SUPPLEMENTAL MATERIAL for Submitted Paper

Table 1 Concentration of species in serum and diffusion rates in normal conditions

Species	**Concentration in Serum (mM)**	**Diffusion Rate (cm^2/s)**
Bicarb	23.8 (Mizumori et al., 2006)	$5x10^{-6}$ (Tanaka et al., 2002)
Glucose	5	$5x10^{-6}$ (Groebe et al., 1994)
O2	0.15	$1.5x10^{-5}$ (Nichols and Foster, 1994)
CO2	1.2 (Mizumori et al., 2006)	$1.5x10^{-5}$ (Tanaka et al., 2002)
H+	$3.98x10^{-8}$ (pH 7.4)	$1.1x10^{-5}$ (Patel et al., 2001)

Table 2 Concentration of species in serum with high bicarbonate concentration (HB)

Species	**Concentration in Serum (mM)**
Bicarb	33.13
CO2	1.66

Table 3 Concentration of species in serum with very high bicarbonate concentration (VHB)

Species	**Concentration in Serum (mM)**
Bicarb	47.88
CO2	2.4

Table 4 Concentration of hypothetical buffer in blood serum with pH= 7.4 for assessment of carbon dioxide effect on bicarbonate buffer effect

Species	**Concentration in Serum (mM)**
A^- (pKa 6.1)	9.32
AH (pKa 6.1)	0.47

Table 5 Diffusion rates of hypothetical buffer in three different scenarios: slower, faster and same diffusion rate as bicarbonate and CO_2

Species	**Diffusion Rate (cm^2/s)**
A^- (Slow)	$2.5x10^{-6}$
AH (Slow)	$0.75x10^{-5}$
A^- (Normal)	$5x10^{-6}$
AH (Normal)	$1.5x10^{-5}$
A^- (Fast)	$7.5x10^{-6}$
AH (Fast)	$2.25x10^{-5}$

Table 6 Uptake constants for O_2 and Glucose

Constant	**Uptake (s^{-1})**
K_{O2}	$9.41x10^{-2}$ (Smallbone et al., 2006)
K_{Glu} (Normal cell)	$1x10^{-5}$ (Patel et al., 2001)
K_{Glu} (Cancer 10x)	$1x10^{-4}$
K_{Glu} (Cancer 50x)	$5x10^{-4}$
K_{Glu} (Cancer 100x)	$1x10^{-3}$

Table 7 Number of healthy and tumor cells for each scenario tested. Despite the loss of tumor cells due to the necrotic core, the scenario with less bicarbonate presents more tumor cells due to invasion of healthy tissue.

Scenario	**Number os Healthy Cells**	**Number of Tumoral Cells**	**Volume of Tumor (mm^3)**
Very High Bicarbonate (VHB)	48,113	15,216	$2.38x10^{-1}$
High Bicarbonate (HB)	48,101	15,228	$2.38x10^{-1}$
NORMAL	45,786	16,332	$3.63x10^{-1}$
Original state	48,113	15,216	$2.38x10^{-1}$

Appendix A- Computer Model Implementation

A.1 Diffusion

The diffusion of species in this model was calculated based on Fick's first law, which relates the diffusion flux through a surface to the difference of concentration of species in volumes separated by this surface (equation S1).

$$J = -D \times \frac{\partial \phi}{\partial x} \qquad \text{(S1)}$$

Where J is the diffusion flux expressed as $\frac{mol}{\mu m^2 \times s}$, D is the diffusion coefficient in $\frac{\mu m^2}{s}$ and ϕ is the concentration of a species in $\frac{mol}{\mu m^3}$.

