Appendix A. Parameter Derivation

An average human weighs about 70 kg, with an average blood volume, V_{blood}, of about 4.7L [17]. In contrast, the average laboratory mouse has a weight of 20g, with an average blood volume of 1.1 mL [35]. Despite these differences in size, and subsequent differences in parameters, humans and mice have identical physiological processes (though different parameters) in the context of our model.

Both humans and mice have the same normal values for blood concentrations of HCO_3^-, CO_2, and H^+ [17, 35]. Crucially, metabolic rate differs between humans and mice. As mice have a very large surface area to volume ratio, they lose heat quickly and have a difficult time maintaining their body temperature. Consequently, their metabolic rate and CO_2 production per gram of body weight is 10-16 times that of a human [35, 36][1]. This leads to different rates of ventilation and kidney filtration, which will be discussed below.

Appendix A.1. Vascular exchange

Blood vessels and capillaries act as sources/sinks for CO_2, H^+, and HCO_3^- via vascular exchange. The vascular diffusive permeability for various ions and molecules, P_i, varies based on molecule size and somewhat between tis-

[1] All citations of The Jackson Laboratory are in reference to The Mouse Phenome Database, the website of the Mouse Phenome Project which is headquartered at The Jackson Laboratory in Maine, USA. The Mouse Phenome Database is a database of laboratory mouse strain measurements of phenotypic and genotypic data. The database is located at http://www.jax.org/phenome.

sues and among vessels, but has been fairly well characterised across a wide range of molecules for normal tissues. Detailed calculations of P_i for tumour vasculature are not as widespread, although it has been found that tumours tend to have a higher vascular permeability than normal tissue [16]. Therefore, for the P_i of H^+ we use an estimate specific for tumour vascular permeability, and with lack of data use standard non-tumour estimates for HCO_3^- [16] and CO_2 [38].

Further complications arise in estimating vessel length per unit area, VAD, (commonly referred to as 'vascular density') as direct imaging of vessel number and surface area can lead to misleadingly high estimates in tumour perfusion, as many vessels are ineffective, poorly perfused, transiently reverse their flow, or are simply dead ends, due to the tortuous and leaky nature of tumour vasculature. In addition, areas of necrosis (dead regions with no vasculature) were present in both control and treated mice (Robey, *unpublished results*). Also, the vessel density tends to decrease with increasing tumour size [16, 42].

Monsky *et al.* [40] measured tumour vessel areal density using human breast cancer cells implanted in the mammary fat pad of female SCID mice (similar to the experimental procedures in [11]). We reduce this measurement for our simulations, as the Monsky *et al.* [40] results were found for small tumours without necrosis (≈ 1 mm in radius). Hence, we decrease their value from 70 cm/cm^2 by 60% in line with estimates of reduction of vessels due to size and necrosis in MDA-MB-231 tumours [41, 42].

In calculating v_T, the blood volume V_{blood} and extracellular tumour volume V_{tumour} are needed. The blood volume of a laboratory mouse is ap-

proximately 1.1 mL (1100 mm^3) [35]. In the Robey *et al.* [11] experiments, the total tumour volume varied from 100-1000 mm^3 in the mice during the course of the experiments. Experimental studies estimate the interstitial volume fraction (as a proportion of total tumour volume) at 20% for MDA-MB-231 breast cancer cells [43, 57], thus the extracellular tumour volume, V_{tumour} varied from about 20-200 mm^3. For simplicity, we assume a constant tumour volume from within the experimental range (V_{tumour} =100 mm^3), hence $v_T = 0.1$. In humans, breast cancer tumours do not reach the same proportional volume as in laboratory mice. For example, in a human with 4.7 L of blood and 20% tumour interstitial volume fraction, a $v_T = 0.1$ would translate to a spherical tumour of over 17 inches in diameter. Therefore, for a human, we choose v_T=0.01, which correlates to a tumour diameter of 2.7 inches, equivalent to a stage 3 tumour [44]. In Section 3.4 we examine the effect of varying the tumour size, and find the system is not sensitive to our choice of v_T.

