed model. The conventional 2-tissue compartment model is fitted to TACs simulated for whole tumours and tumour regions using the spatial-temporal model. A 3-tissue compartment model, which is mechanistically closer than the 2-tissue model to the tumour uptake process and fits measured HNSCC TACs better (Bertoldo et al 2001, Liu et al 2014), is also fitted to the simulated TACs. The impact on compartment model fits of the sizes of regions analyzed and the heterogeneity of the vasculature within and around them is explored.

2. Methodology

2.1. Multi-scale spatial-temporal models of FLT dynamics

FLT enters the tumour interstitium from the vasculature, is transported through it by diffusion down concentration gradients and convection from regions of high to low interstitial fluid pressure (IFP), and exits via cellular uptake and backflow into the vasculature. Consequently changes in the interstitial activity concentration (Bq ml$^{-1}$) of FLT, $C_{FLT_i}(x,y,t)$, can be described by a reaction-convection-diffusion equation as

$$\frac{\partial C_{FLT_i}}{\partial t} = \nabla \cdot (D_{diff} \nabla C_{FLT_i}) - \nabla \cdot (R_f U C_{FLT_i}) + \phi_{FLT} - \phi_{bFLT}$$

(1)

where $\partial C_{FLT_i}/\partial t$ is the rate of change of the local FLT concentration, $D_{diff}$ is the diffusivity of FLT (assumed to be constant in this paper), $R_f$ is the movement coefficient between a molecule and its carrier, the terms $\nabla \cdot (R_f U C_{FLT_i})$ and $\nabla \cdot (D_{diff} \nabla C_{FLT_i})$ describe changes in FLT concentration due to convection and diffusion, $\phi_{FLT}(x,y,t)$ is a source term describing net FLT entry from capillaries into the tumour microenvironment, $\phi_{bFLT}(x,y,t)$ is a term describing potential binding of FLT within the interstitium, and $\phi_{FLT}(x,y,t)$ is a term representing net FLT movement from the interstitium to the intracellular space.

The source term $\phi_{FLT}(x,y,t)$ is modelled according to Starling’s Law (Baxter and Jain 1989) as

$$\phi_{FLT}(x,y,t) = \left( C_p(t) - C_{FLT_i}(x,y,t) \right) M(x,y)$$

(2)

where $C_p(t)$ is the FLT activity concentration within plasma in capillaries, and $M(x,y)$ is a function or matrix specifying the permeation of FLT through microvessel walls at each position $(x,y)$, which is normalized as

$$\frac{\int \int M(x,y) \; dx \; dy}{\int \int dx \; dy} = \mu_{bi} \left( \frac{S_i}{V_i} \right)_{average}$$

(3)

or in discrete element form

$$\frac{1}{N_i \sum_{j=1}^{N_i} \sum_{i=1}^{N_j} M_{ij}} = \mu_{bi} \left( \frac{S_{ib}}{V_i} \right)_{average}$$

(4)
Here $\mu_{bi}$ is the average vascular wall permeability of FLT, $(S_b/V_i)_{\text{average}}$ is the average ratio of capillary surface area to interstitial volume, $N_c$ and $N_i$ are the total numbers of elements along the $x$ and $y$ axes, and the simulated 2D geometry is viewed as having unit thickness. Letting

$$M(x, y) = \alpha B(x, y)$$

(5)

where $B(x, y)$ is a vascular matrix generated by assigning each $(x, y)$ element a value of 0 or 1 to include or exclude a blood vessel, then from equations (3)–(5) the constant $\alpha$ is given by

$$\alpha = \mu_{bi} \left( \frac{S_b}{V_i} \right)_{\text{average}} \left( \frac{N_{\text{out}}}{N_{\text{blood}}} \right)$$

(6)

where $N_{\text{out}}$ and $N_{\text{blood}}$ are the total number of matrix elements and the number containing blood vessels.

The term $\phi_{c, FLT}(x, y, t)$ is expressed using first-order kinetics as

$$\phi_{c, FLT}(x, y, t) = \omega_1 Q_{c, FLT-i} - \omega_2 Q_{c, FLT-c} = \omega_1 C_{c, FLT-i} - \omega_2 C_{c, FLT-c}$$

(7)

where $C_{c, FLT-i}$ and $C_{c, FLT-c}$ are concentrations of free 18F-FLT within the tumour interstitium and cells; $V_i$, $FV_i$, $V_c$, and $FV_c$ are absolute and fractional volumes of tumour interstitium and cells respectively within a voxel; $\omega_1$ and $\omega_2$ are rate-constants describing FLT transport into and out of tumour cells; and $Q_{c, FLT-i}$ and $Q_{c, FLT-c}$ are the total quantities of FLT contained in the tumour cells and interstitium of the voxel, equal to $V_c C_{c, FLT-c}$ and $V_i C_{c, FLT-i}$ respectively.

In common with analyses of FLT uptake kinetics carried out by Muzi et al. (2005a, 2012) and Menda et al. (2009) we assume there is no long-term uptake due to FLT binding within the tumour interstitium ($\phi_{b, FLT}(x, y, t) = 0$) and thus that the full form of equation (1) is

$$\frac{\partial C_{c, FLT-i}}{\partial t} = D_{\text{diff}} \nabla^2 C_{c, FLT-i} - \nabla \left( R_i \vec{U} C_{c, FLT-i} \right) + \left( C_p - C_{c, FLT-i} \right) M - \omega_1 C_{c, FLT-i} \frac{Q_{c, FLT-c}}{V_i}$$

(8)

where $R_i$ denotes $FV/V_i$. Then demanding mass conservation and assuming first-order kinetics, rates of change of intracellular free and phosphorylated FLT concentrations can be written as

$$\frac{\partial C_{c, FLT-c}}{\partial t} = \omega_1 R_i C_{c, FLT-i} - (\omega_2 + \omega_3) C_{c, FLT-c} + \omega_4 C_{c, FLT-Pc}$$

(9)

$$\frac{\partial C_{c, FLT-Pc}}{\partial t} = \omega_3 C_{c, FLT-c} - \omega_4 C_{c, FLT-Pc}$$

(10)

where $C_{c, FLT-Pc}(x, y, t)$ is the concentration of phosphorylated FLT in tumour cells, and $\omega_3$ and $\omega_4$ are rate-constants describing intracellular phosphorylation and dephosphorylation respectively.

2.2. Tumour liquid inflow and convection

Tumour capillaries are leakier than normal capillaries (Shieh and Swartz 2011) and so liquid carrying the tracer can easily enter the tumour extracellular matrix, influencing the tracer distribution within the tumour interstitium. The flow velocity, $\vec{U}$, in the tumour extracellular
matrix can be modelled using Darcy’s law which describes low speed liquid flow in porous media as

\[ \mathbf{U} = - \mathbf{K}_p \nabla p_i \]  

(11)

where \( p_i \) is the interstitial hydrostatic/fluid pressure (IFP) in the tumour interstitium and \( \mathbf{K}_p \) is the hydraulic conductivity, assumed constant in this work. From mass conservation it follows that

\[ \nabla \cdot \mathbf{U} = - \mathbf{K}_p \nabla^2 p_i = \varphi_i(x, y) \]  

(12)

where \( \varphi_i(x, y) \) is a source term describing liquid leakage into the tumour interstitium through the capillary wall. According to Starling’s law (Jain 1987b) \( \varphi_i(x, y) \) is given by

\[ \varphi_i(x, y) = \frac{1}{N_{\text{tot}}} \left[ (p_c - p_i) - \sigma_T (\pi_c - \pi_i) \right] G(x, y) \]  

(13)

where \( p_c \) is the capillary hydrostatic pressure, \( \sigma_T \) is the reflection coefficient, \( \pi_c \) and \( \pi_i \) represent capillary and tumour interstitial oncotic pressures respectively, and \( G(x, y) \) is a matrix describing the liquid leakage capability of microvessels at each grid element \((x, y)\), given in discrete element form as

\[ G(x, y) = \beta B(x, y) \]  

(14)

In equation (14) \( B(x, y) \) is the vascular matrix and

\[ \beta = L_p \left( \frac{S_b}{V_i} \right) \frac{\left( \frac{N_{\text{tot}}}{N_{\text{blood}}} \right)}{L_{\text{blood}}} \]  

(15)

so that \( G \) is normalized as

\[ \frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} G_{ij} = L_p \left( \frac{S_b}{V_i} \right) \frac{\left( \frac{N_{\text{tot}}}{N_{\text{blood}}} \right)}{L_{\text{blood}}} \]  

(16)

where \( L_p \) is the hydraulic permeability of the microvascular wall. Fluid flow into the tumour and associated pressure gradients are calculated from equations (13)–(15) and included in the spatial-temporal model. The fluid inflow contributes a small additional tracer source term \( C_p \varphi_i(x, y) \) to equation (8).