Two cubic adjacent volumes in this computer model share a contact surface $S = 25\mu m \times 25\mu m$, the distance between centers the two volumes is $d = 25\mu m$ and the time step used in this calculation is $\Delta t = \frac{1}{10} s$. Under these conditions, the variation in concentration of the two volumes due to diffusion can be approximated as being the product of the flux through the contact surface, during the time step, divided by the volume V:

$$C_{t+1} - C_t = \frac{J \times S \times \Delta t}{V} \qquad \text{(S2)}$$

In the general case, each volume in the model is surrounded by six neighboring volumes, thus the general expression used to calculate the diffusion (equation 1) can be derived from S3:

$$C_{t+1} - C_t = \frac{-D \times \sum_{i=1}^{6} \left(C_t - C_{it} \right) \times S \times \Delta t}{d \times V \times 6} = -\sum_{i=1}^{6} \left(C_t - C_{it} \right) \times D_N \quad \text{(S3)}$$

Where D_N is a dimensionless constant used to simplify the final expression.

A.2 Cell Metabolism

The metabolism of cells in this model, both healthy and cancerous, was based on Smallbone (2007). Considering that we search a steady-state solution for our system, we ignore the possible transients from the kinetics of the metabolic reactions, and focus on the stoichiometry of the metabolism of glucose and oxygen into carbon dioxide, lactate, hydrogen ions and ATP.

This simplified mechanism consists on the diffusion of oxygen and glucose from the extracellular environment at fixed rates (simple Fickian diffusion for oxygen and facilitated transport for glucose, equations 2 and 3). Glucose is then metabolized preferentially through respiration (equation 4) and any excess is metabolized anaerobically (equation 5).

This implementation of the model suggests that tumor cells metabolize glucose and produce lactic acid, even in presence of oxygen, not because of malfunctions in mitochondria but due to excess of glucose metabolism, compared to healthy cells.

Representative simulations showing distribution of pHe and CO_2 in and around tumors are shown in Figure S1. The effects of increased serum buffers on those distributions is shown in Figure S2.

A.3 Buffer

The combined buffer effect of bicarbonate and the hypothetical buffer was calculated as below:

In equilibrium the concentrations of both buffers are defined by:

$$\frac{[CO_2]}{[HCO_3^-] \times [H^+]} = 10^{pKaBicarb} \quad (6)$$

$$\frac{[AH]}{[A^-] \times [H^+]} = 10^{pKaHypotheticBuffer} \quad (7)$$

When certain amounts of H^+ (dH) and CO_2 (dCO_2) are added to the solution by glycolysis and respiration, there is an unbalance that must be corrected by the transfer (dX_1, dX_2) of mass from the exceeding species to the rest of the buffer:

$$\frac{[CO_2 + dCO_2 - dX_1]}{[HCO_3^- + dX_1] \times [H^+ + dH + dX_1 + dX_2]} = 10^{pKaBicarb} \quad (8)$$

$$\frac{[AH - dX_2]}{[A^- + dX_2] \times [H^+ + dH + dX_1 + dX_2]} = 10^{pKaHypotheticalBuffer} \quad (9)$$

The solution of this system of equations, where the variables are dX_1 and dX_2, provides the concentrations of species in the new equilibrium.

A.4 Tool Implementation

The model was implemented in the software TSim (www.i-genics.com), written in Java. This tool works by simulating the system, one slot at a time (blood vessel, cell or empty space) and stores the resulting simulation space in a file which is next used to compute the following simulation step. The result is a series of snapshots that can be played as a movie or analyzed one frame at a time.

Each simulation was run on a SGI Altix supercomputer and took four hours on four processors to complete with an average requirement 1GB of memory.

Increases in the precision of the simulation are possible by adding more steps for the diffusion and metabolism. However this slows down the processing time in direct proportion to the number of steps simulated.

Appendix B- A case report

GL is a 79 year old man followed in the GU clinic at the Moffitt Cancer Center. He presented with hematuria in January 2004 and was found to have a large right renal cancer with clot extending into the inferior vena cava (stage T3b, N2, Mx). He underwent a nephrectomy with clot removal at the Moffitt Cancer Center in February, 2004. In June, 2005, he developed metastatic disease in his liver. He was treated with Sutent, but the tumor progressed with metastases developing in the subcutaneous tissues and retroperitoneal lymph nodes. He was unable to tolerate ALT-801. In September 2007 he elected to discontinue all conventional therapy and began a self-administered regimen of vitamins, supplements, and 3 "heaping tablespoons" of sodium bicarbonate in water per day (about 60 grams total). As of the date of this submission, he has maintained this therapy with no complications. His weight is stable. He walks 2 miles every day and had cataract surgery in March 2008 without complications. CT scans from Dec 5, 2007 and April 18, 2008 are shown in figure S3. These images are representative in that some of the liver lesions have increased in size, some have decreased, and some have remained stable. Interestingly, the tumors that were necrotic in the initial scan became much less so on the follow-up study. At the time of this submission the patient remains clinically well.