Appendix A.2. H^+ production

H^+ production rate varies between tumours, and probably within a tumour due to environmental heterogeneity. In addition, the acid production rate of the body's tissues is heterogeneous, depending on the type of muscle and metabolic activity. Several studies [45, 8, 9] attempt to estimate acid production rates in tumour and normal tissues. Martin *et al.* [45] found an acid production rate in tumours of about 3.3×10^{-7} mol/litre/sec.

In contrast, Gatenby & Gawlinski calculate a per cell tumour production rate of 4.4×10^{-17} mol/cell/sec, which, multiplied by the tissue carrying capacity of 5×10^7 cells/cm^3 (used in [8] and converted from cm^3 to litres),

gives a tumour tissue acid production rate of 1×10^{-6} mol/litre/sec.

In Gatenby *et al.* [9] paper, the authors use a carrying capacity of 5×10^8 cells/cm^3, which would correspond to a tumour tissue acid production rate of 1×10^{-5} mol/litre/sec. It is likely that with different tumour types the upper limit of the range is even higher than this. However, an acid production value from this range is used in our simulations (7.8×10^{-6}), which produces a tumour of pHe of 7.0 as seen in the window chamber.

In calculating the body production of H^+, we multiply the normal cell acid production rate found by [45] (1×10^{-7}mol/litre/sec) by the volume of the body (V_{body}) to obtain a total body production of H^+, and then divide it by the volume of the blood (V_{blood}) to obtain the rate of acid produced in the blood by the body. Hence, $\phi_3 = \frac{(1\times10^{-7})\times V_{body}}{V_{blood}}$, where V_{body} is about 70L in a human, and about 13 mL in mice [35]. As mentioned previously, V_{blood} is about 4.7 L in human, and 1.1 mL in mouse [17, 35]. Hence, $\frac{V_{body}}{V_{blood}}$ is estimated to be 15 in humans and 12 in mice.

Appendix A.3. CO_2 production

Human CO_2 production rate from the body tissues (except the tumour) into the blood, ϕ_4, was taken from the value used in [46]. As mentioned previously, mouse CO_2 production per gram of body weight is 10-16 times that of a human, hence we increase the mouse CO_2 production accordingly [35, 36].

Tumour CO_2 production rate varies between cell lines, and without exact values, this has been estimated by assuming the tumour has a normal cellular production rate. We calculate this for a human by taking the body production rate of CO_2 in the blood (in moles/second/litre of blood), mutiplying

it by the volume of blood (V_{blood}) to get the total moles of CO_2 produced per second, and dividing it by the volume of body tissues (V_{body}) to obtain a rate in moles/second/litre of tissue. Hence, $\phi_5 = \frac{\phi_4 V_{blood}}{V_{body}}$. This gives a CO_2 production rate in moles/second/litre of tissue, which we use as an estimate for the production of the tumour.

As the implanted cell line in the mice is a human breast carcinoma, we use the human estimate for ϕ_5 as the estimate for the mouse CO_2 production rate. This assumes that the human xenograft will produce the same amount of CO_2 in humans as in mice, which is a reasonable assumption, but could in reality differ.

Appendix A.4. Ventilation parameters

Humans have a resting ventilation rate of about 0.1 L/sec at a partial pressure of CO_2 (pCO_2) of 40 mmHg [19]. In times of exercise or stress, both the volume and the rate increase, to a maximum of about 1 L/sec. The minimum ventilation rate is about 0.02 L/sec [20, 21]. These parameters were used to calculate the ventilation system parameters, as experiments have shown it is approximately linear with minimum and maximum thresholds [19].

In mice, the average tidal volume is about 0.10-0.15 mL, and the resting respiratory rate is around 160 breaths/minute [35, 36, 22, 58]. This leads to a resting ventilation rate of about $2.7 \times 10^{-4} - 4 \times 10^{-4}$ L/sec. For the mouse system ventilation parameters we have chosen to use the data from [22], which indicate a ventilation rate of 2.8×10^{-4} L/sec at a blood pCO_2 of 36 mmHg, or 1.08×10^{-3} M/L. From a linear fit, the resting ventilation rate at 40 mmHg is calculated as 3×10^{-4} L/sec. The maximum ventilation

rate is less than in humans, about 5.5×10^{-4} L/sec at a blood pCO_2 of 64 mmHg, or 1.9×10^{-3} M/L. The minimum ventilation is around 1.4×10^{-4} L/sec.