2.3. Dimensional analysis of convection versus diffusion

The molecular weight of FLT is 244 a.m.u., suggesting that its diffusion through the tumour extracellular matrix may be faster than its convection. We have performed a dimensional analysis to assess the contribution made by each term of the model described by equations(8)–(10). Dimensionless variables are denoted by caret as

\[ \tilde{C}_p = \frac{C_p}{C_0}, \quad \tilde{C}_{\text{FLT} - c} = \frac{C_{\text{FLT} - c}}{C_0}, \quad \tilde{C}_{\text{FLT} - \text{PC}} = \frac{C_{\text{FLT} - \text{PC}}}{C_0} \]

\[ \hat{\lambda} = \frac{L}{T_0}, \quad \hat{x} = \frac{x}{L}, \quad \hat{y} = \frac{y}{L}, \quad \hat{\nabla} = L \nabla, \quad \hat{U} = U \frac{T_0}{L}, \quad \hat{D}_{\text{diff}} = \frac{T_0 D_{\text{diff}}}{L^2} \]

where \( C_0, T_0 \) and \( L \) are fundamental dimensions for tracer activity, time and length respectively. From clinical PET scans and estimates of diffusivity it is known that FLT is distributed throughout tumours within a few hours, and so we take \( T_0 \) as 1 h. And for the fundamental
dimension of length \( L \) we choose a value of \( 30 \mu m \), a typical tumour capillary diameter. The dimensionless equivalents of the governing equations (8)–(10) are then

\[
\frac{d\hat{C}_{\text{FLT}}}{dt} + R_f \hat{V} \left( \mathbf{U} \cdot \nabla \right) \hat{C}_{\text{FLT}} = \left( \frac{T_{0\,\text{diff}}}{L^2} \right) \nabla^2 \hat{C}_{\text{FLT}} + (M T_0) \left( \hat{C}_p - \hat{C}_{\text{FLT}} \right) - \left( \omega_1 T_0 \right) \hat{C}_{\text{FLT}} + \left( \frac{\omega_2 T_0}{R_c} \right) \hat{C}_{\text{FLT},c}
\]

\[
\frac{d\hat{C}_{\text{FLT},c}}{dt} = \left( \omega_1 T_0 R_c \right) \hat{C}_{\text{FLT},i} - \left( \omega_2 T_0 \right) \hat{C}_{\text{FLT},c} - \left( \omega_3 T_0 \right) \hat{C}_{\text{FLT},c} + \left( \omega_4 T_0 \right) \hat{C}_{\text{FLT},pc}
\]

\[
\frac{d\hat{C}_{\text{FLT},pc}}{dt} = \left( \omega_3 T_0 \right) \hat{C}_{\text{FLT},c} - \left( \omega_4 T_0 \right) \hat{C}_{\text{FLT},pc}
\]

and the magnitudes of the terms on the right side of equation (17) are

\[
O \left( \frac{T_{0\,\text{diff}}}{L^2} \right) \sim O(10^3); \quad O(M T_0) \sim O(10^5); \quad O(\omega_1 T_0) \sim O(\omega_2 T_0/R_c) \sim O(10^{-10})
\]

(see section 2.4 for estimates of \( \omega_1, \omega_2, R_c \) and \( D_{\text{diff}} \). The relative movement constant \( R_f \) has a value \( \leq 1 \) and so the limited influence of convection is confirmed, being \( O(10^{-1}–10^{-2}) \) of cell FLT uptake and phosphorylation, and \( O(10^{-3}) \) of diffusion.

2.4. Estimates of spatial-temporal model parameter values

Table 1 lists tumour and normal tissue physiological parameter estimates deduced from the literature, while table 2 summarizes the parameter values used in our modelling and non-dimensionalization analysis. The rationale for the choice of parameter values is discussed below.

2.4.1. Blood vessel diameter. Hashizume et al (2000) measured blood vessels in MCA-IV mouse mammary tumours, reporting a mean diameter of \( 39 \mu m \) and a median of \( 25 \mu m \). For C3H/Bi mammary carcinomas, vessel diameters range from \( 10 \mu m \) in \( 35 \text{mm}^3 \) tumours to \( 30 \mu m \) in \( 800 \text{mm}^3 \) tumours (Hilmas and Gillette 1974). In Fisher 344 rats tumour vessels have a median diameter of \( 53 \mu m \), around twice as large as those in granulating tissues which have a median diameter of \( 20 \mu m \) (Dewhirst et al 1989). Vessel diameters of \( 20 \) and \( 30 \mu m \) were reported at the edge and centre of human colon tumours (Konerding et al 2001). Most exchange occurs within capillaries and post-capillary venules (Jain 1987b), and in normal tissues these structures have diameters of \( 10–30 \mu m \) (Fung 1997). Overall, then, \( 20 \) and \( 30 \mu m \) are reasonable estimates of capillary diameters in normal tissues and tumours respectively.

2.4.2. Fractional tumour volumes occupied by the vasculature, interstitial space and cells. Capillary surface areas of \( 50–260 \text{cm}^2 \text{g}^{-1} \) (typically \( 100 \text{cm}^2 \text{g}^{-1} \)) have been reported for mouse mammary carcinomas, adenocarcinomas and hepatomas (Jain 1987b). The surface area-to-volume ratio (\( S_p/V_p \)) of a cylindrical blood vessel of diameter \( d \) is \( 4 \pi d \) and so for a typical diameter of \( 30 \mu m \) equals \( 1.3 \times 10^3 \text{cm}^{-1} \). Thus, for these tumours the fractional volume of the vasculature \( F_{V_b} \) is \( (50 – 260) / 1.3 \times 10^3 \text{cm}^3 \text{g}^{-1} \), or \( 4–15\% \) assuming a tumour mass density of \( 1 \text{g cm}^{-3} \) (Montelius et al 2012). Yuan et al (1993) have reported a similar \( F_{V_b} \) value of \( 9\% \) in LS174T human tumour xenografts implanted in mice, while an \( F_{V_b} \) value of \( 17\% \) was measured for C3H/Bi mammary carcinomas by Hilmas and Gillette (1974), and values of 8 and
12% were measured for tumour and normal tissue fractional vascular volumes in SCID mice by Jennings (2008). Here we estimate \( F_{V_b} \) as 10% and 12% in tumours and normal tissues.

The fractional volume of Walker carcinomas occupied by the interstitial matrix, \( F_{V_i} \), is around 40% (Gullino et al 1965, Jain 1987a). And as tumours largely comprise the fractional vascular volume \( F_{V_b} \), interstitial volume \( F_{V_i} \) and cellular volume \( F_{V_c} \), it follows that \( F_{V_c} \) is (100% − \( F_{V_b} − F_{V_i} \)) or roughly 50% in tumours, similar to the interstitial fractional volume