Supplement Figures

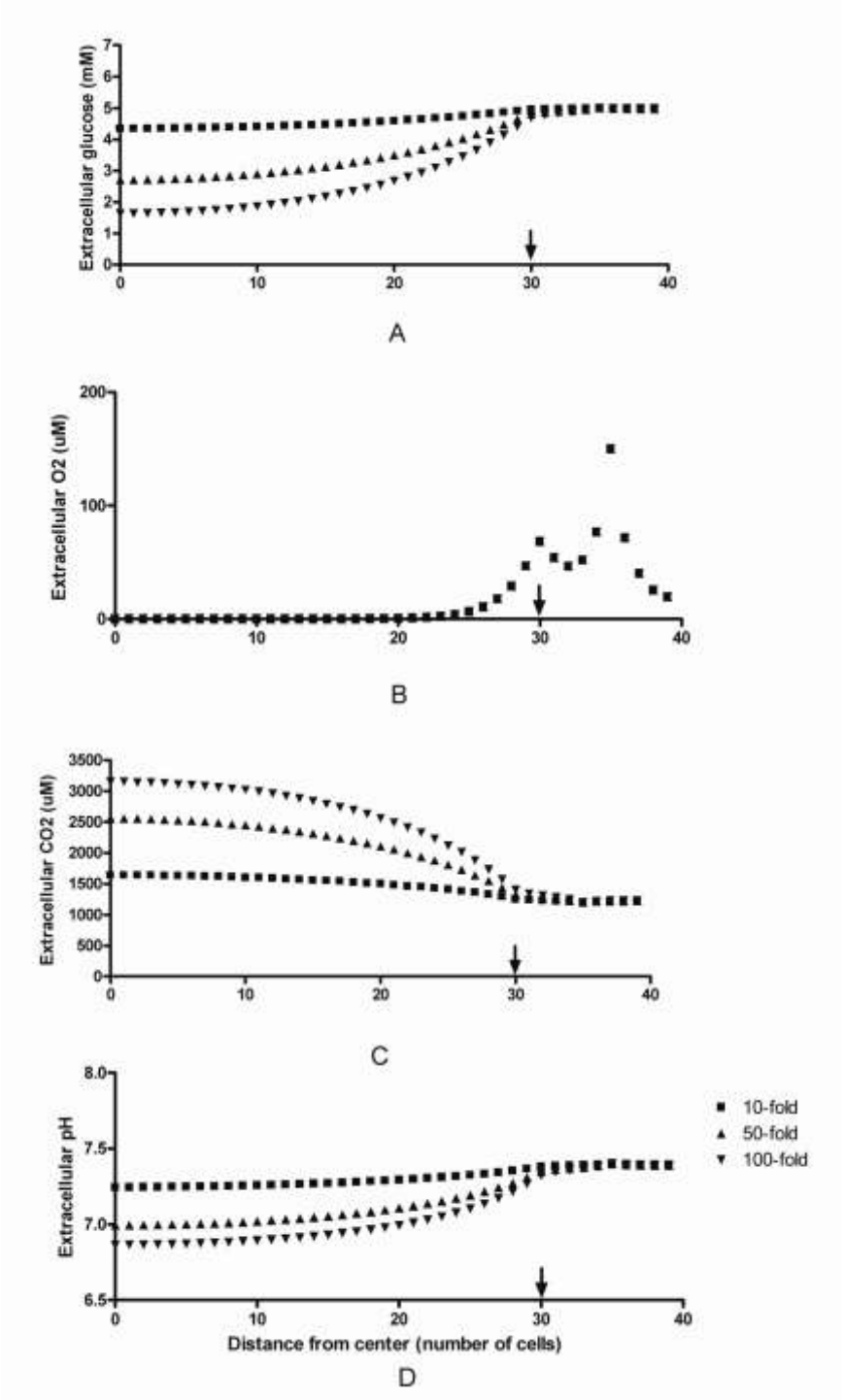


Figure 1

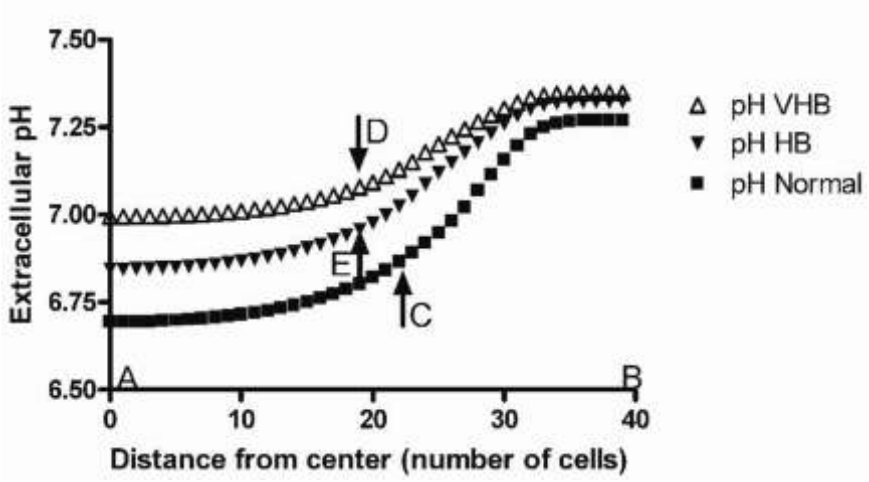


Figure 2

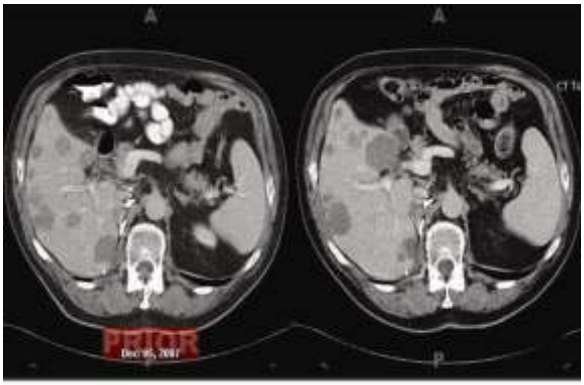
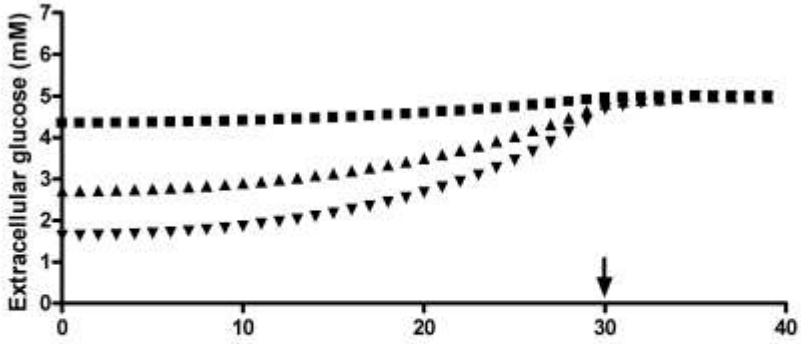
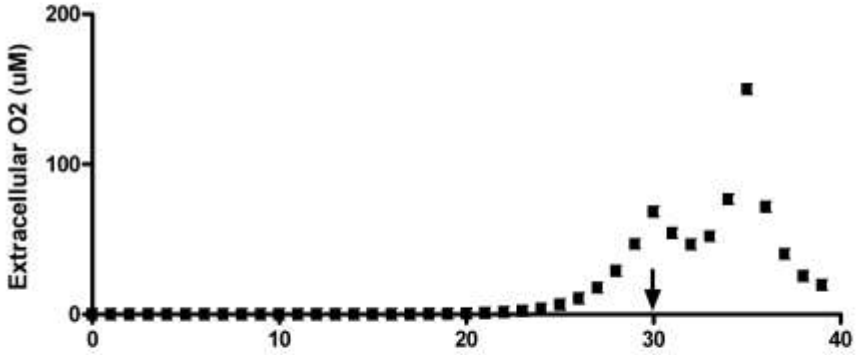


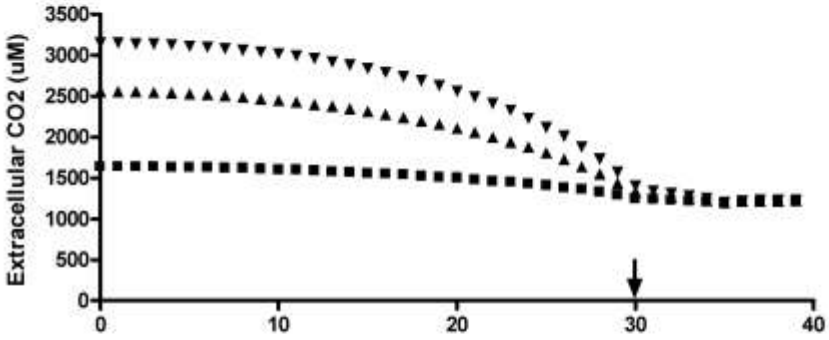
Figure 3


Extracellular glucose (mM)
0
1
2
3
4
5
6
7
0
10
20
30
40

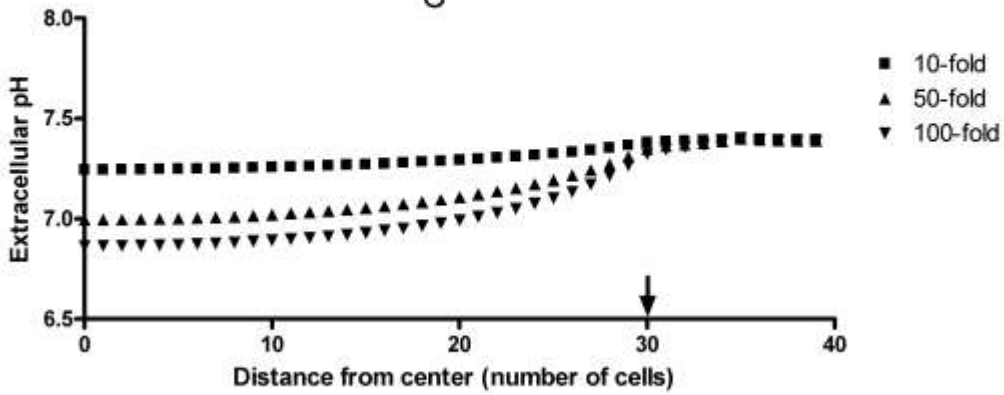
A


Extracellular O2 (uM)
0
100
200
0
10
20
30
40

B


Extracellular CO2 (uM)
0
500
1000
1500
2000
2500
3000
3500
0
10
20
30
40

C


Extracellular pH
6.5
7.0
7.5
8.0
0
10
20
30
40
Distance from center (number of cells)
10-fold
50-fold
100-fold

D

Figure S1 Gradients of extracellular glucose, O_2, CO_2 and pHe for three different tumor phenotypes (10-, 50- and 100-fold increase in glucose metabolism) and normal serum bicarbonate concentration. The vertical arrows mark the tumor-host interface. The spikes of O_2 concentration are due to the presence of a blood vessel in the position 37 and a second blood vessel at position 31 on an adjacent plane.