The parameter λ_2, which governs the overall influence of ventilation on carbon dioxide regulation, is dependent on blood volume and gas exchange dynamics in the lungs, and can be calculated experimentally. Despite incorporating several processes, it is possible to calculate the net effect by using the following equation:

$$\frac{\text{Cardiac output}}{\text{Blood volume}} \times ([\mathrm{CO}_2^{\text{venous}}] - [\mathrm{CO}_2^{\text{arterial}}]) = \lambda_2 [\mathrm{CO}_2^{\text{arterial}}] \times \text{Ventilation rate},$$

where the right hand side is the CO_2 loss through the ventilation term in our model, and the left hand side is the actual observed loss rate between the arterial and venous blood (both sides are in moles/sec/litre of blood). The difference in concentration between venous and arterial blood (in mol/litre) is multiplied by the amount of blood circulated through the lungs (cardiac output, in litres/sec), giving the total moles of CO_2 lost per second. Dividing this by the blood volume results in the loss of CO_2 from the blood in moles/litre/sec.

Appendix A.5. Renal physiology

The amount of bicarbonate lost from the bloodstream to the kidney through filtration is proportional to the blood concentration and the glomerular filtration rate (GFR). The GFR is a combined rate of the amount of bicarbonate filtered from the blood in all of the nephrons in the kidney.

As detailed, the net rate of bicarbonate reabsorption is equivalent to the net rate of total acid excretion [17]. Net total acid excretion rate (TAER) is

commonly found experimentally, and can be used to indirectly find the H^+ secretion rate using [18],

$$\begin{aligned} \text{TAER} = \frac{H^+_{\text{excretion rate}}}{V_{\text{blood}}} &= \frac{H^+_{\text{secretion into nephron}} - HCO^-_{3\text{ filtration}}}{V_{\text{blood}}} \\ \therefore \frac{H^+_{\text{excretion rate}}}{V_{\text{blood}}} &= \frac{H^+_{\text{secr. rate}}[CO_2] - GFR[HCO^-_3]}{V_{\text{blood}}} \\ \therefore \frac{H^+_{\text{excretion rate}}}{V_{\text{blood}}} &= \phi_2[CO_2] - \lambda_1[HCO^-_3] \end{aligned} \tag{A.1}$$

where V_{blood} is the volume of blood.

Mice have smaller kidneys with fewer nephrons than in humans (≈10,000/kidney in mice *vs.* ≈1 million/kidney in humans) and their single nephron glomerular filtration rate (SNGFR), is about 1/6 that of a human (60 nl/min in a human, and 10 nl/min in a mouse [59, 48]). However, as mice have a much smaller volume of blood than humans ($1.1 \times 10^{-3} L$ vs $4.7L$), mice filter their blood more times per day than humans.

With these estimates, the filtration parameter, λ_1, can be calculated by the following equation:

$$\lambda_1 = \frac{\text{total GFR}}{\text{blood volume}} = \frac{\text{SNGFR} \times \text{Nephron number}}{\text{blood volume}}. \tag{A.2}$$

So, using λ_1 we can then calculate ϕ_2 from Equation A.1.

Appendix A.6. Kinetic parameters

The HCO^-_3/CO_2 buffering reaction is accelerated by the presence of the enzyme carbonic anhydrase (CA). The apparent pK_a (where pK_a=-$\log_{10}(k_2/k_1)$) of the HCO^-_3/CO_2 is 6.1 at human body temperature [17, 54]. Hence, we use this formula to calculate the dehydration rate, k_1, from experimentally derived values of the hydration rate, k_2.