### Table 1. Summary of relevant tumour and normal tissue physiological parameters.

| Subject                                      | Measurements                                                                 |
|----------------------------------------------|-------------------------------------------------------------------------------|
| Blood vessel diameter                        |                                                                               |
| Tumour in Fisher 344 rats                    | Median diameter 53 µm (Dewhirst et al 1989)                                   |
| MCa-IV mammary carcinoma                     | Mean diameter 55 µm (Vacok et al 2009)                                        |
| MCa-IV mammary carcinoma                     | Mean diameter 39 µm, median diameter 25 µm, range 8–220 µm                    |
| Human colon cancer                            | 30 µm in the centre and 20 µm at the edge (Konerding et al 2001)              |
| C3H/Bi mammary carcinoma                     | Mean diameter 11 µm in 35 mm³ tumours and larger than 30 µm in > 800 mm³ tumours (Hilmas and Gillette 1974) |
| Granulating tissues                          |                                                                               |
| Capillary or post-capillary venules in normal tissue |                                                                               |
| Vascular volume fraction                      |                                                                               |
| LS174T tumour xenograft                      | 9% (Yuan 1993)                                                               |
| Tumours in mice normal                       | 8% (Jennings 2008) 12% (Jennings 2008)                                       |
| tissue in mice                               |                                                                               |
| C3H/Bi mammary carcinoma                     | 17% (Hilmas and Gillette 1974)                                               |
| Interstitial volume fraction                  |                                                                               |
| Walker carcinoma                             | 40% (Jain 1987a)                                                            |
| Striated muscle                              | 10–30% (Kim et al 2004)                                                     |
| Diffusivity of micromolecules                |                                                                               |
| [3H]-thymidine (MW = 242) in adult bovine cartilage | 3.2 \( \times \) 10^{-6} cm² s⁻¹ (Byun et al 2010, Garcia et al 1996)         |
| Empirical diffusivity equation               | 1.778 \( \times \) 10^{-4} \( \times \) (MW)^{0.75} cm² s⁻¹, for 32 < MW < 69 000 (Swabb et al 1974) |
| Microvessel density (MVD)                    |                                                                               |
| Normal brain tissue in rat                    | 312 vessels mm⁻² (Bosomtwi 2008)                                             |
| Human head-and-neck                          | Mean 143 vessels mm⁻², ranging from 26–732 vessels mm⁻²                      |
| malignant melanoma                           | (Kerr et al 2011)                                                           |
| Surface area of vessels per unit tissue volume |                                                                               |
| Muscle                                       | 70 cm⁻¹ (cat hindlimb), 175–820 cm⁻¹ (other) (Pappenheimer et al 1951)         |
| Tumours in Mouse and Rat                     | 50–570 cm⁻¹ (Jain 1987b)                                                     |
| C3H/Bi mammary carcinoma                     | ≤150 cm⁻¹ for tumours ≥0.5 cm³ and 265 cm⁻¹ for tumours of 0.035 cm³ volume (Hilmas and Gillette 1974) |
| Vessel wall permeability                     |                                                                               |
| Mouse Fc (MW = 25 000)                       | 3.7 \( \times \) 10^{-7} cm s⁻¹, tumour (Yuan et al 1995)                     |
| Doxorubicin (MW = 543)                       | 8–37 \( \times \) 10^{-7} cm s⁻¹, tumour (Eikenberry 2009)                    |
| Azure C (MW = 277)                           | 1.1 \( \times \) 10^{-4} cm s⁻¹, frog mesentery (Jain 1987b)                  |
| FMISO (MW = 189)                             | 2–9 \( \times \) 10^{-3} cm s⁻¹, tumour (Kelly and Brady 2006)                |
| Sacrose (MW = 342)                           | 1–4 \( \times \) 10^{-3} cm s⁻¹, non-fenestrated normal tissue capillaries (Jain 1987b) |
| (Jain 1987b)                                 | 100 \( \times \) 10^{-3} cm s⁻¹, fenestrated normal tissue capillaries (Jain 1987b) |
| Fab (MW~50 000)                              | 1.5 \( \times \) 10^{-3} cm s⁻¹, tumour (Gerlowski and Jain 1986)             |
|                                              | 1.9 \( \times \) 10^{-6} cm s⁻¹, normal tissue (Gerlowski and Jain 1986)      |
In striated muscle the interstitial volume fraction is between 10% and 30% (Kim et al. 2004), and consequently we estimate \( F_Vi \) as 30% and \( F_Vc \) as 58% for normal tissues.

2.4.3. Microvessel density (MVD) and vascular surface area per unit tissue volume. Modelling blood vessels as cylinders of diameter \( d \) running perpendicular to a surface (Dalah et al. 2010), the microvessel density (number of microvessels per surface area) is \( \frac{F_Vb}{\pi \frac{d^2}{4}} \).

Thus for fractional vascular volume and vessel diameter estimates of 10% and 30\( \mu \)m in tumours and 12% and 20\( \mu \)m in normal tissues, the corresponding MVDs work out as 142 and 382 vessels mm\(^{-2} \) respectively – in good agreement with measured MVDs of 143 vessels mm\(^{-2} \) in human head-and-neck tumours (Kerr et al. 2011) and 312 vessels mm\(^{-2} \) in normal rat brain (Bosomtwi 2008). Then the average vascular surface area per unit tissue volume is

\[
( \frac{S_b}{V_b} )_{\text{average}} = \text{MVD} \times \pi \frac{d}{2} \quad (21)
\]

equal to 133 cm\(^{-1} \) and 240 cm\(^{-1} \) in tumours and normal tissues, in line with a measured average of less than 150 cm\(^{-1} \) in tumours larger than 0.5 cm\(^3 \) (Hilmas and Gillette 1974) and a range of 70–820 cm\(^{-1} \) in muscle (Pappenheimer et al. 1951). Given our estimates of 40% and 30\% for the interstitial volume fractions \( F_Vi \) in tumours and normal tissues, the vascular surface areas per unit interstitial space \((S_b/V_i)_{\text{average}}\) work out as 333 cm\(^{-1} \) and 800 cm\(^{-1} \) in these structures.

2.4.4. FLT diffusivity. Diffusivity has not been reported for FLT, and so we have estimated it using approaches based on molecular weight (MW). Swabb et al (1974) proposed an empirical equation

\[
D_{\text{diff}} = 1.778 \times 10^{-4} \times (\text{MW})^{-0.75} \text{cm}^2\text{s}^{-1} \text{ where } 32 \text{ a.m.u.} < \text{MW} < 69000 \text{ a.m.u.} \quad (22)
\]

from which the diffusivity of FLT (244 a.m.u.) can be estimated as \( 2.9 \times 10^{-6} \text{cm}^2\text{s}^{-1} \) – similar to the value of \( 3.2 \times 10^{-6} \text{cm}^2\text{s}^{-1} \) measured in adult bovine cartilage for \( [3H]\)-thymidine.
(242 a.m.u.) (Garcia et al 1996, Byun et al 2010). By extrapolating diffusivity measurements made in carcinomas for substances of high molecular weight (Netti et al 2000) down to a molecular weight of 244 a.m.u., an estimate of the order of $10^{-5}$ cm$^2$ s$^{-1}$ can be obtained for FLT. And from measurements made by Nugent and Jain (1984) for sodium fluorescein (332 a.m.u.), a further estimate of $9.6 \times 10^{-6}$ cm$^2$ s$^{-1}$ can be obtained for FLT tumour diffusivity. Here, then, we use values of 3.2 and $9.6 \times 10^{-6}$ cm$^2$ s$^{-1}$ for diffusivity in normal tissues and tumours.

2.4.5. Vessel wall FLT permeability. No measurements of tumour vascular permeability, $\mu_{bi}$, have been reported for FLT, but measurements of vessel wall permeability have been made for other molecules (Jain 1987b, Yuan et al 1995, Dellian et al 2000, Eikenberry 2009). Wall permeability has been measured as $1.1 \times 10^{-4}$ cm$^2$ s$^{-1}$ for Azure C (277 a.m.u.) in frog mesentry (Jain 1987b), and as 1–4 and $100 \times 10^{-5}$ cm s$^{-1}$ for transport of sucrose (342 a.m.u.) through non-fenestrated and fenestrated capillary walls (Jain 1987b). For this study we estimate FLT wall permeability as $10^{-5}$ cm$^2$ s$^{-1}$ in normal tissues, and $10^{-4}$ cm$^2$ s$^{-1}$ for tumour capillaries whose networks comprise both fenestrated and non-fenestrated vessels.

2.4.6. Cell membrane permeability and the phosphorylation rate of FLT. Cells absorb thymidine and FLT by a combination of passive diffusion across the cell membrane, facilitated diffusion via human equilibrative nucleoside transporters (hENT1-3), and active transport via human concentrative nucleoside transporters (hCNT1-3) (Soler et al 2001, Farré et al 2004, Molina-Arcas et al 2009). Plotnik et al (2010, 2011) have measured total FLT uptake and the fraction of mono-phosphorylated FLT in proliferating TK6 (B-lymphoblast) and plateau-phase A549 (adenocarcinoma) cells located in a medium to which FLT was added 45 or 60 s before the measurements, along with the fraction of mono-phosphorylated cellular FLT. From these data the rate of FLT transport into cells, $\omega_1$, and the intracellular phosphorylation rate, $\omega_3$, can be estimated as

$$\omega_1 \sim 1 \text{ min}^{-1}; \quad \omega_3 \sim 1.5 \text{ min}^{-1}$$

TK6 cells: $\omega_1 \sim 0.25 \text{ min}^{-1}; \quad \omega_3 \sim 0.07 \text{ min}^{-1}$

A549 cells: $\omega_1 \sim 1 \text{ min}^{-1}; \quad \omega_3 \sim 1.5 \text{ min}^{-1}$

The FLT dephosphorylation rate, $\omega_4$, can be deduced from the rate of FLT loss from cells and works out as 0.02–0.08 min$^{-1}$ for proliferating A549 cells (Grierson et al 2004, Muzi et al 2005b).

Here we use the parameter values obtained from fits of the 3-tissue model described in section 2.7 to tumour and neighbouring normal tissue regions in dynamic FLT-PET images of patients with HNSCC (Liu et al 2014). These $\omega_1$, $\omega_3$, and $\omega_4$ values are of the same magnitude as the literature-derived values, allowing for a mixture of proliferating and non-proliferating cells in tumours. The rate-constant $\omega_2$ describing outflow of FLT from cells is around 3–10 fold lower than $\omega_1$, in line with the finding of Plotnik et al (2010) that efflux rates are 4–5 fold slower than influx rates for A549 cells.

2.5. The simulated region and vasculature map

A two-dimensional (2D) computational region of size $6 \times 6$ cm$^2$ was set up on a $2001 \times 2001$ grid with a modelled tumour located at its centre. The diameter of the modelled tumour was
3 cm, concordant with the head-and-neck tumours we have imaged. A map of the vasculature was generated, based on the tumour and normal tissue microvessel densities specified in table 2. Similar to Kelly and Brady (2006) and Dalah et al. (2010) we assumed that vessels were cylindrical and ran perpendicular to the 2D surface, and thus that the probability \( V \) of a vessel lying in a tissue element \((x, y)\) was

\[
V = \frac{MVD \times S_{\text{region}}}{N_{\text{tot}}}
\]

(23)

where \( S_{\text{region}} \) is the area of the simulated region and \( N_{\text{tot}} \) is the total number of grid points in the region. Then the vascular map \( B(x, y) \) was generated by randomly assigning 1’s, indicating the existence of a vessel, to grid elements with a probability \( V \), and 0 otherwise. The resulting map is shown in figure 1.

2.6. Spatial-temporal simulation techniques

The spatial-temporal model was solved numerically for a measured arterial input function, \( C_p(t) \), generating spatially and temporally varying values for \( C_{\text{FLT,i}} \), \( C_{\text{FLT,c}} \) and \( C_{\text{FLT,Pc}} \). By weighting these concentrations and that of FLT in the blood, \( C_b \), by the fractional volumes of the interstitium, cells and vasculature within the region, the total tracer concentration, \( C_T \), was calculated. In blood samples taken from patients diagnosed with stage II-IVa squamous cell head and neck carcinoma (HNSCC) and imaged using FLT-PET, we observed no significant differences between concentrations of FLT in plasma and whole blood (Liu et al. 2014), concordant with findings of Muzi et al. (2005a), and so we have modelled \( C_b \) as equalling \( C_p \). At the interface between the tumour and surrounding normal tissue, the interstitial fluid pressure \( p_i \), velocity \( U_{xy} \), FLT activity concentration, and FLT flux were assumed to be continuous. At the outside boundary of the computational region, the IFP and FLT concentrations were assumed to reach an equilibrium level, i.e. net transport at the edge was set to zero (Neumann boundary condition) as normal tissues contain many blood and lymphatic vessels.

Fluid and pressure distributions were determined first by numerically solving the Poisson equation (12) using the SOR (Successive Over-Relaxation) algorithm (Anderson 1995, Morton and Mayers 2005). Then the governing equations (8)–(10), which describe the transport and uptake of FLT in the tumour, were solved numerically using the forward-time central-space (FTCS) scheme of the finite difference method. For convection-diffusion equations the FTCS scheme provides reasonable accuracy \( (O(\Delta t, \Delta x^2, \Delta y^2)) \), comparable to that of the alternating direction implicit (ADI) and predictor-corrector methods when the Reynolds’ number is low (Zhang et al. 1980). A von Neumann stability analysis was carried out when selecting the time-step of the FTCS scheme, ensuring that the Courant–Friedrichs–Lewy condition was satisfied (Anderson 1995). Due to the very low fluid velocity within the tumour interstitium (Baxter and Jain 1989) the influence of the convection term \( V (\nabla C_{\text{FLT,Pc}}) \) of equation (8) on numerical stability is small, and the spatial-temporal model is close to being a linear diffusion-reaction equation (Zhang et al. 1980). A time-step of 0.2 s was adopted to ensure a convergent solution.

2.7. Description and fitting of the 3-tissue compartment model

Alongside the 2D spatial-temporal (2Dst) model, FLT uptake can also be described by a 3-tissue compartment model (3Tiss) initially used by Bertoldo et al. (2001) to analyse the kinetics
of fluorodeoxyglucose (FDG) uptake in skeletal muscle (see figure 2). The model describes FLT activity within blood vessels, extracellular interstitium and cells. Within the cells FLT can be free or phosphorylated.

Typically compartment models assume that tracer is homogenously distributed within each compartment – from which it follows that in any particular region the net quantity, \(Q_1\), of tracer delivered from plasma to the interstitium should equal the total quantity, \(Q_2\), within the extracellular interstitium and cells (including phosphorylated tracer) (see appendix A). In reality the tracer distribution within the tumour interstitium is heterogeneous, since shortly after injection its concentration will be higher in locations adjacent to blood vessels than in other regions, while at late time-points it will be lower close to blood vessels due to back-flow into them. Consequently diffusive flows will generate mismatches between \(Q_1\) and \(Q_2\).

Following the approach of Bertoldo et al (2001) and Liu et al (2014), the governing equations of the 3-tissue compartment model (3Tiss) are

\[
\frac{\partial C_{\text{FLT-i-cpt}}}{\partial t} = k_1 \frac{FV_b}{FV_i} C_p - k_2 C_{\text{FLT-i-cpt}} + k_3 C_{\text{FLT-i-cpt}} + k_4 \frac{FV_c}{FV_i} C_{\text{FLT-P-cpt}} \tag{24}
\]

\[
\frac{\partial C_{\text{FLT-c-cpt}}}{\partial t} = k_3 \frac{FV_c}{FV_i} C_{\text{FLT-i-cpt}} - k_4 C_{\text{FLT-c-cpt}} + k_5 C_{\text{FLT-c-cpt}} + k_6 C_{\text{FLT-P-cpt}} \tag{25}
\]

\[
\frac{\partial C_{\text{FLT-P-cpt}}}{\partial t} = k_5 C_{\text{FLT-c-cpt}} - k_6 C_{\text{FLT-P-cpt}} \tag{26}
\]

\[C_{T-cpt} = C_i FV_b + C_{\text{FLT-i-cpt}} FV_i + \left(C_{\text{FLT-c-cpt}} + C_{\text{FLT-P-cpt}}\right) FV_c\tag{27}\]

where \(C_{T-cpt}\) is the average FLT activity concentration within a region; \(C_{\text{FLT-i-cpt}}\) is the average FLT concentration in the extracellular interstitium; \(C_{\text{FLT-c-cpt}}\) and \(C_{\text{FLT-P-cpt}}\) are the average activity concentrations of free and phosphorylated FLT within cells; \(C_b\) is the average FLT concentration in blood; \(FV_b\), \(FV_i\) and \(FV_c\) are average fractional volumes of whole blood,
interstitium and cells within the region; $k_1$ and $k_2$ are rate-constants for FLT flow from capillaries to the extracellular interstitium and back; $k_3$ and $k_4$ are rate-constants describing FLT transport into and out of cells; and $k_5$ and $k_6$ are intracellular FLT phosphorylation and dephosphorylation rate-constants. As for the spatial-temporal modelling, $C_b$ is taken to equal $C_p$.

Figure 2 provides a visual comparison between the 2Dst and 3Tiss models, while an algebraic comparison of equations (8)–(10) with equations (24)–(27) suggests the following correspondence between their parameters:

$$k_3 \sim \omega_1,$$  
$$k_4 \sim \omega_2,$$  
$$k_5 \sim \omega_3,$$  
$$k_6 \sim \omega_4$$

Transport across blood vessels walls is largely passive (Michel and Curry 1999) and therefore the wall permeability to FLT flow from the interstitium back into vessels, $\mu_{ib}$, should be the same as the permeability of the wall to flow out into the interstitium, $\mu_{bi}$. Thus (appendix B) it might be expected that:

$$k_1 \approx \mu_{ib}(S_p/V_b)$$  
$$k_2 \approx \mu_{ib}(S_b/V_i) \approx \mu_{bi}(S_p/V_i) \approx k_i(V_b/V_i)$$

We have fitted the 3Tiss model to TACs generated by the 2Dst model, using the methodology described by Bertoldo et al. (2001) and Liu et al. (2014), but equally weighting each simulated TAC point.

### 2.8. Statistical simulation of 3-tissue compartment model fits

In the Results we compare noise-free TACs generated by the 2Dst model with those obtained by fitting the 3Tiss computational model directly to the 2Dst TACs, exploring whether the assumption of spatial homogeneity made by the compartment model biases estimates of FLT.
uptake kinetics obtained by fitting the model to the data. Alongside this work we have also
explored the degree of bias and uncertainty introduced into the fitted parameters by the noise
seen on real images.

Briefly, we initially generated a noise-free TAC using the 3tiss compartment model with
parameter values set to those of the underlying 2Dst model for region A and the whole tumour
(table 3). This simulated TAC is then split into 24 temporal frames spanning a total dura-
tion of 60 min (table 3), following a standard dynamic imaging frame sequence (Young et
al 1999). From the underlying noise-free TAC, 250 further TACs were generated by repeat-
edly adding to each time-frame a noise contribution drawn from a normal distribution whose
standard deviation was specific to the frame-length and average activity-level, and reflected
the levels of statistical noise seen in TACs obtained for whole head-and-neck squamous cell
carcinomas (HNSCC) in FLT-PET imaging studies (Bertoldo et al 2001, Wang et al 2009,
Liu et al 2014). The 3Tiss model was refitted to each of the 250 TACs, weighting each TAC
point according to the inverse of the noise-level. From these fits, the uncertainty on the fitted
parameters (coefficient of variation, COV) and fractional bias (percentage difference between
the true parameter value and the average fitted value) due to noise were calculated. Finally
the whole process was repeated, adding noise at the level seen in single tumour voxels of size
0.55 × 0.55 × 0.33 cm³ rather than the whole tumour.

3. Results

3.1. Interstitial fluid pressure and velocity profiles

Figure 3 shows interstitial fluid pressure and velocity profiles calculated along a line running
through the centre of the modelled tumour. The calculated IFP is nearly uniform throughout
most of the tumour but falls steeply at its edge, consistent with data of DiResta et al (1993).
The calculated IFP in the middle of the tumour centre is 11.5 mmHg, also consistent with a
value of 12.3 ± 2.6 mmHg measured by DiResta et al at the centre of a 3 cm tumour, and with
IFP measurements of 3.4–20 mmHg in animal and human tumours (Boucher et al 1990, Jain
et al 2007, Shieh and Swartz 2011). High IFPs result from both the absence of functional
drainage in tumours and the formation of irregular networks of highly permeable blood ves-
sels (Li et al 2007, Roskoski 2007), and have been reported as a major barrier for drug delivery
(Heldin et al 2004).

The sharp pressure drop at the tumour border causes liquid to flow from the tumour inter-
stitium into surrounding tissues (figure 3(c) and Shieh and Swartz 2011). However due to the
near-uniform interstitial pressure throughout the rest of the tumour, the outflow is limited to
a narrow peripheral region of the tumour; and its influence on FLT transport is very small as
the fluid velocity at the edge is $O(10^{-6} \text{cm s}^{-1})$, in agreement with data of Baxter and Jain
(1989). The asymmetry of the velocity profile shown in figure 3(c) results from a stochastic
difference between the simulated microvessel densities at either edge of the tumour along the
profile studied.

3.2. FLT transport and uptake within the tumour region

Figure 4 shows the distribution of total (free and phosphorylated) FLT activity within the tumour
at 100 s and 1 h after injection, calculated according to the dynamic 2D spatial-temporal model.
Initially the FLT is blood-borne but very rapidly it starts to appear in the interstitium adjacent to
capillaries. Over longer time-scales FLT is further distributed via diffusion and convection, and
is taken up by cells and then phosphorylated. Consequently the uptake of FLT depends on the spatial map of the vasculature and proliferating cells, as well as the heterogeneous transport of FLT within the extracellular matrix. Here the FLT diffusion coefficient and distribution of cells are modelled as being uniform within the tumour—the only source of spatial non-uniformity being the simulated vascular map, whose microvessel density varies binomially around a fixed mean.

The input intravascular arterial FLT TAC $C_p(t)$ used in the study is shown in figure 5(a). This curve is a fit of the algebraic formula (Feng et al 1993)

$$C_p(t) = \begin{cases} 0 & t < \tau \\ (\eta_1 (t-\tau) - \eta_2 - \eta_3) e^{\lambda_3(t-\tau)} + \eta_2 e^{\lambda_2(t-\tau)} + \eta_3 e^{\lambda_1(t-\tau)} & t \geq \tau \end{cases}$$

(30)

to arterial blood sample measurements made with fine temporal sampling for a head-and-neck cancer patient imaged using FLT (Liu et al 2014). In equation (30) $\eta_{1-3}$, $\lambda_{1-3}$ and $\tau$

### Table 3. A comparison between parameter values of compartment models fits to TACs generated using the 2Dst spatial-temporal model, and values expected on the basis of the parameters used in the 2Dst modelling.

| 2Dst          | $\mu_0(S_0/V_0)$ (min$^{-1}$) | $\mu_b(S_b/V_b)$ (min$^{-1}$) | $\omega_{f}$ (min$^{-1}$) | $\omega_{2}$ (min$^{-1}$) | $\omega_{3}$ (min$^{-1}$) | $\omega_{4}$ (min$^{-1}$) | FV$_{b-2D}$ |
|---------------|-------------------------------|--------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------|
| Whole tumour  | 8                             | 2                              | 0.47                        | 0.14                        | 0.12                        | 0.02                        | 0.100         |
| Region A     | 8                             | 2                              | 0.47                        | 0.14                        | 0.12                        | 0.02                        | 0.100         |
| Region B     | 8                             | 1.91                           | 0.47                        | 0.14                        | 0.12                        | 0.02                        | 0.096         |
| Region C     | 8                             | 0.23                           | 0.47                        | 0.14                        | 0.12                        | 0.02                        | 0.013         |
| Region D     | 8                             | 14.25                          | 0.47                        | 0.14                        | 0.12                        | 0.02                        | 0.442         |
| 3Tiss        |                               |                                |                             |                             |                             |                             |               |
| $k_1$        |                               |                                |                             |                             |                             |                             |               |
| $k_2$        | 7.11                          | 1.81                           | 0.480                       | 0.143                       | 0.121                       | 0.020                       | 0.100         |
| $k_3$        | 7.16                          | 1.82                           | 0.478                       | 0.142                       | 0.121                       | 0.020                       | 0.100         |
| $k_4$        | 7.40                          | 1.78                           | 0.475                       | 0.141                       | 0.120                       | 0.020                       | 0.095         |
| $k_5$        | 34.01                         | 0.78                           | 0.299                       | 0.086                       | 0.100                       | 0.023                       | 0.000         |
| $k_6$        | 2.14                          | 3.09                           | 0.606                       | 0.170                       | 0.130                       | 0.019                       | 0.487         |
| $\Delta$Tiss|                               |                                |                             |                             |                             |                             |               |
| $\Delta k_1$| −11%                          | −9%                            | 2%                          | 2%                          | 1%                          | −1%                         | 0%            |
| $\Delta k_2$| −10%                          | −9%                            | 2%                          | 2%                          | 1%                          | 0%                          | 0%            |
| $\Delta k_3$| −7%                           | −7%                            | 1%                          | 1%                          | 0%                          | 0%                          | −1%           |
| $\Delta k_4$| 325%                          | 23%                            | −36%                        | −39%                        | −17%                        | 1.5%                        | −100%         |
| $\Delta k_5$| −73%                          | −78%                           | 29%                         | 21%                         | 8%                          | −5%                         | 10%           |
| $\Delta$k$_{1-2tiss}$ |                      |                                |                             |                             |                             |                             |               |
| $\Delta$k$_{2-2tiss}$ |                          |                                |                             |                             |                             |                             |               |

3Tiss and 2Tiss denote results obtained for the 3- and 2-tissue compartment models. The radius of the whole tumour is 1.5 cm. Regions A, B, C and D are shown in figure 4(b) and have areas of $0.45 \times 0.45$ cm$^2$, $0.18 \times 0.18$ cm$^2$, $0.024 \times 0.021$ cm$^2$, and $0.024 \times 0.006$ cm$^2$. $\Delta$ indicates the difference between fitted values of compartment model parameters and those expected from the spatial-temporal model, expressed as a percentage of the expected values.
are fitted parameters, \( \tau \) accounting for any offset between tracer injection and the start of blood sampling.

TACs simulated according to the spatial-temporal model for the whole tumour, and for poorly- and well-perfused regions C and D, are shown in figure 5(b)–(d). TACs simulated for the larger regions A and B are very similar to the whole tumour TAC and so are not plotted. The simulated whole tumour TAC has the same broad features as TACs obtained from clinical PET images of tumours—an early peak followed by an approach to a near-plateau region created by a trade-off between rising levels of intracellular phosphorylated FLT and falling levels of intracellular and interstitial free FLT, which decrease along with the concentration of FLT in the blood. The TACs simulated for the poorly- and well-perfused regions C and D also comprise an early peak and a near-plateau: for the well-perfused region the peak is higher but the plateau is lower, since the total FLT concentration of this region is heavily weighted by the blood tracer concentration which is high at early time-points but low later on. These shape variations are also seen amongst TACs of voxels in clinically imaged HNSCC tumours; some of these TACs, with shapes similar to the simulated ones, are included in figure 5(b)–(d) for comparison. Standardized uptake values (SUVs) of 2.7–3.5 can be calculated from the TACs simulated for the whole tumour and regions A–D and the

Figure 3. Calculated interstitial fluid pressures: (a) the IFP in the simulated region; (b) the pressure profile along line 1; and (c) the modulus of the velocity profile along line 1.
injected activity (2.59 MBq kg⁻¹) associated with the arterial TAC shown in figure 5(a); these SUVs are in line with the range of 1.8–12 (mean 5.7 ± 3.0) seen in 43 patients with head-and-neck tumours (Hoshikawa et al 2011).

**Figure 4.** Total FLT spatial distributions calculated at (a) 100 s and (e) 60 min after injection. Plots (b) and (f) show the regions outlined in (a) and (e), respectively, while (c) and (d) enlarge regions C and D of plot (b).
3.3. Recovery of simulated spatial-temporal kinetics by the 3-tissue compartment model

Table 3 presents a comparison between 2Dst spatial-temporal model parameter values and analogous 3Tiss model parameter values obtained from fits to total TACs generated for the whole tumour and for tumour subvolumes A–D using the 2Dst model. In figure 6 the TACs obtained directly from the spatial-temporal model are plotted alongside the 3Tiss model fits. Total TACs are graphed for each region, together with TACs of the individual compartments–interstitial free FLT, and intracellular free and phosphorylated FLT.

The 3Tiss model recovers the kinetics of the underlying spatial-temporal simulations well for the whole tumour and for subvolumes A and B. For these regions the TACs generated for the different compartments using the 3Tiss model fits match those obtained directly from the spatial-temporal model, while the values of fitted 3Tiss model parameters match those of their corresponding 2Dst parameters, except perhaps for $k_1$ and $k_2$ which are around 10% out—a result attributable to differences between the concentration of free FLT in the immediate vicinity of blood vessels (high early on but low later) and the average concentration throughout the interstitium. On the other hand for subvolumes C and D the 3Tiss model does not work well: compartment TACs generated using the 3Tiss model fits do not match those generated directly from the spatial-temporal model.

Figure 5. (a) An FLT arterial blood curve derived from measured arterial blood samples. (b)–(d) FLT TACs calculated for the whole tumour and for tumour regions C and D according to the spatial-temporal model, shown together with similarly shaped TACs obtained from voxels of clinical FLT-PET images of HNSCC tumours. The areas under the curves of the imaged TACs have been scaled to best match the simulated TACs; however the SUVs of the simulated TACs broadly concord with those observed clinically.
by the spatial-temporal model, and fitted 3Tiss model parameters values differ substantially from those expected, by around 100% for $k_1$ and $k_2$, 30% for $k_3$ and $k_4$, and 10% for $k_5$ and $k_6$.

Compartment models have difficulty describing the kinetics of tracer uptake in regions which experience substantial net diffusive inflows from, or outflows to, the surrounding interstitium. This effect is described more fully in appendix A, in which a formalism is developed.
based on the quantities $Q_1$ and $Q_2$ described in section 2.7. The net diffusive flow of tracer activity into the region from the surrounding interstitium is denoted as $Q_{\text{var}} = Q_2 - Q_1$, and intuitively might be expected to distort the description of uptake kinetics obtained from compartment modelling if $Q_{\text{var}}$ is a significant fraction of the total activity $Q_T$ within the region.

Activities $Q_1$, $Q_2$ and $Q_{\text{var}}$ were scored for the whole tumour and subvolumes A–D during the spatial-temporal simulation of FLT uptake. Values of $Q_{\text{var}}/Q_T$ calculated at 60 min after injection are listed in table 4 for the different regions, along with $Q_2/Q_1$ ratios. The criterion that $|Q_{\text{var}}|$ be much less than $Q_T$ is met for the tumour and for subvolumes A and B – those regions for which the 3Tiss model accurately recovers the kinetics generated by the spatial-temporal simulation. However for regions C and D the magnitude of the net diffusive inflow $Q_{\text{var}}$ is similar to or larger than the total activity $Q_T$, and the 3Tiss model distorts the uptake kinetics.

Parametric maps of tumour tracer kinetics can be generated by carrying out voxel-by-voxel compartment modelling of dynamic PET scans (Muzi et al 2006, Liu et al 2014). When interpreting these maps it would be useful to be able to identify those voxels or tumour regions in which the tracer kinetics may not have been accurately estimated by the compartment modelling. This is challenging as the PET images provide no direct information about tracer diffusion or $Q_{\text{var}}$. However in appendix A a criterion for establishing whether the kinetics are likely to be accurately estimated is derived, namely

$$\frac{X}{\psi} \ll \frac{r}{X}$$

where $r$ is the length-scale (width) of the region being investigated; $X$ is the tracer diffusion length, equal to $\sqrt{4D_{\text{diff}} T_0}$ where $T_0$ is the duration of dynamic imaging; $\psi$ is the average value within the region of a measure of tracer concentration (e.g. total concentration) or tumour function (e.g. microvessel density) that is tightly linked to the interstitial tracer concentration; and $\frac{\partial \psi}{\partial n}$ is the average over the region’s surface of the gradient of $\psi$ perpendicular to the surface.

Inequality (31) requires the average change in concentration at the edge of the region, evaluated over the diffusion length $X$ and expressed relative to the mean concentration within the region, to be less than the ratio of the region’s width $r$ to $X$. Physically $X$ represents the root mean square (rms) distance through which a tracer diffuses over a time $T_0$ in 2D, or $\sqrt{2/3}$ of the rms distance through which the tracer diffuses in 3D (Krissinel and Agmon 1996). For a scan time of one hour and a value for $D_{\text{diff}}$ of $9.6 \times 10^{-6}$ cm$^2$ s$^{-1}$ in the tumour interstitium (table 2) $X$ works out as 3.7 mm. PET image voxels have similar widths, typically 2.5–5 mm, and so inequality (31) can be understood to mean that compartment modelling of a voxel’s kinetics will be accurate provided

$$\left| \frac{I_{\text{voxel}} - I_{\text{neighbours}}}{I_{\text{voxel}}} \right| \ll 1$$

where $I_{\text{voxel}}$ is the intensity of the voxel, and $I_{\text{neighbours}}$ is the average intensity of immediately neighbouring voxels. For a larger region of width $W$ voxels, the requirement becomes

$$\left| \frac{I_{\text{edge}} - I_{\text{neighbours}}}{I_{\text{region}}} \right| \ll W$$

Where $I_{\text{region}}$ and $I_{\text{edge}}$ are the average intensities of all the voxels within the region and of those immediately inside the region’s edge.
3.4. Uncertainty and bias of 3Tiss compartment model parameters due to noise

Whole tumour-level noise generates biases of up to 10% and uncertainties (1 standard deviation, s.d.) of up to 17% in 3Tiss model parameters. For the parameters $k_3$–$k_6$, the bias is very low, less than 2% (table 5). These figures are much lower than the mismatches reported in section 3.3 between fitted 3Tiss parameters and corresponding 2Dst parameters due to non-modelling of diffusion by compartment models. The higher degree of noise at the voxel-level generates biases of up to 13% into 3Tiss model parameters, and in particular up to 6% bias on $k_1$–$k_3$, $k_5$ and $k_6$. These biases are still substantially lower than the inaccuracies resulting from the exclusion of diffusion from compartment models, although the voxel-level noise does add around 40% uncertainty (1 s.d.) into parameter values.

4. Discussion

The transport and uptake of FLT in tumours has been simulated using a multi-scale spatial-temporal model, generating TACs of similar magnitude and shape to those obtained from clinical PET images. The kinetics of the spatial-temporal model can be recovered well by fitting a 3-tissue compartment model to TACs simulated for regions that can be considered as approximately closed – the net quantity of FLT diffusing into or out of these regions through the interstitium being very much less than the total FLT within them. ($|Q_{var}| \ll |Q_T|$).

For comparison, we have also used a conventional 2-tissue compartment (2Tiss) model (Muzi et al 2005a) to estimate the FLT kinetics from the simulated TACs. This model is simpler than 3Tiss, but is less directly linked to the underlying spatial-temporal model: specifically, the 2Tiss model describes tracer flow between the capillaries and cells in a single step (with forwards and backwards rate-constants $k_{1,2\text{tiss}}$ and $k_{2,2\text{tiss}}$, see figure 7), whereas the 3Tiss model breaks this step into two–flow between the vasculature and interstitium (rate-constants $k_1$ and $k_2$) and between the interstitium and cells ($k_3$ and $k_4$). Thus $k_{1,2\text{tiss}}$ might be thought to be related to $k_1$ and $k_3$ of the 3Tiss model, and $k_{2,2\text{tiss}}$ related to $k_2$ and $k_4$. On the other hand, $k_{3,2\text{tiss}}$ and $k_{4,2\text{tiss}}$ describe intracellular FLT phosphorylation and FLT-MP dephosphorylation alone, and thus might be expected to be equivalent to $k_5$ and $k_6$ of the 3Tiss model:

$$k_3 \sim k_3 \sim k_5 \sim k_4$$

Fitted 2Tiss model parameter values are listed alongside 3Tiss values in table 3. As expected $k_{1,2\text{tiss}}$ lies between $k_1$ and $k_3$, while $k_{2,2\text{tiss}}$ lies between $k_2$ and $k_4$. Surprisingly, however, fitted values of $k_{3,2\text{tiss}}$ and $k_{4,2\text{tiss}}$ differ substantially from $\omega_3$ (or $k_5$) and $\omega_4$ (or $k_6$) – by around 90% and 20% respectively. These differences are substantially greater than the parameter bias introduced by either tumour-or voxel-level noise and stem from the poorer description of the simulated TACs provided by the simpler 2 tissue models, which mirrors the poorer fits.
to clinically observed HNSCC TACs achievable using these models (Liu et al. 2014). When trying to infer tumour function from kinetics parameters, then, it is useful to note that parameter values depend on the compartment model used in their estimation: for the case described here, the most accurate (least biased) kinetics values are obtained using the compartment model most closely related to the underlying spatial-temporal dynamics of tracer uptake, although the precision of kinetics values estimated from noisy TACs is better for simpler models which include fewer parameters (Liu et al. 2014).

To further check the influence of convection on FLT transport and uptake we have evaluated the index

$$\Delta C_T = 100\% \times \left| C_{T_{-\text{convection}}} - C_{T_{-\text{diffusion-only}}} \right| / C_{T_{-\text{diffusion-only}}}$$

(34)

at points throughout the simulated tumour. In equation (34) $C_{T_{-\text{convection}}}$ and $C_{T_{-\text{diffusion-only}}}$ denote FLT voxel activities calculated using spatial-temporal models which include and exclude the effect of convective transport. Changes due to convection prove to be negligible, $\Delta C_T$ having a mean value of less than 0.01% and a maximum of less than 0.8% – a finding that concords with our earlier dimensional analysis (Methods 2.3).

The present work has a number of limitations: in particular, the influences of O$_2$ concentration, nutrient supply and pH on cellular FLT uptake have not been considered. Irregularities in the vascular network lead to spatial and temporal variations in the oxygen distribution, creating chronic (diffusion-limited) and acute (perfusion-limited) hypoxic regions (Gulliksrud et al. 2008) in which cells die or cease to proliferate. Similarly, tumour acidity or a lack of nutrients can cause tumour cells to enter G$_0$ phase or to die (Patel et al. 2001, Ribba et al. 2005, Smallbon et al. 2005). And because cellular FLT uptake is greater in proliferating cells, it follows that the $\omega_3$ FLT phosphorylation rate of the spatial-temporal model potentially varies spatially – a factor not included in the modelling presented here, but which will be built into future work.

Likewise, FLT permeabilities of the capillary wall ($\mu_{bi}$) and cell membrane ($\omega_1$ and $\omega_2$) are modelled here as being constant but can also vary spatially and temporally, as can FLT diffusivity ($D_{\text{diff}}$) in the tumour interstitium. Intermittent opening and closing of tumour capillaries has also been observed (Faye et al. 2011). And while we have simulated short-range spatial variations in microvessel density by distributing vessels according to a simple spatially-invariant binomial distribution, variability also exists on longer length-scales and can be simulated by allowing the probability that a blood vessel runs through a tissue element to change with location.

Finally, 18F-FLT is slowly metabolized to 18F-FLT-glucuronide (FLT-glu) in the liver (Visvikis et al. 2004, Liu et al. 2014) and returns to the blood circulation. However, we have not modelled the contribution of 18F-FLT-glu to the total tumour FLT uptake, because FLT-glu is not absorbed into cells (Muzì et al. 2006) and by the time it comprises a significant fraction of the total circulating activity (around 5% and 25% at 10 and 60 min post-injection) the large

| Noise        | $k_1$ (%) | $k_2$ (%) | $k_3$ (%) | $k_4$ (%) | $k_5$ (%) | $k_6$ (%) | FV$_{b-3tiss}$ |
|--------------|-----------|-----------|-----------|-----------|-----------|-----------|----------------|
| Tumour level | bias (%)  | -5.7      | -7.4      | -0.7      | -0.4      | 0.2       | 2.3            | 8.8            |
|              | COV (%)   | 6.3       | 9.2       | 9.6       | 16.3      | 11.5      | 8.2            | 10.2           |
| Voxel level  | bias (%)  | -0.2      | 1.4       | 5.5       | 12.6      | 5.6       | 0.6            | 3.9            |
|              | COV (%)   | 24.7      | 39.9      | 34.6      | 58.7      | 42.0      | 36.3           | 34.0           |
majority of the tumour activity is intracellular rather than intravascular or interstitial (Liu et al. 2014). Consequently little error ensues if the contribution of the metabolite to tumour TACs is ignored.

5. Conclusions

A mechanistic spatial-temporal model of FLT uptake, based on a convection-diffusion-reaction equation with parameter values estimated from the biological literature, has been used to generate tumour FLT time activity curves whose magnitudes and shapes agree with those observed clinically. Tumour TACs generated by the spatial-temporal model can be matched almost exactly by fits of the 3-tissue compartment model, which in turn describes clinical FLT TACs well for HNSCC patients (Liu et al. 2014).

In this work the spatial-temporal (2Dst) model has been used to study the accuracy with which the known modelled kinetics of tracer uptake can be recovered by fitting the 3-tissue model to 2Dst-simulated FLT TACs. The kinetics can be recovered accurately, but only for regions that can be considered to be approximately closed – those for which the net quantity of FLT diffusing into or out of the region, $Q_{\text{var}}$, is much less than the total quantity of FLT within the region $Q_{\text{Tiss}}$. Over the course of an hour the estimated diffusion length of FLT in the tumour interstitium is 3.7 mm, from which it follows for a PET voxel of width 2.5–5 mm that the condition $|Q_{\text{var}}| \ll Q_{\text{Tiss}}$ is roughly equivalent to the requirement that the relative difference in tracer uptake between the voxel and its neighbours is very much less than one. If this condition is not satisfied, parametric maps of the kinetics, estimated from compartment model fits, may be distorted in the vicinity of the voxel by unmodelled diffusive effects.

While the rate-constant of intracellular FLT phosphorylation is estimated accurately by fitting the 3-tissue compartment model to TACs generated by the spatial-temporal model, substantially less accurate (although more precise) estimates are obtained using a 2-tissue compartment model, despite the close relationship between the 2Tiss and 3Tiss models and the identical role played by the phosphorylation rate-constant in the two models. When interpreting rate-constant values obtained from compartment model fits, then, it is useful to note...
that the resulting values are model-dependent, and that in this study the most accurate estimates were obtained using the (3Tiss) model most closely linked to the underlying spatial-temporal process of tracer uptake.

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Appendix A. Compartment models and diffusion

The total quantity \( Q_T \) of FLT in a tumour region \( R \) at time \( T_0 \) is the sum of free FLT in the interstitium \( (Q_{\text{FLT,i}}) \), cells \((Q_{\text{FLT,c}})\) and capillaries \((Q_b)\), together with phosphorylated intracellular FLT \((Q_{\text{FLT,pc}})\)

\[
Q_T = Q_{\text{FLT,i}} + Q_{\text{FLT,c}} + Q_{\text{FLT,pc}} + Q_b = Q_2 + Q_b \quad (A1)
\]

where \( Q_2 \) is the combined quantity \((Q_{\text{FLT,i}} + Q_{\text{FLT,c}} + Q_{\text{FLT,pc}})\) of FLT in the tissue interstitium and cells, which in a closed system would equal the net quantity of FLT delivered from the capillaries to the tumour \((Q_1)\).

In reality neither the whole tumour nor subvolumes of it are completely closed, and the quantity of FLT they contain is influenced by inflows \((Q_{\text{in}})\) and outflows \((Q_{\text{out}})\) of FLT via diffusion and convection through the tumour interstitium and normal tissues. Hence in a real tumour volume the total quantity of FLT \((Q_T)\) is the sum of that delivered into the region by capillaries \((Q_1)\) and the difference \((Q_{\text{var}})\) between the integrated FLT interstitial flow-rate into \((F_{\text{in}})\) and out of the tumour volume \((F_{\text{out}})\), as well as the activity remaining in capillaries \((Q_b)\), i.e.

\[
Q_T = Q_1 + Q_{\text{var}} + Q_b = Q_2 + Q_b \quad (A2)
\]

where \( Q_{\text{var}} = \int_0^{T_0} [F_{\text{in}}(t) - F_{\text{out}}(t)] dt \) \quad (A3)

\[
= Q_1 - Q_2 \quad (A4)
\]

Changes in \( Q_{\text{var}} \) over time represent net inflows or outflows from the region, excluding vascular flows, and are described directly by spatial-temporal models, but not by compartment models which assume that the tracer is distributed uniformly throughout each compartment and ignore diffusive and convective effects. Consequently compartment models are forced to account for these spatial flows indirectly by distorting their kinetics to describe the uptake dynamics, thus biasing rate-constants and modelled concentrations within different compartments. However there will be little distortion provided

\[
\left| \frac{dQ_{\text{var}}}{dt} \right| \ll \left| \frac{dQ_{\text{FLT,i}}}{dt} \right| \quad (A5)
\]

As the effect of convection is small compared to that of diffusion, the net rate of FLT influx to a region from the surrounding interstitium is given approximately by

\[
\frac{dQ_{\text{var}}}{dr} = \int_R D_{\text{diff}} \cdot \nabla \cdot C_{\text{FLT,i}} \, dV \quad (A6)
\]

where \( R \) denotes integration over the region. Consequently inequality \( (A5) \) will be met if
where \( V_i \) is the absolute volume of tumour interstitium in the region, over which the rate of change of concentration on the right-side is averaged. Then from Gauss’ theorem (A7) can be written as

\[
D_{\text{diff}} \left| \int_R \nabla^2 C_{\text{FLT}-i}(t) \, dV \right| \ll V_i \left| \frac{dC_{\text{FLT}-i}(t)}{dt} \right|
\]

(A7)

where \( S \) denotes integration around the surface of the region and \( n \) is the normal vector to the surface. Inequality (A8) will hold if

\[
D_{\text{diff}} \left| \int_S \frac{\partial C_{\text{FLT}-i}(t)}{\partial n} \, dS \right| \ll V_i \left| \frac{dC_{\text{FLT}-i}(t)}{dt} \right| \left( \frac{V_i}{\bar{r}} \right)
\]

(A8)

(\( \bar{r} \) is the maximum of the preceding quantity over the scan duration \( T_D \)) since the left-side of (A9) is less than the time-average of the right-side of (A8). Considering the tumour region to be a sphere of radius \( r \), (A9) can be rewritten as

\[
\left| \frac{\partial C_{\text{FLT}-i}(t)}{\partial n} \right|_{\text{max}} \ll \frac{V_i}{4\pi r^2 D_{\text{diff}}} \frac{\bar{C}_{\text{FLT}-i}(t)_{\text{max}}}{T_D} \sim \frac{V}{4\pi r^2 D_{\text{diff}}} T_D \frac{\bar{C}_{\text{FLT}-i}(t)_{\text{max}}}{T_D}
\]

(A10)

From equation (A12), then, diffusive effects will not disrupt the ability of compartment models to recover the kinetics of tracer uptake in a region, provided that the mean gradient of the tracer concentration over the surface of the region is very much less than the mean concentration within the region multiplied by the length-scale \( r \) of the region and divided by the square of the tracer diffusion length in the interstitium. Finally, denoting by \( \psi \) any facet of tracer concentration (total concentration, for example) or of tumour function that is tightly linked to the interstitial concentration of FLT, one may further approximate (A12) as

\[
\left( \frac{\partial C_{\text{FLT}-i}(t)}{\partial n} \right)_{\text{max}} \ll \frac{r}{\bar{X}} \frac{\bar{C}_{\text{FLT}-i}(t)}{\bar{C}_{\text{FLT}-i}(t)}
\]

(A11)

(neglecting a factor of \( \frac{3}{4} \) on the right-side). Observing that the magnitude and gradient of \( C_{\text{FLT}-i} \) are likely to reach their maximum values at similar times, (A11) can be approximated as

\[
\left( \frac{\partial C_{\text{FLT}-i}(t)}{\partial n} \right)_{\text{max}} \ll \frac{r}{\bar{X}} \bar{C}_{\text{FLT}-i}(t)
\]

(A12)

\[
\bar{C}_{\text{FLT}-i}(t) \ll \frac{r}{\bar{X}} \bar{C}_{\text{FLT}-i}(t)
\]

(A13)

\[
\bar{X} \left| \frac{\partial \psi}{\partial n} \right| / \psi \ll r / \bar{X}
\]
meaning that interstitial diffusion should not be problematic for compartment analysis of a tumour region provided that the average across the region’s surface of the relative change in tracer concentration (or related function) evaluated over the diffusion length is very much less than the ratio of the width of the region to the diffusion-length.

Appendix B. Links between parameters describing diffusion from one compartment to another

Movement of a substance across a wall of area $S_{XY}$ and permeability $\mu_{XY}$ from compartment $X$ (volume $V_X$) to compartment $Y$ (volume $V_Y$) can be approximated either in terms of a compartment model or via Starling’s Law, leading to the relations

$$V_Y \frac{dC_Y}{dt} = K_{XY} V_X C_X; \quad V_Y \frac{dC_Y}{dt} = \mu_{XY} S_{XY} C_X \quad (B1)$$

and thus

$$\mu_{XY} = \frac{K_{XY} V_X}{S_{XY}} \quad (B2)$$

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