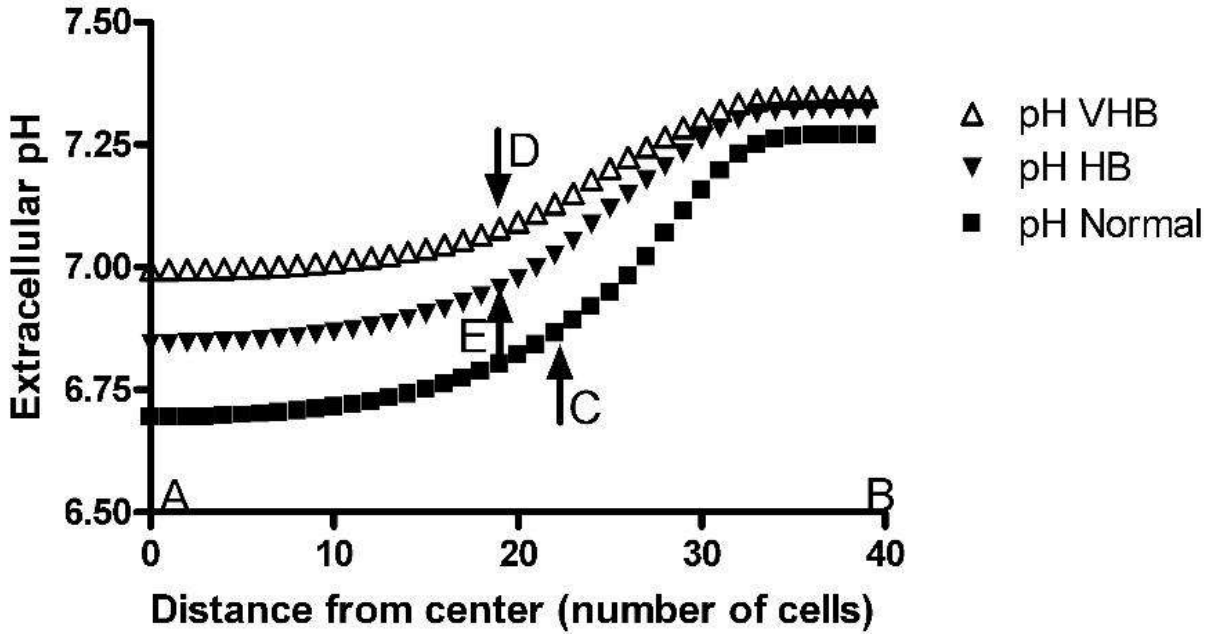


Figure S2. pHe gradients for three tumors of original diameter of 60 cells as described in figure 5. After 20 generations the untreated tumor (Normal) presents lower pH curve and invasion of healthy tissue (C) when compared to tumors treated with bicarbonate (E and D). Concentrations of bicarbonate administered for HB and VHB as described in text.

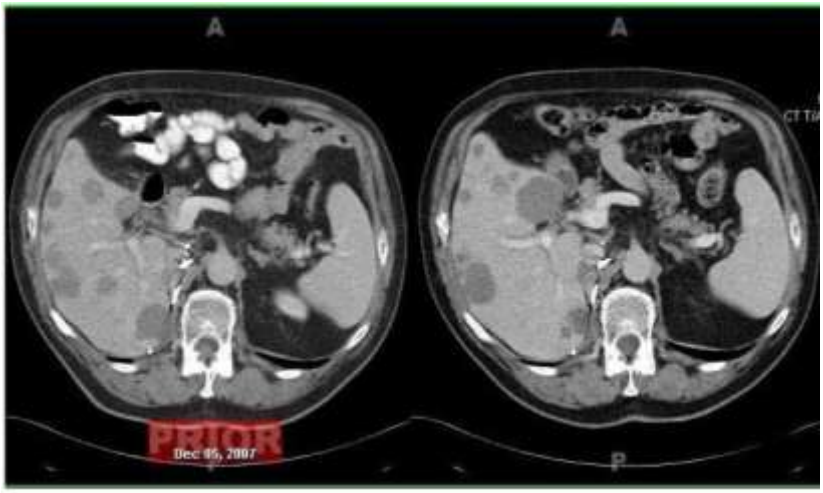


Figure S3. CT scans of the liver from patient with metastatic renal cancer self-administering 40grams of NaHCO3 daily and receiving no conventional therapy since September 1, 2007. Scans from Dec 5, 2007 (left) and April 18, 2008 (right) are shown. These images are representative in that some of the liver lesions have increased in size, some have decreased, and some have remained stable. Interestingly, the central necrosis seen in several of the tumors on the initial scan was no longer present on the follow-up study.