In the blood, isozyme CA II can accelerate the hydration reaction (represented by k_2) 50,000 to 1,000,000 fold over the uncatalyzed rate at human body temperature [13]. In the tumour, CA II and CA IX are present [14], and the activity of CA IX has recently been found to be as high as CA II [15]. Hence, we assume for simplicity that the catalytic rates in the blood and tumour tissue are equal. We assume in our simulations that carbonic anhydrases accelerate the hydration reaction by approximately 200,000 times.

Appendix B. Sensitivity coefficients

The sensitivity coefficients can be found in Table B.3.

Table B.3: Human and mouse sensitivity coefficients with treatment (human: $\theta_1 = 6 \times 10^{-7}$, mouse: $\theta_1 = 7.6 \times 10^{-6}$). The table indicates how sensitive a variable is to a particular parameter, as detailed in the text. Coefficients in bold highlight the parameters to which the blood and tumour $[H^+]$ is most sensitive (selected by an absolute value greater than 1). Notably, the parameters involved with renal function $\hat{\Phi}_2$ and $\hat{\xi}_1$ are the most important for both mouse and human. Furthermore, parameters involved with tumour proton production ($\hat{\Phi}_1$), ventilation ($\hat{\Delta}_1$ and $\hat{\Delta}_2$), and pK_a ($\hat{\alpha}_2$) significantly affect tumour pHe in humans. Note the small impact of the treatment term ($\hat{\Theta}_1$) at this dose, in line with the numerical simulations. Compared with the same relative dose in humans, the mouse displays a higher sensitivity to treatment ($S_{h_t,\hat{\Theta}_1}$ is -0.17 in a mouse, -0.09 in a human). The parameters comprised of only initial conditions involved in the nondimensionalisation scaling are neglected as discussed in the text.

Variable	$\hat{\Gamma}_1$	$\hat{\Phi}_1$	$\hat{\Gamma}_2$	$\hat{\Gamma}_3$	$\hat{\Phi}_2$	$\hat{\xi}_1$	$\hat{\Theta}_1$	$\hat{\Phi}_3$	$\hat{\Phi}_4$	$\hat{\Delta}_1$	$\hat{\Delta}_2$	$\hat{\alpha}_2$	$\hat{\Phi}_5$	v_T
Human $\tilde{h}_t$	-0.84	**1.10**	0	-0.25	**-1.94**	**1.85**	-0.09	0.15	-0.05	**1.12**	**-1.05**	**-1.0**	0.01	0.01
Human $\tilde{h}_b$	0	0.01	0	0	**-1.05**	**1.0**	-0.05	0.09	0	0.05	-0.05	**-1.0**	0	0.01
Human $\tilde{b}_t$	0.84	-0.85	0	0	1.94	-1.85	0.09	-0.14	0.08	-1.82	1.71	0	0	-0.01
Human $\tilde{b}_b$	0	0	0	0	1.05	-1.0	0.05	-0.07	0.04	-0.99	0.93	0	0	0
Human $\tilde{c}_t$	0	0.24	0	-0.24	0	0	0	0.01	0.03	-0.70	0.66	0	0.01	0
Human $\tilde{c}_b$	0	0	0	0	0	0	0	0.02	0.04	-0.94	0.88	0	0	0
Mouse $\tilde{h}_t$	-0.76	**1.01**	0	-0.25	**-1.64**	**1.75**	-0.17	0.02	-0.36	0.50	-0.11	**-1.0**	0.01	0.01
Mouse $\tilde{h}_b$	0	0.01	0	0	-0.93	**1.0**	-0.10	0.02	0.03	-0.04	0.01	**-1.0**	0	0.01
Mouse $\tilde{b}_t$	0.76	-0.76	0	0	1.64	-1.75	0.17	-0.01	0.67	-0.93	0.21	0	0	0
Mouse $\tilde{b}_b$	0	0	0	0	0.93	-1.0	0.10	0	0.38	-0.53	0.12	0	0	0
Mouse $\tilde{c}_t$	0	0.25	0	-0.25	0	0	0	0.01	0.31	-0.42	0.10	0	0.01	0.01
Mouse $\tilde{c}_b$	0	0.01	0	0	0	0	0	0.02	0.41	-0.56	0.13	0	0	0.01

Table B.3: